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EDITORIAL COMMUNICATION

The objective of the special issue of Bioscience Biotechnology Research Communications Vol 13 No (12) 2020 on "**Recent Research on Intelligent Systems, Data Science Communication and Computing**" is to provide a platform to researchers to publish original research work in different avid areas related to Intelligent Systems, Data Science, Communication and Computing.

We are happy to share that quality research work addressing important issues in the field of data science, communication systems, computational intelligence, machine vision, robotics and smart systems etc. are published in this special issue. This Special issue also has articles related to Intelligent System, Communication, Computing, Data Science and applications, COVID-19 have been published in this issue.

This special issue aims to foster the growth of a new research community, acting as an international forum for researchers and practitioners in academia and industry to present research that will definitely play a very important role in changing the landscape of our near future.

The published research articles have been aimed to motivate the next generation researchers working in various emerging research areas. The articles published in this issue will be helpful for the researchers working in these new emerging areas. We express our heartfelt gratitude to all the contributors from different colleges and universities of India and Abroad for giving us an opportunity to publish their research work in this Special Issue on Recent Research Intelligent Systems, Data Science, Communication and Computing.

Guest Editors

Current Research Trends in Management, Science and Technology

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Anti-Nutritional Factors in Plant-Based Aquafeed Ingredients: Effects on Fish and Amelioration Strategies

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ABSTRACT

Limited supply and high demand of fish meal made it imperative to use plant derived feed ingredients such as seeds of legumes (lupin and peas), oil seed cakes (soy bean, cottonseed, and rape seed), cereals (corn, rice and wheat), meal of protein rich leaves, concentrates and isolate of non-edible oil seeds (jatropha, castor, karanj and neem) as a fish feed ingredients. However, the major challenge in utilising the protein rich plant derived ingredients as fish feed is the presence of anti-nutritional factors. The most widely distributed anti-nutritional factors among potential alternatives are protease inhibitors, phytic acid, saponin, tannin, cyanide, oxalate, gossypol, non-starch polysaccharides, phytoestrogens, mimosine. We need to remove or ameliorate the effects of these anti-nutritional factors for the incorporation of the plant ingredients. There exists a species-specific tolerance limit to each anti-nutrients which needs to consider before determining their amelioration techniques. The effects of these anti-nutrients along with the techniques employed to remove them have been discussed in this article.

KEY WORDS: ANTI-NUTRITIONAL FACTORS; AMELIORATION STRATEGIES; FISH; PLANT INGREDIENTS

INTRODUCTION

Aquaculture has become an important sector for improving food security, raising nutritional standards and alleviating poverty, especially in the light of increasing global population which is projected to reach 10 billion by 2050 (FAO, 2016). Global aquaculture sector grows faster than any other food producing sector with a growth rate of 5.8% during 2001-2016 (FAO, 2018). To sustain such high rates of increase in aquaculture production, a matching increase in the levels of production of fish feeds is required.The most immediate challenge to aquafeed industry is the availability of quality feed ingredients that not only meet the nutritional requirements of fish but also minimise production cost, limit environmental impacts and enhance products quality. In this regard, fish meal is the most utilised traditional dietary protein ingredient in aquaculture diets because of its high protein content, balanced amino acid profile, high digestibility and palatability (Drew et al., 2007). However, inconsistent supply, localised production, depleted fish stocks, greater demand, and rising prices have made it imperative for fish feed industry to look for more economical and sustainable alternatives.

In search of ingredients to replace totally of partially the fish meal, soybean meal is the most commonly used alternative in many aquaculture species (Gopan et al., 2019b). The nutrient quality such as amino acid profile and high digestibility are the reasons for choosing soyabean as the alternative for fish meal especially in

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freshwater species. However, using soyabean as a fish meal replacer is also not sustainable as it has high demand and competition from other sectors holds high cost and its incorporation in fish feed will erode the expected profit. Similarly, most commonly used other plant products such as ground nut, mustard, rice bran and other legumes seeds possess same problem. Thus, finding alternatives to commonly used plant derived ingredient is a major concern for nutritionists. Another option is to utliise the by-product of commonly used plant ingredients. The plant-derived nutrient sources and their by product may become very good alternatives but they are known to contains a wide variety of anti-nutritional substances. The presence of this naturally occurring anti-nutritional substance are the sole factor which limit their utilisation in the fish feed (Gopan et al., 2019a, b). These anti-nutritional factors are the part of defense system of plants against different diseases and pests. However, they pose several adverse effects on animals, especially on the non-ruminants.

Table 1. Important anti-nutritional factors present in plant derived nutrient source

Plant-derived nutrient source	Anti-nutritional factors
Ground nut oil cake	Protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, NSP
Mustard oil cake	Glucosinolates, tannins
Sunflower oil cake	Protease inhibitors, saponins, arginase inhibitor
Linseed oil cake	Cyanogens, Phytic acid, phytoestrogen, anti-vitamins (anti-thiamin& anti-pyridoxine)
Rubber seed oil cake	Cyanide, Phytic acid, Tannin, Trypsin inhibitor
Neem seed oil cake	Azadirachtin, Cyanide, Phytic acid, oxalate, Tannin
Karanja oil cake	Karanjin, phytic acid, Tannin, Trypsin inhibitor
Caster seed oil cake	alkaloid ricinine, tannin, phytate and oxalate
Soybean meal	Protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, anti-vitamins, allergens
Cottonseed meal	Phytic acid, phytoestrogens, gossypol, anti-vitamins, cyclopropenoic acid
Sesame meal	Phytic acid, protease inhibitors
Rapeseed meal	Protease inhibitors, glucosinolates, phytic acid, tannins
Lupin seed meal	Protease inhibitors, saponins, phytoestrogens, alkaloids
Pea seed meal	Protease inhibitors, lectins, tannins, cyanogens, phytic acid, saponins, anti-vitamins
Leucaena leaf meal	Mimosine
Alfalfa leaf meal	Protease inhibitors, saponins, phytoestrogens, anti-vitamins
Jatropha curcas meal	Phorbol esters, Trypsin Inhibitors, Lectin, Cyanogenic glycosides, glucosinolates, amylase inhibitors, Saponins, Tannins, Phytates, Non-starch polysachharides
Sweet potato leaf meal	Phytic acid, tannin, oxalate, saponin, trypsin inhibitor, alkaloid, cyanide
Hygrophila spinosa leaf meal	Phytic acid, oxalate, tannin, alkaloids
Sesbania aculeata leaf meal	Phytic acid, tannin, alkaloids, oxalate Rice Protease inhibitors, lectins, phytic acid, anti-vitamins
Wheat	Protease inhibitors, lectins, phytic acid, NSP
Corn	Protease inhibitors, lectins, NSP
Duckweed	Protease inhibitors, Cyanogens, tannin, gossypol
Grass pea	Protease inhibitors, phytic acid, tannin, lathyrogen
Azola	Protease inhibitors, phytic acid, tannin, gossypol
Pistia	Protease inhibitors, saponins, tannin
Eichornia	Protease inhibitors, phytic acid, cyanogen, saponins, tannin
Chick pea	Protease inhibitors, phytic acid, cyanogen

Anti-nutritional factors: The anti-nutritional factors (ANFs) may be defined as substances which by themselves or through their metabolic products arising in living systems, interfere with nutrient utilisation and affect the health and production of animals (Makkar, 1993). The substances which produce direct health effects are called toxic principles. In nature, plants can synthesise certain antimetabolites which exert a deleterious effect upon ingestion by animals. The plant synthesises these chemical substances for self-defence.

Plants contain a wide range of compounds which can have beneficial or harmful effects on organisms consuming them by influencing the metabolic pathways. ANF causes sublethal effects such as lower feed intake, poor feed conversion, reduced growth rate, hormonal alterations, and organ damage (Francis et al., 2001). Thus, the anti-nutritional factors restrict the use of the plant ingredients in the feed. Although plant-based ingredients are deficient in some essential amino acids, it can be rectified by supplementing them in the diet. However, the ANF should be eliminated from the ingredients to use different plant-derived materials as alternative feed ingredients.

Classification of Anti-nutritional Factors of Plant ingredients

According to the chemical nature, ANFs in plant ingredients can be classified into four major groups such as,

1. Proteins: includes protease inhibitors, hemagglutinins, toxic amino acids and food allergens
2. Glycosides: which includes goitrogens, cyanogens, saponins and estrogens
3. Phenols: Gossypol and tannin
4. Other phytochemicals, such as phytic acids, anti-vitamins, and anti enzymes.

According to the mode of action ANFs are broadly divided into four groups such as 1) which affect protein utilisation and digestion example protease inhibitors (trypsin inhibitor, chymotrypsin inhibitor), tannins, and lectins (haemagglutinin) 2) Which affects mineral utilisation such as phytates, gossypol, oxalates, glucosinolates 3) Which affects utilisation of anti-vitamins such as tannins 4) miscellaneous substances like mycotoxins, mimosine, cyanogens, nitrate, alkaloids, photosensitising agents, phytoestrogens and saponins and phorbol esters(Francis et al., 2001)

Based on the ability to withstand the thermal processing, which is commonly employed to destroy them the ANFs are classified into heat-labile factors represented by protease inhibitor, phytates, lectins, goitrogens and anti-vitamins whereas heat-stable factors such as saponin, non-starch polysaccharides, antigenic protein, estrogens and phenolics compounds. ANFs may also be classified as per the source such as endogenous, which includes ANFs which is present in the feed ingredient itself. Such as protease inhibitor, tannin, lectin, phytate, gossypol, oxalate. Exogenous ANFs which includes substance produced by extraneous agents represented by mycotoxins produced by the fungus. Aflatoxins, zearalenone, deoxynivalenol, fumonisins, ochratoxin and trichothecene, are examples for mycotoxins. Aflatoxins produced by Aspergillus flavus is the most common and dangerous one in fish feed. Important ANFs present in alternative fish feed ingredients are depicted in the table 1.

Table 2. Important anti-nutritional factors and their harmful effects, dietary tolerable level and amelioration techniques.

Anti-nutritional factors	Harmful effects	Dietary tolerable level	Amelioration techniques
Protease Inhibitors	i)Pancreatic hypertrophy /hyperplasia ii) Reduced protein digestion and amino acid utilization iii) Reduced growth	i) Salmonids: <5 mg/g ii) Nile tilapia: < 1.6 mg/g iii) Carp: > 8.3 mg TI/g iv) Channel catfish: 2.2 mg/g	i) Moist heat treatment (autoclaving) for 15–30 min ii) Extrusion or steam cooking iii) Fermentation iv) Germination v) Supplementation of essential amino acids, especially S-containing amino acids, to compensate the unavailable amino acids.
Phytic acid	i) Reduced protein, carbohydrate and minerals utilisation and growth in ii)reduced muscle ash content iii)skeletal deformity iv) reduced thyroid function v)Promotion of cataract formation vi) Abnormal pyloric caecal structure resulting in depressed absorption of nutrients vii) Increased mortality.	i)Carps, tilapia, trout, fish salmon and shrimp: <5 g/kg or <0.5%	i) Dietary phytase supplementation ii) Fermentation of feed ingredients yeast or lactic acid bacteria iii) Milling of outer layer iv) Supplementation of mineral premix v) Microwave irradiation & E-beam irradiation vi) The additional supplementation of Zn to prevent cataract vii) Aqueous extraction (18h) viii) Moist heating (120oC for 2h)

Table 2 Continue

Glucosinolates	i) Progoitrin and epi-progoitrin impair palatability & reduced feed intake ii) The thiocyanates, allyl isothiocyanate and Goitrin interfere with iodine availability being the most potent antithyroid agents iii) Antithyroid activity leads to less production of T3 & T4 that affects metabolism resulting in depressed growth iv) Nitriles are known to affect liver and kidney functions with severe damage	i) Common carp (Cyprinus carpio) and other fish: <0.4 mg/g diet or 3.6 µmol/g diet ii) The carnivorous fish species are generally more susceptible to glucosinolate toxicity than omnivorous/herbivorous fish species	i) Water Extraction is a cost-effective method however leaching of essential nutrients is a problem ii) Heat treatment (extrusion cooking & wet pressure -cooking) iii) Readily removed by extraction with dilute alkali or organic solvent mixtures iv) Treatment with Copper sulphate solution v) Microwave irradiation & E-beam irradiation vi) Iodine supplementation in the diet of and ruminants vii) Selective breeding programmes to yield low glucosinolate rapeseed/ mustard varieties.
Saponins	i)Reduce palatability and feed intake ii)reduced feed efficiency & growth iii)reduced reproductive performances iv) respiratory distress v) death.	i) Carp and other fish: < 1 g/kg of diet ii) iii) Ornamental fish: yet to be established.	i) Aqueous extraction but leaching of nutrients should be taken care of ii) Extraction with ethanol iii) Cholesterol supplementation.
Tannins	i) Due to undesirable bitter taste they reduce palatability and feed intake ii) Reduced feed efficiency & growth iii) Damage of liver and kidney lead to death.	i) Ruminants: up to 15000 mg/kg feed (1.5%) ii) Laying hens: up to 10000 mg/kg feed (1%) iii) Rabbit: up to 10000 mg/kg feed (1%) iv) Pig: up to 1500 mg/kg feed (0.15%) v)Up to 15 mg/kg feed is safe for all animal species	i) Dehulling of seeds to remove the tannin-rich outer layer ii) Treatment with alkali iii) Treatment with ferrous sulphate (oxidising agent) iv) Treatment with tannin complexing agents polyethene glycol v) Soaking and drying and heat treatment (autoclaving) vi) Fermentation with lactic acid bacteria vii) Microwave radiation and E-beam irradiation.
Cyanogens	i) reduced feed efficiency and growth ii) death in excess dose	i) Livestock: up to 100 mg/kg feed on DM basis (0.01%). The lethal dose of HCN for cattle and sheep is 2.0-4.0 mg per kg body weight ii) Human: The lethal dose of HCN taken by mouth in humans has been estimated to be between 0.5 and 3.5 mg/kg body weight	i)water soaking(24h) followed by sun-drying ii) Boiling followed by sun-drying iii) Roasting iv)Microbial fermentation v) Grinding followed by sun-drying vi)Microwave radiation and E-beam irradiation.

Table 2 Continue

Gossypol	i) Constipation ii) Depressed appetite iii) Loss of weight iv) The toxic, systemic effects of gossypol exhibited through reduction of haematocrit, haemoglobin, reproductive capacity as well as lesions in the liver, kidney, spleen and gonads may develop v) Death.	i) Fish: <20 mg/kg feed recommended for all animals but some fish can tolerate the level between up to 100-<300 mg/kg diet	i) Heat treatment: Roasting, extrusion ii) Irradiation: Gamma or E-beam irradiation iii) Fungal fermentation iv)Nutritional supplementation: a) Deficient amino acids, such as cysteine, lysine, and methionine b) Ferric sulphate c) Sodium selenite d) Vitamin E
Haemagglutinins or lectins	i) agglutination of RBC ii)reduction in the absorption of nutrients from the gut or alimentary canal iii)Internal haemorrhages iv) reduction in growth.		Heat treatment. Moist heat treatment (autoclaving) is more effective than dry heat treatment.
Oxalate or oxalic acid	i) formation of calcium magnesium oxalate stones in the kidney concomitant kidney failure ii) growth and immunity depression	ruminants < 2% dietary oxalates and for non-ruminants < 0.5% to avoid oxalate poisoning	Heat treatment
Phorbol ester	i) Feed rejection ii) growth reduction, severe damage of intestine liver and kidney, death at high dose	Common carp above 3.75 ppm caused an adverse effect	Autoclaving, Fermentation,

Major ANFs and their effects and amelioration: Based on the chemical nature of the ANFs and amount present in the ingredients, their efficacy against nutrient utilisation and fish health. Possible mode of action for different anti-nutrients are discussed below based on the available information. Harmful effects of ANFs dietary tolerable level and their amelioration process are discussed in table 2.

Protease Inhibitors: The factors which cause an adverse effect on the nutritional value of proteins are known as protease inhibitors. These are the entities that exhibit the ability to inhibit the proteolytic activity of some enzymes. They are found in the entire plant kingdom, particularly among the legumes, such as soybean (Norton, 1991). Biochemically, they fall under two categories such as i) Kunitz inhibitors with a molecular weight of 20,000 to 25,000 Da with few disulfide bonds which inhibit trypsin and ii) Bowman-Birk inhibitors with a molecular weight of only 6000 to 10,000 Da, rich in cysteine and inhibit chymotrypsin as well as trypsin at the independent binding site (Makkar et al., 2007). Commercial soybean products mostly show trypsin inhibitors (TI) in the range of 2–6 mg/g, averaging 4 mg/g. Protease inhibitors inhibit the activity of enzymes within the gastrointestinal tract of animals by binding with chymotrypsin and/or trypsin to form stable complexes thus preventing access to the active site of the enzyme leading to decreased protein digestibility.

Phytic acid or Phytate: Phytates or Phytic acid is one among the most potent anti-nutritional factors in plant feedstuffs. It is a common storage form of phosphorus in plant seeds. Phytate (hexaphosphates of myo-inositol) can chelate with di- and trivalent mineral ions such as phosphorus, calcium and magnesium, trace elements such as iron and zinc, and protein and amino acids (D'Mello et al., 1991). Commonly used and potentially usable plant-derived fish feed ingredients such as soybean meal, rapeseed meal, and sesame meal contain 10–15, 50–75 and 24 g/kg phytate, respectively (Francis et al., 2001). Inclusion of phytate containing ingredients in the diet has negatively affected the growth in commonly cultured fish species as most of the fishes do not have endogenous enzymes to break down phytate and release nutrients. Thus, they pass through the gut undigested. Therefore, more significant proportions of valuable nutrients from plant sources are becoming unavailable for aquatic animals and are wasted as excreta. Phytates also form meagrely digestible phytate–protein complexes, resulting in reduced digestibility and availability of protein for

muscle growth (Richardson et al., 1985; Makkar et al., 2007). Growth and nutrient utilisation efficiency of different fish species fed phytate-containing diets were reported to be adversely affected (Francis et al., 2001).

Saponins: Saponins are triterpenoid or steroidal glycosides which are characterised by their hemolytic and foam producing properties and impart a bitter taste. They are found in feed ingredients like oilseed cakes, legumes etc. Mostly, the leguminous seeds contain 18-41 mg/kg, and defatted soya-bean meal contains 67 mg/kg of saponin (Fenwick et al., 1991). Even though saponins are found in many of the potential alternative fish feed ingredients, they are highly toxic to fishes due to their interaction with cellular membrane component. It can hemolyse red blood cell with nonspecific interactions with membrane proteins. Saponin is the active ingredient in the mahua oil cake which is used as a fish poison to kill unwanted fishes in the culture system.

Tannins: Tannins are secondary compounds of various chemical structures widely occurring in the plant kingdom and are divided into hydrolysable and condensed tannins. They interfere with the digestive processes either by binding the enzymes or by binding to feed components like proteins or minerals and reduce the absorption of vitamin B12 (Francis et al., 2001). Plant ingredients tested as alternative nutrient sources in fish feed such as rapeseed meal, pea seed meal, mustard oil cake, do contain tannin. Hydrolysable tannin can be broken down by acid, alkali, and some hydrolytic enzymes present in the biological systems, thereby forming smaller compounds that can enter the bloodstream (Francis et al., 2001). Feeding on diets containing high levels of hydrolysable tannins causes toxicity to vital organs and lead to the death of the animal (Francis et al., 2001; Makkar et al., 2007). Contrarily, condensed tannins are highly resistant to degradation. Consumption of plant ingredients containing condensed tannins can cause a reduction in feed intake nutrient utilisation and growth (Makkar et al., 2007).

Oxalates: Oxalate is an anti-nutritional factor which affects mineral utilisation; it is present in appreciable amounts in fish feed ingredients (Francis et al., 2001). Oxalic acid is a metabolic product formed through several pathways in plants and animals. .Oxalates of monovalent ions, such as sodium, potassium or ammonium are well soluble in water however those oxalates formed with divalent ions, such as calcium, magnesium and iron are almost insoluble (Libert and Franceschi, 1987; Savage et al., 2000).Oxalates chelates with dietary calcium and with other divalent metals with specific concentration (Abara et al., 2000). Thus calcium is limited, unavailable for absorption, making it less bioavailable for bone formation and various metabolic activities as well as for the cofactor requirements of many enzymes (Hajra et al., 2013). In mammals, long-term exposure to a high-oxalate diet may lead to the formation of calcium magnesium oxalate stones in the kidney, which can cause urine flow problems or kidney failure (Noonan and Savage, 1999). According to Rahman et al. (2013), oxalate consumption should be less than 2% for ruminants dietary to avoid oxalate poisoning and less than 0.5% for non-ruminants.

Cyanides: Hydrogen cyanide also called prussic acid, is an organic compound with the chemical formula HCN. It is a colourless, extremely poisonous liquid that boils slightly above room temperature, at 25.6°C. Cyanides are one of the most common anti-nutritional factors in plant kingdom which exist in the form of cyanogenic glycosides (or cyanogen) (Makkar et al., 2007). Cyanogens are found in high concentrations in several pulses, root crops, such as cassava, and some oil seeds, such as linseed, which have been tried as fish feed ingredients (Francis et al., 2001). Cyanogens are glycosides of sugar and cyanide containing aglycon, which generally taste bitter. Intact cyanogenic glycosides are not toxic, but when hydrolysed by an intracellular enzyme β-glucosidase, produces toxic products such as hydrogen cyanide and some carbonyl compounds.

These compounds suppress natural respiration and cause cardiac arrest (Davis, 1991) Cyanide can alter glucose metabolism. Sadati et al. (2013) reported that cyanide exposure at the dose of 0.2 mg/L caused a significant increase in glucose concentration in common carp. Cyanide can also cause a reduction ATP/ADP ratio and shift towards anaerobic metabolism resulting in elevated lactate levels (Way, 1984). Sadati et al. (2013) reported increased activity of LDH enzyme in the serum, as a characteristic feature of lactic acidosis and an indication of anaerobic glycolysis when common carp exposed to a sublethal dose of cyanide (0.1-0.2 mg/l).

Gossypol: Gossypols are polyphenolic pigments, which are seen to be concentrated in the pigment glands of the genus Gossypium (cotton plant). Glandless cottonseed meal has significantly low gossypol (< 0.01%) but the glanded varieties contain about 1.3% of gossypol that appears to be toxic to fish. Gossypol also forms gossypol protein complex and may form deficiency of some amino acids like the 'methionine', which plays a role in fat metabolism. Gossypol reduces the bioavailability of another limiting amino acid, 'lysine'. Feeding formulated diets to fish containing appreciable amounts of gossypol, like cottonseed meal causes growth depression, intestinal and other internal organ abnormalities and anorexia. Gossypol reacts with iron and forms an inactive ferrous gossypolate complex. As a result, dietary iron can be successfully used to neutralise or counter the undesirable effects of gossypol in formulated fish feeds used for gossypol sensitive fish species.

Non-starch polysaccharides: Non-starch polysaccharides and oligosaccharides are present in a wide variety of plants such as grains, legumes and cereals. The principal oligosaccharides reported from soybean are sucrose, raffinose and stachyose. Pectin and cellulose are also non-starch polysaccharides which leads to a decreased nutrient utilisation of nutrients (Choct et al., 2010). High NSP in the feeds found to reduce the digestibility of fat by binding with minerals in the intestine (Storebakken et

al., 1998). Sunflower, lupin and soybean contain pectins, galactans, cellulose and lignin. The diets containing the above substances have decreased feed intake and feed digestibility in hybrid striped bass and rainbow trout (Gallagher, 1994; Sanz et al., 1994).

Phytoestrogens: Non-steroidal estrogenic substances distributed in plants are known as phytoestrogens which present mostly in soybean, cottonseed, linseed and sunflower seed. Plant estrogens are chemically isoflavones that exist in the form of glycosides they can bind directly to estrogen receptors or convert into compounds that have estrogenic effects (Francis et al., 2001). Dietary estrogen has wide-ranging effects on many physiological processes in fishes like the induced vitellogenesis or an increase in plasma vitellogenin level (Kaushik et al. 1995).

Mimosine: It is an unusual amino acid, which affects the production of thyroxine and hence the growth of the organism. Although structurally resembles the amino acid, tyrosine, it functions as an antagonist to this amino acid. The deleterious properties of mimosine include, disruption of reproductive processes and teratogenic effects (D'Mello, 1991). Mimosine is reported from Leucaena leucocephala (ipil ipil) and contains about 3–5% of the total protein on a dry weight basis (Liener, 1989). This was used as alternative feed ingredients in fish feeds. Soaking in water was found to be effective in removing the mimosine content.

Azadirachtin: Azadirachtin is a triterpenoid, which is the main active component in the neem seed cake. Azadirachtin is concentrated in the kernel around 4-6 g of azadirachtin is found in 1 kg of seed. In neem, azadirachtin is considered as the main agent for controlling insects. It is soluble in polar organic solvents and slightly soluble in water (Morgan, 2009; Gopan et al., 2019a). Nisbet (2000) has detailed the antifeedant and toxic effects of azadirachtin in insects. A feeding deterrent or antifeedant has been defined as a chemical which inhibits feeding but does not kill the insect directly, the insect often remaining near the treated plant and possibly dying through starvation (Munakata, 1975; Morgan, 2009). The presence of azadirachtin hampers the utilisation of neem seed cake in livestock and aqua feeds, make it non-edible. Thus removal of this active principle is imperative to utilise the seed cakes. It is highly soluble in organic compounds; thus extracting with solvents is an effective method to remove it. Isolating protein from the seed cake also found to be adequate to remove the ANFs in the neem seed cakes (Gopan et al., 2019a).

Amelioration techniques: Several techniques are employed for ameliorating ANFs in plant-derived feed ingredients, which can be broadly classified as i) physical methods such as heating washing, pressure and dehulling. ii) Chemical methods include the use of alkali, acids, solvent, urea, ammonia, ions, salts etc. iii) Biotechnological techniques which include the application of microorganisms and genetics. Lastly, a combination of several techniques such as water soaking, heat treatment, fermentation, irradiation, supplementation of enzymes, essential amino acids and genetic modifications are used to ameliorate ANFs. Some of the commonly used amelioration processes are discussed below.

Soaking: It is an age-old practice used to eliminate ANFs from plant-derived feed ingredients. Soaking in water have been used by many researchers to improve the digestibility of the plant feed ingredients. Among the pre-processing methods to eliminate ANFs soaking was found to be effective method in many feedstuffs (Nwosu, 2010) Soaking can be done in a variety of solvents based on the solubility of the anti-nutrients to be removed. However, the major drawback is the nutrient leaching, i.e., loss of valuable nutrients in the soaking medium.

Extrusion cooking: It is another widely used method to remove anti-nutrients from plant-derived feed ingredients. During the extrusion process, high temperature (90-150°C) is applied along with pressure and moisture. Thus it eliminates all the heat-labile ANFs in the feedstuff along with other pathogens if present. At the same time, it improves the nutritional quality of the feedstuff by enhancing the digestibility. Extrusion treatment has been applied to improve the quality of some legume seeds such as soybean or rapeseed, alone or blended with peas.

Fermentation: It is an innovative approach by which nutrient digestibility bioavailability can be improved. Solid-state fermentation is mainly used for aquafeed ingredients. It is also an integral part of the food detoxification process, which significantly lowers the anti-nutritional content, also improves the nutritive value of food grains (Yuan et al., 2013). Fermentation of the oilseed meal with lactic acid bacteria resulted in a reduction of the tannin content, thus increased feed efficiency and contributed to enhanced growth response. Controlled fermentation encourages the multiplication of particular organisms and their metabolic activities in food. Apart from the inactivation of anti-nutrients, fermentation with probiotic organisms may contribute to bio-enrichment.

CONCLUSION

Quest for alternate feed ingredients to replace fish meal and fish oil addressed the need for utilising the plant-derived feed ingredients. Recently more efforts have been made in the field of utilising non-conventional feed ingredients to minimise the competition from others feeds producing sectors. As the presence of ANFs is the main factor which affects the utilisation of the non-edible plant seeds in the feed industry, it is the high time to remove it from the ingredient to improve their utilisation. In the case of conventional plant feedstuffs, the presence of anti-nutrients will not lead to mortality at the level they exist. However, it can produce an adverse effect on the fish but varies with different factors such as species, kind, level of the anti-nutrients and the culture systems. Hence, there is a need for a species-specific approach

to investigate the effect of plant-derived anti-nutrients before employing effective elimination strategies.

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Fish Oil's Beneficial Effects to Human Health: A Review

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ABSTRACT

Since our body cannot de novo synthesise fatty acids, they be consumed by diet. Fish oil is a major source of essential fatty acids, Omega 3 (ω–3) and Omega 6 (ω–6). This fatty acid prevents major heart diseases, cancer, diabetes, mental disorders, arthritis, and many other health problems. Fish oil therefore contributes a large number of beneficial effects to us. It must be used according to the recommended dosage. Consumption of 1.0g of fish oil per day will help the human being fight coronary heart disease. Fish oil supplements have many advantages over the direct consumption of fish.

KEY WORDS: FISH OIL, CORONARY HEART DISEASE, POLYUNSATURATED FATTY ACIDS (PUFA), EICOSAPENTAENOIC ACID (EPA), DOCOSAHEXAENOIC ACID (DHA).

INTRODUCTION

Fatty acids are aliphatic single-carboxylic acids derived in animal or vegetable fat (oil or wax) or contained in esterified form. One type of fatty acid is Essential Fatty Acids (EFA). In our body, they cannot be de novo synthesised. Because our body lacks the enzymes needed to make certain fatty acids, they must be obtained from plant or animal sources through a diet. However, as they are involved in important biological processes, they are very important to us. The essential fatty acids, ω–6 EFAs and ω–3 EFAs, are polyunsaturated and grouped into two families. For instance, the body can convert one ω–3 to another ω–6, but cannot create a ω–3 from ω–6 or saturated fats. Essential fatty acids are so called because we can't survive without them. From the tissues of oily fish, fish oil is derived. For a healthy diet, it is recommended because it contains ω–3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), precursors to eicosanoids that reduce inflammation throughout the body, as reported by Sargent (1997). For optimum health benefit, cellular metabolism and normal physiological functions, fish oils must be added in minute quantities (Sau and Paul, 2004).

In the early 1970s, when Danish doctors observed that, despite consuming a high-fat diet, Greenland Eskimos had an exceptionally low incidence of heart disease and arthritis, scientists learned about many advantages of fish oils. Intensive research soon found that EPA and DHA, two of the fats they consumed in large quantities, were actually extremely beneficial. There are numerous beneficial effects of fish oil on the human body. Atherosclerosis, angina, heart attack, congestive heart failure, arrhythmias, stroke and vascular peripheral disease are prevented. Clinical studies have shown that many disorders, including rheumatoid arthritis, diabetes, cancer, etc., are also effective in treating (Connor and William, 2000). It is observed that due to imbalance of intake of ω–3 and ω–6 fatty acids a number of serious diseases occur. So by changing dietary habits by taking fish oil these problems can be avoided.

Omega-3 Fatty Acids (ω–3): EPA and DHA are important two ω-3 fatty acids. They have vital biochemical functions. EPA is converted into biochemical intermediates that are anti-inflammatory. DHA can be produced from EPA and is the most abundant ω-3 fatty acid in most tissues,

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present in the brain and retina in large amounts. For brain development and function, central nervous system growth, and optimum visual acuity, DHA is required. In a series of enzymatic steps, alpha-linolenic acid (ALA) is converted to EPA and DHA (Steffens and Wirth, 1997).

Omega-6 Fatty Acids (ω–6): Although sometimes ω–6 fatty acids are seen as pro-inflammatory mediators, the physiological effect depends on the type and amount of omega-6 fatty acid consumed. With cardioprotective benefits, linoleic acid is an essential fatty acid. In adipose tissue, the linoleic acid content was found to be inversely associated with the risk of cardiovascular disease; the greater the linoleic acid content, the lower the risk of cardiovascular disease. Through enzymatic steps, linoleic acid converts to arachidonic acid, which is the major omega-6 fatty acid.

Ratio of ω-3 and ω-6 fatty acids: A dietary equilibrium between the two fatty acids must exist. In inhibiting inflammatory responses, the ratio of ω-3 to ω–6 fatty acids appears to be critical. Depending on enzyme activity, the body has the capacity to convert ω-3 fatty acids into anti-inflammatory mediators. Because ω-6 fatty acids require the same enzymes for their prostaglandin and thromboxane equivalents to be converted. Sargent (1997) reported that the fatty acid ratio of ω-3: ω-6 should be 5:1. Nevertheless, diets with a ratio of about 1:1-6 were consumed by early ancestors with a ratio of about 1:1-3. (Simopolons, 1991).

Bio chemistry of PUFA: The mechanism of action of fish oil reflects the action of these EFAs, since fish oil contains ω-3 and ω-6 fatty acid types. Both must be obtained by diet, because vertebrates do not synthesise them de novo. Major physiological and developmental processes can be supported by both types. Eicosanoids can be formed, such as prostaglandins, leukotrienes, lipoxins, etc. It is possible to esterify these ω-3 and ω-6 fatty acids. A variety of highly unsaturated fatty acids can be metabolically elongated and desaturated and hydrolyzed from tissue glycerolipids and (Hazra et al., 2003). The conversion slowly occurs. A competition exists between alpha lenolenic and linoleic acids. High-level linoleic acids inhibit the conversion of alpha linolenic to its long-chain derivatives. These fatty acids with a longer chain are very important. Because they are eicosanoid precursors. Eicosanoids are capable of influencing platelet aggregation, blood pressure, blood clotting, inflammation, etc. In cases of inflammation, platelet aggregation, eicosanoids from ω-3 fatty acids are less potent than ω-6 fatty acids. As a component of the cell membrane, ω-3 and ω-6 fatty acids compete with each other for incorporation into it. Since there is a competition between the two fatty acid families, their intake must be properly balanced.

Advantages of consumption of fish oil over whole fish:

- Fish oil supplements have numerous advantages over directly consuming fish. Fish oil can be consumed directly by eating fish or by drinking fish oil, often in the form of supplements such as tablets, capsules, pills, soft gels, etc. Some of the fish oil benefits are listed here:
- Regular consumption of fish oil can lead to a decrease in the body's antioxidants such as vitamin E. As they contain added vitamin E in them, certain fish oil supplements help us to address this problem.
- Sometimes in severe cases, when a large dose of fish oil is required for treatment and when we try to eat fish oil by eating fish, we have to eat a lot of fish. Fish oil supplements offer us this convenience because they contain a concentrated form of fatty acid.
- Supplements with fish oil are very handy. We just have to open the jar, pick a tablet and gulp it if we want to consume fish oil on a daily basis. Cooking fish, on the other hand, becomes quite troublesome every day.
- What's more, we need to know the exact dosage of fish oil.

Positive Effects of Fish Oil:

A. Cardiovascular disease: It is well established that populations with high oily fish consumption have a lower incidence of heart disease, and several studies have confirmed that the protective components are EPA and DHA fish oils. Omega-3s are effective in reducing the incidence of heart disease (CVD). Therefore, fish oil, which is abundant in ω-3 fatty acids, reduces the risk of heart diseases and arrhythmias of the heart. It helps to maintain the elasticity of the artery walls, to prevent blood clotting, to lower blood pressure, to stabilise the rhythm of the heart and to help fight inflammation. It lowers the levels of bad cholesterol, which is LDL cholesterol, and raises the levels of good cholesterol, which is HDL. Fish oil prevents triglyceride accumulation and reduces the levels of excess triglycerides further. Silver carp arachadonic acid has a significant effect on blood pressure, serum lipid function, and platelet function (Wirth et.al., 1992).

Fish oil can therefore be used to prevent atherosclerosis in patients with coronary artery disease, and it can also be effective in the treatment of heart strokes and the regular use of fish oil can help prevent numerous sudden cardiac deaths. To maintain a healthy heart, an adequate daily intake (about 1 gramme) of EPA and DHA is vital. Fish oil is believed to have the ability to enhance blood circulation along with reducing levels of triglycerides and serum cholesterol. Patients with haemodialysis have an exceptionally high incidence of death due to cardiovascular problems. It is due to lipid and platelet abnormalities. Eskimos have a low incidence of myocardial infarction and a high dietary intake of fish rich in ω-3 polyunsaturated fatty acids (Rylance, 1986). Thus, fish oil prevents myocardial infarction as well. The risk of stroke and heart attack is increased by atherosclerosis, because part of the plaque on the inner wall of the arteries can dislodge and block the heart's smaller arteries and thus cut off the vital oxygenated blood supply.

Strokes are caused by a blood clot or a blood vessel that has burst. Consumption of fish and fish oils was first associated with a decreased risk of cardiovascular disease nearly 50 years ago. A number of epidemiological studies have since evaluated whether their intake is specifically linked to stroke. In general ecological/ cross-sectional and case-control studies have shown an inverse association between fish and fish oil consumption and stroke risk (Skerrett and Hennekens, 2003). The anti arrhythmic effects of fish oils help prevent blood clotting. Supplementation with fish oil will enhance the stability of plaque and thus help prevent heart attack and stroke. So the consumption of fish oil helps protect against stroke.

B. Brain development: One of the largest consumers' of DHA is the human brain. 15 to 20 percent of the cerebral cortex and 30 to 60 percent of the retina are made up of DHA. So it is absolutely necessary for the foetus and baby to develop normally. An adequate intake of DHA and EPA is vital during pregnancy and lactation. During this time, the mother must provide DHA and EPA for all the baby's needs. This is because it can not synthesise these essential fatty acids on its own. There is some evidence that the risk of premature birth and abnormally low birth weight may be increased by insufficient intake of ω-3 fatty acids. Experts recommend that during pregnancy and lactation, females get at least 500-600 mg of DHA every day.

The easiest way is to get a good supplement of fish oil every day. More than 20 grammes of DHA is contained in a normal adult human brain. Low levels of DHA have been associated with low levels of serotonin in the brain, which is again associated with an increased tendency to depression, suicide, and violence. A high intake of fish has been associated with a significant decrease in age-related memory loss and impairment of cognitive function and a lower risk of Alzheimer's disease (Kalmijn et.al., 1997). A recent study found that patients with Alzheimer's who received an omega-3-rich supplement had a substantial improvement in their quality of life.

C. Diabetes: Fish oil has been associated with glucose reduction and the level of insulin is controlled by it. People with type II diabetes often have high triglyceride levels in the blood and are therefore susceptible to coronary heart disease (McManus, et al., 1996). Fish oils are known to be effective in reducing levels of triglycerides, but concern has been expressed that they may also increase levels of low-density lipoprotein (LDL) and is harmful to the control of glucose. Type 1 diabetes is an illness that occurs frequently during childhood. In this high-risk group of children, ω-3 fatty acids could safely prevent the development of type 1 diabetes.

D. Immunity: Fish oil has the potential to improve humans' immune system. Regular fish oil consumption increases our immunity. It therefore helps to withstand the incidence of common diseases such as cold, cough and flu. Fish oil is also beneficial to patients suffering from lupus, which is a disease characterised by the attacks of the body's immune system on different organs and tissues. ω-3 fatty acids present in fish oil benefit the immune system by affecting cytokines and eicosanoids present in our body. Fish oil helps to reduce the pain and swelling of the joints, eyes, kidneys, heart, blood vessels, lungs, nerves, etc. It also helps to reduce the fever, skin rashes and fatigue that are associated.

E. Prevent Cancer: Cancer has now become the most dangerous illness. A huge number of people die each year from different types of cancers, such as kidney, colon, pancreas, etc. Several in vitro and animal experiments have clearly shown that the main components of fish oil, the long chain ω-3 polyunsaturated fatty acids (PUFAs), EPA and DHA, help inhibit cancer promotion and progression. In hormone-dependent cancers such as breast and prostate cancer, their beneficial impact is particularly pronounced. The steps that follow are how cancer is prevented:

1. They suppress arachidonic acid's synthesis of proinflammatory eicosanoids and thus produce an overall anti-inflammatory effect.
2. They have a positive effect on the expression of genes or on the activities of signal transduction molecules involved in cell growth control, apoptosis differentiation, angiogenesis and metastasis.
3. During chronic inflammation, they suppress excessive production of nitrogen oxide (NO) and thus help avoid DNA damage and impaired DNA repair.
4. They reduce the production of oestrogen and thus decrease the oestrogen- stimulated growth of hormone-dependent cancer cells.
5. Fish oils improve the sensitivity of insulin and cell membrane fluidity and, through these effects, may help prevent metastasis.

Other studies have shown that a daily intake of EPA + DHA in excess of 2.3 grammes reduces the production of a potent cancer promoter, superoxide. In addition to fish oil, the risk of colon cancer is reduced by lowering the level of COX-2 enzymes, which leads to colon cancer over-expression. In order to improve the quality of life and survival of patients with end-stage cancer, chemotherapy and other conventional medical treatments have proven ineffective. Greek medical scientists now report that supplementation with fish oil significantly increases the survival time of cancer patients with generalised malignancy. In cancer patients with end-stage metastatic disease, supplementation with dietary ω-3 polyunsaturated fatty acids, particularly fish oils containing antioxidants such as vitamin E, may provide significant palliative support.

F. Inflammation: Fish oil has anti-inflammatory properties; it is therefore, effective in reducing blood and tissue inflammation. For those suffering from chronic inflammatory diseases, regular consumption of supplements, tablets, pills and capsules of fish oil is helpful. In the treatment of gastrointestinal disorders, sprue, short bowel syndrome and inflammatory bowel disease (IBD), including Crohn's Disease and ulcerative colitis, which are typical intestinal disorders, fish oil is

effective. The absorption of vitamins, fats and essential supplements is difficult for patients suffering from Crohn's disease. For such patients, fish oil supplements are an effective diet. In the case of ulcerative colitis, fish oil prevents leukotriene from accumulating in the colon. It should be noted that fish oil's anti-inflammatory properties are restricted to reducing inflammation. Fish oil has little influence on the prevention of inflammation. In addition to other dietary supplements and drugs, research is also being conducted to enhance the anti-inflammatory action of fish oil.

G. Arthritis: In the treatment of arthritis, rheumatism, Raynaud's symptoms and similar conditions, fish oil is useful (Sales et al., 2008). In the case of osteoarthritis, fish oil can help reduce the impact of cartilage-depleting enzymes. The production of inflammatory eicosanoids is suppressed by ω-3 fatty acid. So they are in a position to reduce pain.

H. Mental disorder: Phospholipids, sphingolipids, gangliosides, and cholesterol are found in the brain. These are involved in the brain's structure and function of cell membranes. A high proportion of polyunsaturated fatty acids contain glycerophospholipids in the brain (PUFA). It is derived from alpha-linolenic acid and linoleic acid. Docosahexaenoic acid (DHA), alpha-linolenic acid, arachidonic acid and docosatetraenoic acid are the main PUFA in the brain. DHA is derived from ω- 3 fatty acids. Experimental animal studies have shown that diets lacking ω- 3 PUFA lead to significant neural function disruptions (Sinclair et al., 2007). The inclusion of ω- 3 PUFA in the diet can restore this neural dysfunction. There has been an emerging interest in the treatment of omega-3 PUFA for neuropsychological disorders (depression and schizophrenia) over the past 10 years. Fish oil is good for relieving depression, sadness, anxiety, restlessness, mental fatigue, stress, decreased sexual desire, suicidal tendencies and other nervous disorders because of the presence of ω- 3 fatty acids. In the treatment of Alzheimer's disease, fatty acids are effective. It helps with Alzheimer's disease, since fish oil is one of the best sources of essential fatty acids, including EPA and DHA.

I. Asthma: Asthma involves airway inflammation (pharynx, larynx and lungs). Epidemiological studies have shown that there is a lower incidence of inflammatory diseases such as asthma in populations with a high intake of fish oil. In the Western world, asthma is an increasingly common affliction. Between 20 and 25 per cent of all children are estimated to suffer from one or more asthma symptoms at some point. In many patients, dietary intake of polyunsaturated fatty acids (PUFAs) may be effective in reducing the symptoms of asthma. A viable asthma therapy may be dietary supplementation with fish oils or other enriched sources of ω- 3 PUFAs.

J. Eye Disorder: Fish oil is well known to be good for its ability to enhance vision. Macular degeneration is one of the most prevalent degenerative eye diseases. It also assists in preventing macular degeneration associated with age. When it comes to continued eye health, as a person ages, DHA in fish oil may be the most significant nutrient. A major component of the retina is ω- 3 fatty acids. As it accounts for 60% of the fatty acids in the retina, DHA is very important.

K. Pregnancy: Fish oil is very useful for pregnant women as it helps the development of the baby's eyes and brain with DHA present in it. It helps to prevent premature births, low birth weight, and miscarriages. Women who do not have a sufficient intake of EPA and DHA in their diet are also believed to suffer from depression after birth as some amount of brain mass is transferred from the mother to the child in the last stages of pregnancy. Low birth weight can be avoided if the right amount of fish oil is consumed at the time of pregnancy. There is some evidence that a lack of ω- 3 fatty acid intake may increase the risk of premature birth and low birth weight.

Fish oil and Vitamin: Fish oil is a rich source of vitamin A and vitamin D, especially one obtained from the liver such as cod liver oil. Excessive dosage of such oils, however can lead to hypervitaminosis, the accumulation of excessive vitamins in the body that can cause side effects. Interrelationship between fish oil and vitamin E:

- The moment it is removed from the fish, fish oil is highly susceptible to oxidation and will go rancid very quickly if preventive measures are not taken. The number of free radicals roaming around in the body can increase by taking an inferior fish oil that has started to become rancid, counteracting any health benefits from the fish oil itself. Good fish oil must contain a strong fat-soluble antioxidant such as tocopherol (vitamin E) to protect the oil from becoming rancid, to protect it and keep it fresh both within the capsule and in the body (Sau et al.,2004 and Paul et al., 2004).
- The excessive dosage of fish oil in the human body leads to a decrease in vitamin E. It is necessary to supplement this loss of vitamin E with external vitamin E supplements.
- In the treatment of many cancers and cardiac diseases, the combination of fish oil and vitamin E may have added to the benefits of being used separately.

Source and requirement: Although the use of fish oil supplements provides the body with many health benefits, the wrong dosage can also have harmful effects. For patients with coronary heart disease, the American Heart Association recommends the consumption of 1g of fish oil daily, preferably when eating fish. The optimal dosage has to do with body weight. In the human diet, nearly all the polyunsaturated fat is from EFA. The fatty acids ω-3 and ω-6 are obtained from alpha linolenic acids. Fish oil and various oily fish are the richest source of very long-chain ω-3 fatty acids. Mackerel, rainbow trout, lake trout, halibut, herring, sea bass etc are the various types of fish which can be a good source of fish

oil. In addition to this, many cyprinid spices, such as silver carp, grass carp and common carp, have higher levels of essential fatty acids, especially ω-3 PUFA (Steffens and Wirth, 1997). The summary requirement is given below for fatty acids.

Population	Recommendation
Patients with Coronary heart disease	They should eat 1 g per day of EPA+DHA, preferably from oily fish. In consultation with the physician, EPA+DHA supplements could be considered.
Patients needing triglyceride lowering	Under the advice of a physician, two to four grammes of EPA+DHA per day are given as capsules.

CONCLUSION

There are a number of beneficial effects that fish oil has. Fish oil is an excellent and usually uncontaminated source of EPA and DHA that is used by the body to make ω- 3 fatty acids "calming" and to keep the brain and heart healthy. The positive effect on cardiovascular health of ω-3 fatty acids is most consistently linked to the use of fish oil. In individuals with known cardiovascular diseases, fish and fish oil supplements should be recognised as a potential treatment choice. Eating a modest amount of fish oil ensures that EPA and DHA are directly supplied. In conclusion, we must say that the consumption of 1.0 g of fish oil every day will help human beings to fight coronary heart disease. Generally, fish oil supplements are safe. But it is very crucial that fish oil is processed and packaged. A balance between the intake of ω-3 and ω-6 fatty acids must be achieved. We can conclude that fish oil provides us with a healthy life. To maximise therapy and extract the maximum benefit from fish oil, health professionals need to educate people about the benefits, side effects, and dosage, duration, and drug-drug interactions.

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An Overview of Geotextiles in Agriculture

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ABSTRACT

Geotextiles are lightly fabric made from jute, coco coir or any natural plant fbers. Geotextiles a natural product are eco-friendly and biodegradable in nature and act as useful ameliorative to eliminate the soil related constrains of crop production. Bio deterioration of cellulose fiber results from the reduction at die polymerization leading to loss textile strength. It also helps to protect the most vital natural resources of soil and water from various degradation processes by erosion of soil and runoff water. It plays a vital role in increasing moisture holding capacity in soil, improving water uptake and drainage capacity. Application of suitable ameliorative thus necessitates for improving various soil conditions towards increasing the crop productivity.

KEY WORDS: GEOTEXTILE, SOIL PROPERTIES, MULCHING AND SOIL EROSION.

INTRODUCTION

Geotextile is totally biodegradable, geotextiles will stabilise the soil along with fostering its fertility. Apart from protecting the soil, the sheets will eventually turn into organic manure, making the soil more nutrient rich. While allowing water to pass through and blocking rapid moisture evaporation, the porous fabric will also protect the soil surface. In the first phase of the project, tubers, including yam, taro, purple yam and tapioca, will be planted along with curry leaves. Naturally occurring jute agro geo-textiles are eco-friendly and biodegradable products which act as surface cover materials and useful ameliorative to eliminate soil related constraints to crop production (Yong et al.,2000; Pain et al., 2013, Pal et al., 2020). It also helps to protect the most vital natural resources against various degradation processes and promotes vegetative cover it through accelerated seed germination and seedling emergence (Bhattacharya et al., 2010).

Natural geotextiles degrades to form organic mulch and held in weak establishment of vegetation. Jute geotextile degrades in 1 to 2 years, dry grasses, coco coir geotextile and Banana leaf fiber, geotextile degrades 1 to 2 to 3 years (Adhikary et al., 2019 Adhikary et al., 2016 and Pal et al., 2020). The use of different types of geotextiles management practices involving organic resources and shows beneficial effect on improving soil fertility, mentioned soil health and sustainable crop production scientific information's in relation to the above are reviewed hear under. Application of geotextiles is location specific so in addition to the characteristics of geotextiles, identification and application of geotextiles depends on soil type, soil compaction, moisture content, liquid limits, plasticity index, bulk density, soil pH, iron/ calcium content, clay / silt and sand composition, land sloping and hydraulic action etc. (Adhikary et al., 2019 and Pal et al., 2019).

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Geotextile in Agriculture: Eco geotextile another similar soil conditioner, are equally effective in erosion control, stabilization of soil slops and increasing water retention capacity also improve crop productivity (Rajgopal and Ramkrishna, 1997). Ahmed (1993) reported that influence of composted coconut coirdust (coirpith) on soil physical properties, growth and yield of tomato. In a field trial with tomato cv. Pusa Ruby on an Alfisol soil, composted coir pith (5-20 t/ha). Improved soil condition and bulk density) and moisture retention capacity compared with 10 t FYM/ha. Fruit yields were greatest (19.01 t/ha) with FYM, followed by 20 t coir pith/ha (16.97 t fruit/ha) and were lowest in controls (11.23 t/ha), which were treated with neither FYM nor coir pith. Hongal et al., (2010) reported that effect of green manures and nitrogen levels on the soil properties. In the field experiment conducted effect of green manures and nitrogen levels in cotton + chilli cropping system was evaluated. Sun hemp recorded higher phyto mass (25.58 ton/ha) followed by cowpea (22.44 ton/ha) and green gram (14.40 ton/ha). Similar trend was observed in biomass production and Accumulation.

Effect of geotextile in soil properties: Sinowski and Auerswald (1992) Studied that fleece used as a geotextile maintained its water holding properties and varied its speed of wetting after each application. The erosion protection of the tested geotextiles was increased by 30%, depending on the effect of covering. Soil infiltration was improved more with geotextiles than with fallowing. Andre and Gerand (1988) reported that geotextiles made from synthetic fibres are used in drainage applications. This function can be assumed for many years if no alteration or changes of their structure results from chemical attack, mechanical deterioration, mineral and bacterial clogging and accumulation of particles, or organic matter between or upstream of the fibres. Thomas et al., (1987) reported that the geotextiles have been employed to reduce lateral deformation of bridge approach embankments and to prevent closure of the expansion devices in the bridges.

This concept will be implemented in an existing bridge undergoing reconstruction. Chen et al., (2009) reported that the water absorption characteristics of geotextile can influence runoff producing process directly, and their decomposition characteristics relate to the geotextile durability for soil and water conservation. Olesen et al., (1995) reported that geotextiles are any textile like material used to enhance soil structural performance. Biobased geotextiles are used for short term (6 months to 10 year) applications where biodegradability is a positive attribute, such as mulching and erosion control. Biswas et al., (1970) reported that the nature of organic matter played an important role in the development soil structure owing to diferential nature of by products produced during the process of decomposition. Rajagopal and Ramakrishna, (1997) describe properly about to improve the soil organic carbon (SOC) and soil by the application of geotextile.

Effect of geotextile on soil erosion control: Smets et al., (2007) conducted a field experiment on palm-leaf geotextiles could be an effective and cheap soil conservation method with enormous global potential. Effectiveness of palm geotextiles reducing soil erosion from water. A field experiment was conducted by Paterson and Barnard (2011) in South Africa Beneficial effect of palm geotextiles on inter-rill erosion. Geotextile mats made of woven palm leaves showed potential using a rainfall simulator for their effectiveness in reducing surface runoff and sediment load from a range of South African soils and mine tailings. Rickson (2000) has done another experiment on geotextiles and reported that geotextiles can be used to control soil erosion and establish vegetation on disturbed landscapes or newly constructed sites. Rickson (2006) reported the controlling sediment at source an evaluation of erosion control geotextiles. This paper presents one method to evaluate the effectiveness or ability of geotextiles in controlling soil erosion. Smets et al., (2009) conducted a field experiment that impacts of soil tilt on the effectiveness of biological geotextiles in reducing runoff and in Terrill erosion. Bhattacharyya et al., (2010) reported that use of palm-mat geotextiles for rain splash erosion control. Hence, the utilization of palm-mat geotextiles as a rain splash erosion control technique was investigated at Hilton.

Effect of geotextile as mulch: Nag et al., (2008) reported that mulch is a layer of material spread on top of the soil to conserve soil moisture, discourage the growth of weeds, help prevent erosion and prevent large fluctuations in soil temperature. Kaku et al., (2007) reported that Geotextile mulch had become popular recently in the installation of landscape ground cover, because it provides both suppression of weeds and maintenance of soil conditions desirable for cover-plant growth. Effect of mulching of plant materials on the growth of ground cover plants and emergence of weeds on levee slope. Otani et al., (2009) reported that ground cover plants are useful for weed suppression on levee slopes. However, weeding is necessary until the slopes has covered with ground cover plants. Walsh et al., (1996) reported that the effects of cultivation, straw mulch, geotextile mulch, grass cover, a cover crop mixture of lupin (Lupinus albus) and wild carrot. Wilen et al., (1999) reported that Weed control efficacy of organic mulches as well as a copper hydroxide-coated geotextile (fabric) disk was examined using Rhaphiolepis indica cultivars Snow White and Pinky or Callistemon citrinus growing in containers.

CONCLUSION

Geotextiles of various natures due to its effects as surface cover materials have potentials for maintaining soil quality and protecting the soil against any form of degradation. Each of the geotextiles increased the higher yield associated with much increase of organic carbon and availability of phosphorous and potassium. Sharp improvements of bulk density, porosity, moisture

use efficiency as well as better aggregation and well stabilization of soil aggregates occurred due to application of each geotextiles, of which jute geotextile showed most prominent effect in all such respect.

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Effect of Fertility Levels and Cytokinin on Growth and Yield of Sunflower (*Helianthus Annuus* L.)

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ABSTRACT

The field experiment was carried out at Campus Farm, M.S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi, Odisha during summer season, 2019. The soil in field was clay loam in texture, slightly acidic in reaction (pH 6.5), low in organic carbon (0.57 %) and available nitrogen (176 kg ha^{-1}), medium in available phosphorus (38.49 kg ha^{-1}) and sulphur (29.52 kg ha^{-1}) and high in available potassium (340.0 kg ha^{-1}). The field experiment was laid out in factorial randomised complete block design with three replications and twelve treatments combination consisted of two factors including factor A (Nutrient management levels) and factor B (Cytokinin levels). The nutrient management treatments for factor A were 100% recommended dose of fertilizer (RDF) i.e. 80:60:40 kg of N: P2O5: K_2O ha^{-1}, 100% RDF + Azotobacter @ 5 kg ha^{-1}, 100% RDF + Azotobacter @ 5 kg ha^{-1} + S @ 30 kg ha^{-1}, 125% RDF i.e. 100:75:50 kg of N: P_2O_5: K_2O ha-1, 125% RDF + Azotobacter @ 5 kg ha^{-1}, 125,% RDF + Azotobacter @ 5 kg ha-1 + S @ 30 kg ha^{-1}. The cytokinin levels included in factor B were cytokinin 50 ppm and no cytokinin. The nutrient management treatments and cytokinin significantly influenced the crop growth parameters in terms of plant height, basal stem girth, number of leaves $plant^{-1}$, dry weight of plant, leaf area index and seed yield. The interaction effect of nutrient management treatments with cytokinin was found positive with respect to seed yield. The crop growth parameters like plant height (151.70 cm), basal stem girth (9.79 cm), number of leaves $plant^{-1}$ (13.93), leaf area index (1.70) and dry matter production (595.08 g m-2) and seed yield (2.43 t ha^{-1}) were obtained from 125% RDF of NPK + Azotobacter @ 5 kg ha-1 + S @ 30 kg ha^{-1}. The corresponding values with application of cytokinin 50 ppm were 145.91cm, 9.44 cm, 13.52, 1.58 and 587.82 g m-2), respectively and seed yield (2.36 t ha^{-1}). The combination of 125% RDF of NPK + Azotobacter @ 5 kg ha^{-1} + S @ 30 kg ha^{-1} with cytokinin 50 ppm found in increasing the seed yield (2.77 t ha^{-1}) which recommended as suitable production technology for sunflower cultivation under South Odisha condition.

KEY WORDS: NPK LEVELS, AZOTOBACTER, SULPHUR, CROP GROWTH, SEED YIELD AND SUNFLOWER.

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INTRODUCTION

Sunflower plays an important role in meeting the shortage of edible oils in our country (Kalaiyarasan et al., 2019). In India, it is cultivated to an area of 0.48 million ha in which 0.29 million ha in rabi season with the total annual production of 0.34 million tonnes (Agricultural research data book, ICAR 2019). Considering the Indian oil economy in point of view, sunflower ranks fourth next to groundnut, soybean and rapeseed. It is one of the fastest growing and important oilseed crop in the world as a chief source of vegetable oil. Due to the national priority of vegetable oil production in India, sunflower has gained popularity in recent times. It is grown throughout the year as a photo-insensitive crop. Though it is a temperate zone crop but it performs well under varying climatic and soil condition (Ijaz et al., 2017).

The farmers are highly interested to grow sunflower. Nitrogen, phosphorus and potassium are the three important major fertilizers used in balanced or unbalanced manner for increasing production (Kalaiyarasan et al., 2019). Nitrogen, phosphorous and potash in balanced manner are most required in crop production in order to boost the yield. Nitrogen is an important nutrient to improve the vegetative growth, yield and quality of sunflower. Phosphorus nutrition is vital for plant growth and involved in energy transfer, photosynthesis, transformation of sugars, starches and nutrient movement within the plant. Potassium improves the crop growth and productivity as well as promotes the tolerance of crops to pest attack (Jehad et al., 2008). The various combination of NPK had greater role in improving the growth and seed yield of sunflower (Ijaz et al., 2017).

Besides nitrogen, phosphorus and potassium, sulphur plays an important role in enhancing the photosynthesis and seed yield of sunflower. Sulphur is responsible for synthesizing of sulphur containing amino acids, proteins and activity of enzymes thus, increases oil content in oil bearing plants. Sulphur deficient plant produces less protein and oil (Gajbhiye et al., 2013). The sulphur is increasingly deficient in Indian soil due to adoption of multiple cropping systems, growing of hybrid and high yielding varieties, substancial use or no use of organic manures and application of high analysis sulphur free fertilizers. The requirement and use of cheap source of sulphur for higher seed and oil yield of sunflower has been reported by Vala et al. (2014). Application of sulphur in conjunction with NPK fertilizer in enhancing the growth and yield (Vala et al. 2014 and Ravikumar et al., 2016) of sunflower is well documented by several agricultural research scientists.

Biofertilizers are the living microorganism that supply the nutrients to plants in symbiotic and asymbiotic way. The beneficial effect of Azotobacter is to fix the atmospheric nitrogen. It increases the seed germination, plant growth and yield (Khandekar et al. 2018). The positive effect of Azotobacter in conjunction with NPK fertilizer in increasing the growth, yield and quality of sunflower has been observed by Pramanik and Bera (2013) and Khandekar et al.(2018). The use of biofertilizer leads to significant improvement in crops yield by 15- 20% and reduces the depletion of soil nutrients (Khandekar et al., 2018). Biofertilizers in an integrated way serves a viable option to improve crop productivity. The plant growth hormone cytokinin plays a vital role in promotion of cell division and differentiation and influences several developmental and physiological aspects in plants including seed germination, apical dominance, growth, flowering time, flower and fruit development and leaf senescence. The benefit of cytokinin results in delayed leaf senescence, better maintenance of photosynthetic rate, increase in plant biomass, higher nitrate influx, increase in post-harvest life in flowers, drought tolerance and higher seed yield (Surya kant et al., 2015). The application of benzyl adenine in augmenting the number of seeds fruit^{-1}, test weight and yield in oil seed crops has been reported by Gora et al. (2018).

MATERIAL AND METHODS

The field experiment was conducted during summer season of 2019 at M.S Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi campus. The experimental plot was clay loam in texture, slightly acidic in reaction and low in organic carbon and available nitrogen, medium in available phosphorus and sulphur and high in potassium with pH of 6.5. The experiment was conducted adopting factorial randomized complete block design comprised of six fertility levels in factor A and two cytokinin levels in factor B which were replicated thrice in the plot size of 4.8 m × 4.2 m. The factor A was comprised of six fertility levels like 100% recommended dose of fertilizer (RDF) i.e. 80:60:40 kg of N: P_2O_5: K_2O ha^{-1} , RDF + Azotobacter @ 5 kg ha^{-1}, RDF + Azotobacter @ 5 kg ha^{-1} + S @ 30 kg ha^{-1}, 125% RDF, 125% RDF + Azotobacter @ 5 kg ha^{-1}, 125% RDF + Azotobacter @ 5 kg ha^{-1} + S @ 30 kg ha^{-1}.

In factor B, two cytokinin levels like without cytokinin and with cytokinin @ 50 parts per million (ppm) were tested with factor A. The experimental field was ploughed properly and YSH 475 sunflower hybrid was sown on 5th January, 2019 at the spacing of 60 cm × 30 cm. The sources of fertilizers were urea, diammonium phosphate and muriate of potash in the nutrient management treatments having no sulphur. For the nutrient management treatments containing sulphur, the chosen fertilizers were complex fertilizer grade 20:20:0:13, diammonium phosphate, urea and muriate of potash. The recommended fertilizer dose of 80:60:40 kg N: P205:K2O ha^{-1} was applied to the sunflower crop to the specific treatments. In the treatments of 125% recommended dose of fertilizer, 100 kg N, 75 kg P_2O_5 and 50 kg K_2O were used for the purpose.

In all the nutrient management treatments, full dose of P_2O_5 and half dose of K_2O were applied as basal. As to the treatments specification of 100% RDF + 30 kg S ha^{-1} + Azotobacter and 125 % RDF + 30 kg S ha^{-1}

+ Azotobacter, 30 kg S was applied to the nutrient management treatments containing sulphur. In the treatment of 100% recommended fertilizer dose + 30 kg S ha^{-1} + Azotobacter, 51.57 kg N ha^{-1} was applied through complex fertilizers at the time of sowing as basal. For the treatment of 125% recommended dose of fertilizer + 30 kg sulphur + Azotobacter, 57. 44 kg N was incorporated to soil through complex fertilizers just before sowing as basal in that specific nutrient management treatment. The basal dose of fertilizers was applied in furrows just before sowing. Afterwards, the fertilizers were incorporated by placing the soil detached from both the sides of the furrows. The bio-fertilizer, Azotobacter was collected from bio-fertilizer unit of M.S. Swaminathan School of Agriculture and applied to the specific treatments in furrows at the time of sowing.

The remaining amount of N along with 50% K_2O as per the specifications of the nutrient management treatments were top dressed followed by earthing up at 5th week of crop age. The growth regulator benzyladenine was applied at 45 days after sowing for the treatments with 50 ppm cytokinin. The crop was free from pest load and grown with all recommended package of practices. The crop harvested when it attained full maturity. At 90 days after sowing, five plants were randomly selected in each treatment for recording growth parameters like plant height, stem girth, number of leaves $plant^{-1}$, leaf area index (LAI), dry weight and yield parameters like grain yield. The grain yield was recorded from each plot after proper drying.

RESULTS AND DISCUSSION

Growth parameters: Data pertaining to growth parameters of sunflower affected by different fertility levels and cytokinin levels was presented in Table 1. The maximum plant height was observed in 125% RDF of NPK + Azotobacter @ 5 kg ha^{-1} + 30 kg S ha^{-1} (151.70 cm) being at par with 125% RDF + Azotobacter @ 5 kg ha^{-1} (147.26 cm). It was followed by 100% RDF + Azotobacter @ 5 kg ha^{-1} + S @ 30 kg ha-1 (144.01 cm) which did not differ significantly from other nutrient managemen treatments. The conjugated use of 125 % recommended dose of NPK with Azotobacter @ 5 kg ha^{-1} and S @ 30 kg ha^{-1} increased the absorption and uptake of N, P, K and S nutrients in more available form thereby facilitated the effective movement of nutrients from source to assimilating organ to promote cell division and enlargement there by increased the internodal length that reflected the plant height.

Table 1. Influence of fertility levels and cytokinin levels on plant height, stem girth and number of leaves $plant^{-1}$ at harvest.

MTreatments	Plant h eight (cm)	Stem girthth (cm)	Number of leaves plant-1	LAI	Dry matter accumulation
100% RDF (80:60:40 kg NPK ha^{-1})	140.72	8.66	12.93	1.42	531.80
100% RDF + Azotobacter @ 5 kg ha^{-1}	142.15	8.77	13.04	1.45	540.12
100% RDF + Azotobacter @ 5 kg ha^{-1} + S @ 30 kg ha^{-1}	144.01	9.18	13.22	1.52	566.17
125% RDF	142.45	9.38	13.11	1.48	576.12
125% RDF + Azotobacter @ 5kg ha^{-1}	147.26	9.44	13.19	1.50	577.48
125% RDF + Azotobacter @ 5 kg ha^{-1} + S @ 30kg ha^{-1}	151.70	9.79	13.93	1.70	595.08
S.Em. (±)	1.27	0.18	0.20	0.03	13.38
CD (P=0.05)	3.74	0.52	0.59	0.09	39.24
Cytokinin					
50 ppm Cytokinin	145.91	9.44	13.52	1.58	587.82
No Cytokinin	143.52	8.96	12.95	1.44	541.10
S.Em. (±)	0.74	0.10	0.12	0.02	7.72
CD (P=0.05)	2.16	0.30	0.34	0.05	22.65
Interaction					
S. Em. (±)	1.79	0.25	0.28	0.04	18.92
CD (P=0.05)	NS	NS	NS	NS	NS

The favourable effect N + Azotobacter + Azospirillum with common dose of phosphorus and potassium (Khandekar et al., 2018), NPK fertilizer with sulphur (Vala et al., 2014) and (Ravikumar et al., 2016) and sulphur with Azotobacter + phosphorus solubilizing bacteria + vesicular arbuscular mycorrhizha along with common

dose of NPK @ 80:100:100 kg ha^{-1} (Patra et al., 2013) in increasing the plant height has been reported by several workers.

Stem girth: The maximum stem girth was observed with 125 % recommended dose of NPK with Azotobacter @ 5 kg ha^{-1} + S @ 30 kg ha^{-1} (9.79 cm) followed by 125% recommended dose of NPK + Azotobacter @ 5 kg ha^{-1} (9.44 cm) and 125% recommended dose of NPK (9.38 cm) which were on par (Table1). The minimum stem girth was observed in 100 % recommended dose of NPK at all the growth stages of crop. Increase in stem diameter is ascribed to positive influence of nutrient management treatments due to better availability and absorption of nutrients that resulted in more translocation of assimilates from source to sink. It favoured the cell division, differentiation and proliferation to enlarge the stem growth. The use of inorganic fertilizer N with Azospirillum + Azotobacter and common dose of P and K (Khandekar et al. 2018) and recommended dose of fertilizer with suphur (Muhammad et al., 2019) in improving the stem diameter of sunflower was recorded by various research workers.

The stem girth of sunflower was increased with application of cytokinin 50 ppm (9.74 cm) over no cytokinin application (8.96 cm). Cytokinin governs the plant growth by regulating the developmental and physiological processes through cell division, proliferation and differentiation facilitating the promotion of shoot growth and elongation. This has been supported by (Kurkawa et al., 2007) and (Schaller et al., 2014). Thus, the favourable effect of cytokinin application resulted in augmentation of stem girth of sunflower.

Table 2. Interaction effect of fertility levels and cytokinin on seed yield

Treatments	Cytokinin 50 ppm	No cytokinin	Mean
100% NPK	2.00	1.76	1.88
100% NPK + Azotobacter @ 5 kg ha^{-1}	1.88	1.91	1.89
100% NPK + Azotobacter + 30 kg S ha^{-1}	2.68	2.00	2.34
125% NPK	2.40	2.04	2.22
125% NPK + Azotobacter	2.41	2.03	2.22
125% NPK + Azotobacter + 30 kg S ha^{-1}	2.77	2.09	2.43
Mean	2.36	1.97	2.16
Fertility levels	Cytokinin	Interaction	
S. Em (±)	0.05	0.03	0.07
CD (P=0.05)	0.14	0.08	0.20

Number of leaves plant^{-1}: The perusal of data (Table 1) on number of leaves per plant indicated that there was a significant difference due to fertility levels. The maximum number of leaves was noticed with use of 125 % recommended dose of NPK with Azotobacter @ 5 kg ha^{-1} + S @ 30 of ha^{-1} (13.93) and other treatments were not significantly different from each other. The lowest number of leaves plant^{-1} was observed with application of 100% recommended dose of NPK. Leaves are the photosynthetic apparatus of the crop. The enhancement of leaves plant^{-1} contributes to increase in source of photosynthates that is mobilized to sink. The nourishment of N, P and K along with S in conjunction with Azotobacter provided the better crop nutrition leading to rapid cell division and elongation resulting in increase in plant height which ultimately facilitated more formation of functional leaves in the plant. Increase in functional leaves plant^{-1} by use of 100 % recommended N with Azotobacter + Azospirillum with common dose of phosphorus and potassium and NPK fertilizer with S was reported by (Khandekar et al., 2018).

It was clearly indicated from data in Table 2 that the plant height of sunflower was significantly enhanced with 50 ppm cytokinin (145.91 cm) over no application of cytokinin (143.52 cm). The application of cytokinin regulates the physiological and metabolic process in plant at all the crop growing period thereby, promotes plant growth through cell division and differentiation leading to activity of shoot apical meristems to enhance shoot growth and elongation of internodes that reflects the plant height. The beneficial effect of benzyl adenine in improving the plant height of oil seed crops was reported by (Gora et al., 2018).

Significant increase in number of leaves was obtained with application of cytokinin 50 ppm giving the values of 13.52 over no cytokinin spray (12.95). Cytokinin application influences many developmental and physiological processes in plants by regulating the cell division, differentiation and proliferation (Kurakawa et al., 2007).

Leaf area index: It is evident from the data (Table 1) that the maximum leaf area index was recorded with 125 % recommended dose of NPK with Azotobacter @ 5 kg ha^{-1} + S @ 30 kg ha^{-1} (1.70) was significantly at par with 100% RDF + Azotobacter @ 5 kg ha^{-1} + S @ 30 kg ha-1 (1.52). The minimum leaf area index was recorded

from treatment with application of 100% RDF 80:60:40 kg NPK ha (1.42).

The foliar application of cytokinin 50 ppm positively enhanced the LAI at 90 DAS with the values of 1.58, respectively. The corresponding values with no application of cytokinin was 1.44, respectively. The key role of cytokinin is to regulate cell expansion by influencing the cell division and differentiation as stated by Schaller et al. (2014). The beneficial effect of cytokinin leads to increase in size of leaf associated with increase in chlorophyll content and photosynthetic efficiency including the activity of apical and axillary meristem that promotes shoot growth thus, accommodates a greater number of leaves plant^{-1}. This led to increase the canopy development that ultimately enhanced the LAI.

Dry matter accumulation: It is observed from the data that the nutrient management treatments exerted the significant influence on dry matter accumulation (Table 1). The dry matter production was recorded the highest in 125% RDF + Azotobacter @ 5 kg ha-1 + S @ 30 kg ha^{-1} (595.08 g m-2) followed by 125% RDF + Azotobacter @ 5 kg ha^{-1} (577.48 g m-2). The minimum dry matter production was noticed in 100% recommended dose of NPK (531.8 g m-2). The increase in crop growth parameters enhanced the vegetative growth which consequently reflected dry matter production. Increase in vegetative growth with application of 100% recommended dose of N + Azospirillum + Azotobacter with common dose of phosphorus and potassium (Khandekar et al., 2018) and dry matter accumulation with bio fertilizer consortia of Azotobacter + phosphorus solubilizing bacteria + vesicular arbuscular mycorrhiza along with uniform dose of 80:100:100 kg NPK ha^{-1} (Pramanik and Bera, 2013) were observed by various workers.

The foliar application of cytokinin 50 ppm positively increased the dry matter with the value of 587.82 g m-2. (Table 1). The corresponding value for no application of cytokinin was 541.10 g m-2, respectively. Ctokinin application influences many developmental and physiological processes in plants by regulating the cell division, differentiation and proliferation (Kurakawa et al., 2007). Thus it favoured its positive influence on enhancing dry matter peoduction.

Seed yield: The perusal of data pointed out that nutrient management treatments had exerted significant influence on seed yield of sunflower (Table 2). Among the nutrient management treatments, application of 125% recommended dose of NPK + Azotobacter @ 5 kg ha^{-1} + 30 kg S ha^{-1} recorded the highest seed yield (2.43 t ha^{-1}) which remained at par with 100% RDF + Azotobacter @ 5 kg ha^{-1} + 30 kg S ha-1 (2.34 t ha^{-1}). The lowest seed yield was noticed under 100% recommended dose of NPK (1.88 t ha^{-1}). Application of 125% recommended dose of NPK + Azotobacter + 30 kg S ha^{-1} was proved to be ideal nutrient management option resulted in increasing the seed yield. Many research workers reported the conducive effect of higher level of nitrogen when used with uniform dose of P and K (Khandekar et al. 2018), biofertilizer consortia (Azotobacter + phosphorus solubilizing bacteria + vesicular arbuscular mycorrhizha) with common dose of NPK (Pramanik and Bera 2013), NPK with S (Shubangi and Patil 2008) in enhancing the seed yield of sunflower.

The data on seed yield represented in table 2 showed that significantly the higher seed yield was noticed with 50 ppm cytokinin (2.36 t ha^{-1}) than that of no application of cytokinin (1.97 t ha^{-1}). It was attributed to benevolent role of cytokinin in increasing the seed yield. Gora et al. (2018) opined the marked effect of foliar application of benzyl adenine in improving the seed yield.

Interaction effect of nutrient management treatments and cytokinin exhibited positive effect on seed yield of sunflower (Table 2). The maximum seed yield was noticed when crop was fertilized with 125% RDF + Azotobacter @ 5 kg ha^{-1} + 30 kg S ha^{-1} along with 50 ppm cytokinin (2.77 t ha^{-1}) followed by 100% RDF of NPK + Azotobacter @ 5 kg ha^{-1} + 30 kg S ha^{-1} with 50 ppm cytokinin (2.68 t ha^{-1}) which were at par. The minimum seed yield was recorded with application of 100% RDF of NPK without application of cytokinin (1.76 t ha^{-1}).

CONCLUSION

Application of 125% recommended dose of NPK + Azotobacter @ 5 kg ha^{-1} + 30 kg S ha^{-1} along with 50 ppm cytokinin exhibited better growth and maximum seed yield compared to other treatments in sunflower which is preferred for South Odisha ecosystem.

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Progress in the Emerging Areas of RNA Biology and Implications in Life Sciences

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ABSTRACT

Recent advances in transriptomics such as Next gen sequencing and the discovery of novel mechanisms for control of gene expression such as microRNAs, RNAi and riboswitches gave a better insight on the complexity of the RNA function within the cell but also provides us with bigger opportunities for applying novel RNA-based engineering strategies in health care, agriculture and clinical diagnostics. Undestanding RNA biology in medical genomics offered new exciting opportunities for the development of novel therapeutic strategies in tackling various forms of cancer, viral infection diseases, deployment of novel antibiotics, and others. Likewise RNAi substantially contributed to crop improvement in obtaining improved cultivars with desirable traits such as biotic stress tolerance, toxin free plant types, seedless fruits, male sterile lines, delayed ripening of fruits, enhanced nutritional content, removal of secondary metabolites and altered floral characteristics.

KEY WORDS: EPITRANSCRIPTOMICS, POLYADENYLATION, ALTERNATIVE SPLICING, RNAI, CRISPR/CAS9.

INTRODUCTION

The various post-transcriptional modifications such as RNA editing, alternative splicing, and alternative polyadenylation greatly increase the transcript heterogeneity and biodiversity of proteins that can be encoded by the genome in humans. While the existence of post-transcriptional regulatory events enhances the scope and breadth of RNA functions at transcriptional, post-transcriptional, translational and post-translational levels and in conjunction with bioinformatic tools offers exciting opportunities for future genomic medicines and inducing plant resistance to various biotic stresses.

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Epitranscriptomics: Recently a new field of study has emerged termed as epitranscriptomics which refers to the study of chemical modifications (mostly methyl groups) to RNA. In recent years it is becoming increasingly evident that RNA is also epigenetically modified. Next Generation Sequencing (NGS) technology revealed more than 170 kinds of biochemical ribonucleic acid (RNA) modifications in almost all living organisms and all kinds of RNAs, including ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), messenger RNAs (mRNAs), circular RNAs (circRNA) and long non coding RNAs. Such epigenetic RNA modifications include N6-methyladenosine (m6A), N6,2′O-dimethyladenosine (m6Am), 5-methylcytosine (m5C), 5-hydroxymethylcytosine (hm5C), N1-methyladenosine (m1A), inosine (I) and pseudouridine (ψ) at promoter sites as well regulate RNA metabolism by influencing RNA structure, RNA stability and splicing factors Lorna (2019). Special classes of proteins that add methyl groups to RNA are termed writers.

Classes of proteins that remove methyl groups from RNA are termed writers. The classes of proteins that enable reading of methyl groups on RNA are termed readers. The RNA modifications most commonly characterized

is N6-methyladenosine (m6A) when "writer" enzyme complex METTL3/14 adds a methyl group to adenosine to form m6A. Two different "eraser" enzymes ALKBH5 and FTO remove the methyl groups and restores adenosine. While the causal link between RNA modifications and disease development has not been established so far, but the elevated levels of METTL3 and ALKBH5 contributing to cancer progression makes this new field of RNA biology a future promising and potential approach in the development of cancer therapeutics vu et al. (2017) and Zhang et al. (2017)).

These epigenetic modifications termed as RNA epitranscriptomic marks are added by a series of writers (methyltransferases (METTL3 and METTL14), wilms tumour 1-associated protein (WTAP), KIAA1429, putative RNA binding proteins (RBM15/15B), and METTL16) and are removed by erasers (fat mass and obesity associated protein (FTO) and α-ketoglutarate-dependent dioxygenase alkB homolog 5 (ALKBH5) (Lorna). Apart from regulating RNA metabolism, these RNA writers and erasers collectively termed as RNA-modifying proteins (RMPs) are also implicated in the pathological progression. This novel area study called epi-transcriptome is linked to the initiation and progression of cancers and other diseases. Accordingly, mutations in the writer or eraser have been associated with cancers such as hepatocellular carcinoma and acute myeloid leukemia (AML), with memory, fertility and metabolic phenotypes Lorna (2019). Currently RNA epigenomic writers and erasers are showing promise as diagnostic markers and future therapeutic strategies for oncology Zhen and Hongjuan (2016)

Non-coding RNAs (microRNAs and long non-coding RNAs) as diagnostic and therapeutic tools: Majority of the transcriptome is represented by noncoding RNAs (ncRNAs) such as ribosomal (rRNA), transfer (tRNA), Piwi-interacting (piRNAs), micro (miRNA), small nuclear (snRNA), small nucleolar (snoRNA) and other types of RNA. Long noncoding RNAs (lncRNAs) are located and transcribed from different genomic locations. Using this criteria lncRNAs are generally placed into five categories; sense, antisense, bidirectional, intronic, and intergenic Lina ey al. (2013).

a) sense lncRNA are transcribed from the sense strand of a protein coding gene and contains exons from protein coding genes. b) Antisense lncRNA are transcribed from the antisense strand of a protein coding gene. c) Bidirectional lncRNA sequence is located on the opposite strand from a protein coding gene whose transcription is initiated less than 1000 base pairs away, but share common promoters with protein coding genes d) Intronic lncRNA – These are transcribed entirely from within an intron of protein-coding genes. e) Intergenic lncRNA – These are transcribed from intergenic regions from both the strands. These RNA sequences are not located near any other protein coding loci.

Using large-scale complementary DNA (cDNA) sequencing projects such as FANTOM5 (Functional Annotation of Mammalian cDNA), more than 19,000 potentially functional long ncRNAs have been identified in various human sources suggesting that these RNAs constitute a heterogeneous group of diverse functions (Lina).

lncRNAs act to regulate chromatin remodeling and gene expression at the epigenetic, transcriptional and translational level. Long ncRNAs attract microRNAs and regulate expression level of transcripts containing common miRNA binding sites. LncRNAs can also form duplexes with target mRNAs and inhibit their translation or disrupting their stability. In addition, some long noncoding RNAs can modulate pre-mRNA splicing, protein localization, telomere replication, RNA interference, beyond transcription and translation regulation. Although few lncRNAs have been functionally characterized so far, it is now clear that alteration in their expression can contribute to various cancer types, neurodegenerative disorders, cardiovascular diseases, conditions associated with genome imprinting, aging, eye diseases, immune response, apoptosis and other pathologies. Since lncRNAs and miRNAs are dysregulated in diseased states, they have the potential to be used as prognostic markers and novel therapeutic targets Ling (2013).

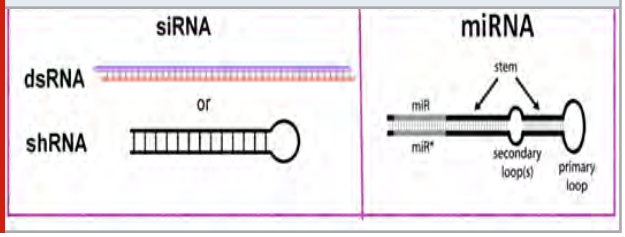
Difference between siRNA vs miRNA:

1. Unlike siRNAs (rarely conserved), many miRNAs are evolutionarily conserved in related organisms, which implies that they have important biological functions.
2. The major difference between siRNAs and miRNAs is that the former inhibit translation of a specific mRNA by degradation while the latter (miRNA) either degrade target mRNA (in plants) or inhibit the translation of multiple mRNAs because of imperfect base pairing (seen in animals).
3. miRNAs and siRNAs are generated in slightly different ways. miRNAs precursors are derived from distinct gene loci by RNA polymerase II transcription and subsequent processing into 23 nucleotide long miRNA by dicer-I enzyme. siRNAs do not have distinct gene loci and are derived from an exogeneous double-stranded RNA uptaken by the cell, and cleaved by dicer 2 enzyme. siRNAs are also encoded by transposable elements, viruses or heterochromatic DNA. Hence siRNAs are abundantly present in cells than miRNAs.
4. siRNAs are believed to be the most ancient form of RNA interference, with miRNAs being a later refinement.
5. miRNA can regulate multiple geens while siRNA mediate silencing of same genes from which they originate.
6. In terms of structural differences miRNA has a heteroduplex structure that includes an imperfect stem loop structure while the siRNA is a single duplex structure or extended hairpins Ian and Michael (2008) (Fig 1).

The first miRNA called lin-4 was discovered in Caenorhabditis elegans and seven years later lin-7 was identifies in the same organism Li and Kowdley

(2012). With the advancement in the areas of functional genomics and bioinformatics, novel miRNAs were implicated in various human diseases and already made a transition from laboratories to clinical research. miRNAs are stable in cells, tissues and body fluids making them as an ideal choice as biomarkers to study disease progression in various human diseases such as cancer, neurodegenerative, cardiovascular and liver disease and viral infection Szelenberger (2019).

Figure 1: Structural difference between siRNA vs miRNA



Engineering Plant Metabolic Pathways through RNAi: The recent understanding of functional genomics in non-coding RNAs that has substantially contributed to crop improvement is RNA interference (RNAi). Small interfering RNAs (siRNAs) and microRNAs (miRNAs) activate RNAi machinery inside the cells, abrogates the specific gene function without affecting other agronomic traits. Several desirable traits improved by RNAi gene silencing were biotic stress tolerance, removal of toxins from improved cultivars, development of seedless fruits, development of male sterile lines, delayed ripening of fruits, nutritional content, removal of secondary metabolites and altered floral characteristics Jagtap et al. (2011). RNAi has been used to modify plant metabolic pathways to enhance nutrient content and reduced toxin production (Table 1). The technique takes advantage of the heritable and stable RNAi phenotypes in plants.

Therapeutic application of alternative splicing: During the final step in formation of a mature, functional mRNA, the introns are removed from precursor mRNA and exons are joined together in a process called mRNA splicing. Each intron usually starts with a (5') GU and ends with an (3') AG (called GU-AG rule). Splicing occurs at these two Short, Conserved Sequences in Pre-mRNAs via Two Transesterification Reactions. In the first transesterification reaction, the ester bond is formed between the 5′ phosphorus of the intron-I and the 2′ OH of the branch-site Adenosine residue. In the second trans-esterification reaction, the ester bond is formed between the 5′ phosphorus of exon 2 and the 3′OH of exon 1, releasing the intron in a loop called lariat structure and joining the two exons. In each reaction, one phosphate-ester bond is exchanged for another. Since the number of phosphate-ester bonds in the molecule is not changed in either reaction, no energy is consumed. The net result of these two transesterification reactions is that two exons are ligated and the intervening intron is released as a branched lariat structure Verma and agarwal (2005).

In certain instances, the use of alternative splicing or alternative RNA splicing, or differential splicing of hnRNA, is a regulated and an evolutionary conserved process during gene expression that results in a single gene coding for multiple proteins. In this process, particular exons of a gene may be included within or excluded from the final, processed messenger RNA (mRNA) produced from that gene. Consequently, the proteins translated from alternatively spliced mRNAs will contain differences in their amino acid sequence and in their biological functions. Notably, alternative splicing allows the human genome to direct the synthesis of many more proteins than would be expected from its 20,000 protein-coding genes. Alternative patterns of RNA splicing result from tissue-specific adaptive and developmental control mechanisms. There are numerous modes of alternative splicing observed, of which the most common is exon skipping. In this mode, a particular exon may be included in mRNAs under some conditions or in particular tissues, and omitted from the mRNA in others.

Alternative splicing is a combinatorial process because many features are involved in the generation of transcript diversity such as splice site strength, intron-exon architecture, RNA secondary structure, splicing regulatory elements, promoter use and transcription speed by RNA polymerase and the presence of post-transcriptional nucleotide modifications. A comprehensive understanding of the factors that influence alternative splicing decisions is necessary to predict disease associated mis-splicing events Hossein and Hertel (2019). It has been estimated that approximately 15% of all mutations that cause genetic diseases result in defective splicing of pre-mRNA. Defects in splicing has been shown to be associated with genetic disorders such as β-thalassemia, cystic fibrosis, Duchenne muscular dystrophy, etc. the defects in alternative splicing contributes to cancer and also induce drug resistance Jhung-Chun (2018) Riboswitches are in vivo RNA aptamers that regulate both gene expression and alternative splicing by binding to small-molecule ligands Kenneth et al. (2009).

Therapeutic application of polyadenylation: Processing of the primary transcript involves the addition of a cap to the 5'-end, removal of introns, and polyadenylation (the addition of a poly(A) tail to the 3'-end) to produce the mature mRNA. Pre-mRNAs are first cleaved at specific 3′ Sites and subsequently polyadenylated. Both cleavage and polyadenylation of a pre-mRNA is carried out by by an enzyme, called poly (A) polymerase, utilizing ATP as a substrate. This step is called polyadenylation. The 3' end of most protein-coding genes and long non-coding RNAs is cleaved and polyadenylated. Control of polyadenylation is mediated by a number of sequence elements such as the polyadenylation site itself, but also a series of upstream (U and UGUA rich) and downstream (U and GU rich) elements. Similar to 5' capping, the 3' poly(A) tail is important for the export of mRNA from nucleus to cytoplasm, protein translation and stability of mRNA. The oligoadenylated RNAs are

efficiently decapped and degraded in 5' to 3' manner than polyadenylated and unadenylated RNAs.

Many genes contain more than one polyadenylation site. The use of alternative polyadenylation (APA) sites from a single transcript results in mRNA heterogeneity by generating mRNA isoforms that differ either in their coding sequence or in their 3' untranslated regions. Differential use of polyadenylation sites may also have impacts on function, stability, localization, and translation efficiency of target mRNAs. APA misregulation has been identified in human diseases. High levels of proliferation and differentiation capacity of cells correlates with increased relative expression of shorter 3'UTR isoforms and indicate UTR-based mRNA regulation Zheng and Tian (2014). Growing lines of evidence have shown that RNA-binding proteins (RBPs) play important roles in regulation of APA. Some RBPs are part of the machinery for cleavage and polyadenylation; others influence polyadenylation choice through binding to adjacent regions. Patterns of alternative polyadenylation are also regulated by differential binding of RNA binding proteins; cleavage stimulation factor (CSTF2) and cleavage factor I (CFI) of the main polyadenylation machinery have been shown to have effects on relative expression of alternatively polyadenylated isoforms Bin and Graber (2012).

Table 1. Improvement of quality traits engineered through RNAi Kamthan et al. (2015)

Trait	Target Gene	Host	Application
Enhanced nutrient content	Lyc	Tomato	Increased concentration of lycopene (carotenoid antioxidant)
	DET1	Tomato	Higher flavonoid and b-carotene contents
	omega-3 fatty acid desaturase [an enzyme converting linoleic acid (18:2) to alpha-linolenic acid (18:3)]	soyabean	Reduced alpha-linoleic acid content (1–3%) compared to non-transgenic soybean seed (7–10%).
	Starch branching enzymes (SBEIIa and SBEIIb)	Wheat, Sweet potato, Maize	Increased in amylose content for glycemic management and digestive health
	FAD2	Canola, Peanut, Cotton	Increased oleic acid content
	SAD1	Cotton	Increased stearic and oleic acid content
	22-kDa maize zein storage proteins (ZLKR/SDH)	Maize	Lysine-fortified maize
	Sucrose phos- phatase (SPP)	Potato	Reduction in conversion of sucrose to hexose sugars
Reduced alkaloid production	CaMXMT1 COR	Coffee Opium poppy	Decaffeinated coffee Production of non-narcotic alkaloid, instead of morphine
	CYP82E4	Tobacco	Six fold reduced levels of the carcinogen nornicotine in cured leaves
Heavy metal accumulation	ACR2	Arabidopsis	Arsenic hyperaccumulation for phytoremediation
Reduced polyphenol production	delta-cadinene synthase gene	Cotton	Lower gossypol levels in cotton seeds, for safe consumption
Ethylene sensitivity	LeETR4 ACC oxidase gene	Tomato Tomato	Early ripening tomatoes Longer shelf life because of slow ripening

Reduced allergenicity	Arah2	Peanut	Allergen-free peanuts
	Lolp5	Ryegrass	Reduced allergen in rye grass pollen
Reduced production of lachrymatory factor synthase (LFS)	Lyce3	Tomato	Reduced in tomato peel
	α-globulin and β-glyoxalase	Rice	mutated α-amylase/ trypsin inhibitor rice line
	lachrymatory factor synthase gene	Onion	Tearless onion

The cytoplasmic polyadenylation element binding protein 1 (CPEB1), an RNA-binding protein that regulates mRNA translation, also controls alternative 3'-UTR processing. CPEB1-mediated 3'-UTR shortening correlates with cell proliferation and tumorigenesis Ran et al. (2013). Other RNA binding proteins involved in regulation of cleavage and polyadenylation are RNA splicing factor hnRNP H1. Since RBPs influence the 3' end processing of alternatively polyadenylated transcripts, they can potentially be used as novel therapeutic agents Zheng and Tian (2014).

CRISPR/Cas9 genome editing: Genome editing technologies have become vital genetic tools in delivering pathogen resistance in plants. Due to higher success rate, easier to implement and less expensive nature of clustered regularly interspaced short palindrome repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) technology, it has overtaken other site directed modification methods such as meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs). This review focuses on the recent advances in plant protection using CRISPR/Cas9 technology in model plants and crops in response to viral, fungal and bacterial diseases. As regards to the development of viral disease resistance, integration of CRISPR-encoding sequences in Arabidopsis and Nicotiana benthamiana genome has been the main approach to prevent viral multiplication. With regards to fungal and bacterial disease resistance in crop species such as rice, tomato, wheat, and citrus, the strategies were based on targeted modification of susceptibility genes using CRISPR/Cas9 Borelli et al., (2018).

FUTURE PROSPECTS

Post transcriptional events such as alternative splicing, alternative polyadenylation and epigenetic modification of transcriptomes contribute to transcript heterogeneity and protein diversity, any misregulation in these events leads to human diseases. This is an exciting era of geneomic medicine where the future RNA-based therapeutics target post-transcriptional regulatory circuits at the interface of genes and proteins. Additionally development of siRNA constructs that can target multiple endogenous genes can usher development of novel plant types with altered phenotypic capabilities. By selectively suppression gene expression enhance the genetic value of crop plants that can be used for breeding for superior plant types that is impossible to be generated by conventional plant breeding methods. RNAi shortens breeding in woody trees by grafting transgenic RNAis on non-transgenic cutting for transmitting trait in a short time.

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The Role of Personalized Nutrition in Human Physiological Disorders

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ABSTRACT

The nutrients in regular diet are considered as environmental factors which have long-term effect on human genome. Personalized nutrition includes nutrigenetics and nutrigenomics studies that explains how food and genes interact and alters the gene expression. The "omics" studies are important tools to study the food-gene interactions. Most of the foods that are included in food habit of an individual exerts some physiological effects on the cells, organs or the whole body. These foods are referred as functional foods and the branch of science which deals with medicinal properties of nutrients is known as "nutraceuticals". The functional foods may affect the genotype of different individuals in a different way and not all the foods are suitable for all the persons having various pathophysiological conditions. Function foods should be should be recommended based on one's health, physiological condition, age and most importantly, their genetic setup. The concept of personalized nutrition was derived from "personal medicine" and in both cases regulation of gene expression is given priority based on the knowledge genomic studies. Understanding the complete molecular mechanism underlying the food-gene interaction and their effect to prevent the diseases like cancer, diabetes, obesity, thyroid, chronic degenerative diseases, etc, is required to assess the importance of personalized nutrition and functional foods as future tools for maintaining human health.

KEY WORDS: PERSONALIZED NUTRITION, NUTRIGENOMICS, FUNCTIONAL FOODS, PHYSIOLOGICAL DISORDERS, GENE EXPRESSION.

INTRODUCTION

Personalized food refers to genetically tailored diet which acts as therapeutics to treat certain physiological disorders in human beings. The concept of nutraceuticals or functional foods, where food is used as medicine paves the path of personalized diet, which mainly deals answers the question how genetic variations occurs due to the effect of consumed food. For example, a single gene mutation can cause phenylketonuria (PKU) and the affected person should refrain from taking foods like eggs, cheese, chicken, beef, pork, which are rich source of phenylalanine. Another such example is lactose intolerance, where the gene responsible for proper functioning of the enzyme lactase is turned off permanently. Polymorphisms in genes coding for the enzyme 5,10-methylene tetrahydrofolatereductase (MTHFR) affect its catalytic activity, which is directly related with individual'smetabolism and nutrient requirements (Reddy et al., 2018). People with lactose intolerance should avoid dairy products in their diet. The term like nutrigenetics and nutigenomics are closely related with personalized medicine. The ultimate goal for personalized food is to recommend a diet in accordance to one's genetic setup.

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To understand the background of personalized medicine, we have to look back in 1989 when Dr. Stephen De Felice coined the term "nutraceutical", a hybrid word derived from the two words "nutrition" and "pharmaceutical". The use of food as medicine is an age old concept which was gained a proper scientific acknowledgement at that time. In 1990, the famous Human Genome Project (HGP) was started aiming the determination of the actual base pairs present in human DNA as well as to identify and map all the genes of the human genome. After the completion of HGP in April, 2003, influence of foods in diet was studied with respect to the change in genetic expression. Sales et al., 2014 pointed out three important questions regarding food-gene regulation:

1. Can metabolic processes regulate the gene expression at cellular level which in turn can affect human health?
2. Do the genotype and nutrient interaction is responsible for regulation of gene expression by metabolic processes?
3. Studying the underlying mechanism of gene-nutrient interaction and ultimately will give rise to specific diet plan for each individual.
4. Nutrigenomics and nutrigenetics addressed all the above queries and emerged as a new topics of research.

Due to genetic polymorphism, the process of transcription in human is variable and regulated by variable factors among which nutrients is most important (Dauncey, 2013). The expressed mRNAundergoes alternate splicing and translation and gives rise to proteins having variations in function and half-life (Heyd and Lynch, 2011).The knowledge of metabolites and translation products will provide a clear idea about any pathophysiological condition. The end product of any metabolic pathwayexplains the expression of a gene at a given physiological scenario. The qualitative and quantitative analysis of all metabolites in a living system with respect to its gene expression is called metabolomics. Thus, personalized nutrition deals withnutritional research which comes under the broad terminology "nutigenomics" which includes nutrigenetics, transcriptomics, proteomics and metabolomics.Nutrigenomics refers to the ability of nutrients toupregulate or downregulate gene expression, and ultimately altering individual phenotype. On the other hand, nutrigenetics refers to polymorphic and mutant genes that can modify the bioactivity a metabolic pathway and its mediators.The basic concept of nutrient-gene interface can be summarized as (Farhud et al., 2010):

1. Nutrients directly or indirectly can alter gene expression
2. In some individuals under certain physiological conditions, food habit can be a determining factor for the onset of some diseases.
3. Certain diet-regulated genes and their variants are responsible for the onset and progression of some chronic diseases.
4. Depending on one's genetic makeup, the food habit regulates the metabolic pathways in healthy and diseased state of body.
5. Dietary intervention based on one's nutritional needs and genotype can be used to prevent, reduce the risk or cure chronic diseases. This explains the concept of personalized nutrition.

Personalized nutrition can be administered for the management of several diseases, though there are some challenges too. The underlying biological mechanisms at a single gene or protein level to study the nutritional effectsneedsdynamic interaction among diet, genes andphysiological condition rather thanhighlighting the interaction of nutrients on specific gene (Verma et al., 2016).There is an urgent need to build up a proper computational infrastructure that will deal with personalized nutrition. Standardization of data and intervention of improved methods to understand the topic in a detailed manner is the need of the hour. This review focuses on the impact of personalized nutrition to prevent, manage and treat physiological disorders as well as points out certain disadvantages/lack of study.... And the need of personalised diet for decreasing the risk factor of health issues due to the daily hectic schedule and unbalanced diet.

2. Interaction between genes and nutrients: The interaction between genes and nutrients is mediated by three probable mechanisms:
a) Direct interaction, where nutrients acts as transcription factors and bind to DNA to regulate the expression of related genes;
b) Epigenetic interactions, where nutrients modify the structure of DNA resulting in altered genetic expression;
c) Genetic variation: single genetic variations like nucleotide polymorphisms (SNPs) can regulate the functionality or expression of genes.

Direct Interaction: There are evidences which show that gene expression can be regulated by cholesterol, carbohydrate and the metabolites can behave as direct effectors of transcription factors. When there is excess intake of simple carbohydrate in diet, a major portion of carbohydrate is converted to triglycerides in liver, regulating hepatic enzymes. Pyruvate kinase (glycolysis), acetyl CoA carboxylase and malic enzyme (fatty acid biosynthesis), glycerol-3-phosphate acyltransferase (triglyceride synthesis) are induced by carbohydrate diet due enhanced levels of mRNA.

Cell membrane biosynthesis in mammals require cholesterol which may derive from diet or synthesized by cells. Low-density lipoprotein (LDL) receptor mediate the cholesterol uptake in cells. Low cholesterol levels induce production of more LDL receptor to uptake more of the cholesterol and vice versa. Low cholesterol upregulates the synthesis of two rate-limiting enzymes responsible for biosynthesis of cholesterol, namely, HMG-CoA synthase and HMG-CoA reductase. The cell ensures that is receives the required amount of cholesterol from diet and maintains the transcriptional regulation of genes that encode HMG-CoA synthase and HMG-CoA reductase.

This was experimentally demonstrated by Goldstein and Brown, 1990. Moreover, the nutrients andmetabolites can regulate the transcription by a well-established pathway.

The genes of steroid family acts as transcription factors and have receptors for steroid hormones, thyroid hormones, retinoic acid, vitamin D3 thatcan directly enter the cell to exhibit their biological activity (Towle, 1997). However, no ligands were identified for activating these receptors and hence they were termed as "orphan receptors". Later it was found that the intracellular nutrients and metabolites behave as their natural ligands. One of the most common examples of orphan receptors are peroxisome proliferator-activated receptors (PPARs) and the fatty acids were detected as their natural ligands. It was reported that consumption of high fat diet leads to selective induction of fibroblast growth factor1 by PPARγ in adipose tissues which is necessary for remodelling of adipose tissue (Jonker et al., 2012).

Epigenetic Interaction: Nutrients with potential bioactive molecules have the capability to induce protective epigenetic modification. The three-dimensional conformation of chromatin is regulated by environmental factors like pollutants, chemicals and nutrients which directly effects gene expression. Complete understanding of molecular mechanism by which environmental factors (nutrients, pollutants, chemicals) exerts their epigenetic effects will lead to the development of personalized nutrition strategies to prevent many diseases including cancer (Tiffon, 2018). The nutritional status at an early stage of an individual have a long term effect on DNA methylation pattern which in turn is related with chronic degenerative diseases (Lillycrop et al., 2014).

The nutrients modify the epigenetics by inhibiting DNA methyltransferases (DNMTs), histone deacetylases (HDACs) or histone acetyl transferases (HATs); or alters the substrate availability for these enzymes to carry out the enzymatic action. This ultimately leads to regulation of gene expression linked with pathophysiological processes like aging, embryonic development and carcinogenesis (Choi et al., 2010). Personalized nutrition and bioactive nutrient compounds can emerge as epigenetic therapeutical agents to combat with type 2 diabetes mellitus, inflammation, obesity, cancer, neuro degenerative diseases. Though there are a handful of studies regarding the preventive measure and disease management with this approach, nutritional epigenetics warrants better understanding of the molecular mechanisms of the bioactive nutrient components. Some examples of bioactive food components and their roles are as follows (Tiffon, 2018):

- Folic acid, vitamin B12, vitamin B6 play role in methionine synthesis
- Choline acts as methyl donor to S-Adenosyl methionine (SAM)
- Methionine plays role SAM synthesis
- Betaine lyses the toxic byproducts formed during synthesis of SAM
- Resveratrol, a well-known compound against breast-cancer, can remove acetyl group from histone
- Diallyl sulphide, butyrate, sulforaphane, turn on the anti-carcinogenic genes by increasing histone acetylation
- Genistein increases DNA methylation and has anti-cancer activities

2.3 Genetic Variation: The majority of the qualities have differences in small sequences– polymorphisms – that fluctuate among people. Single nucleotide polymorphisms (SNPs) are the most well-known sort of variety (Debusket al., 2005). The single nucleotide polymorphisms consortium is mapping polymorphic areas of the genome that control individual phenotypic contrasts among the human populace. The significance of this hereditary variety to the fluctuating requirements for and physiological reactions to the specific supplements was expressed by Ames (Afman and Müller, 2006). Missense single nucleotide polymorphisms happen around 1 in each 1000 bases in communicated qualities, so one anticipates that there will be a lot more polymorphisms to be found in micronutrient and dietary investigations. Explicit hereditary polymorphisms in human populaces change their metabolic reaction to slim down and impact the hazard examples of infection as SNPs are like varieties in a formula. Every quality is a formula for a particular protein or gathering of proteins that either manage organic capacities or fill in as basic structure hinders for tissues (e.g., collagen). A few SNPs change the formula for the quality so that either an alternate amount of the protein is created or the structure of the protein particle is modified (Schneider et al., 1998)

3. Dietary Habit Affects Gene Expression: These hereditary polymorphisms lead to modification of the reaction to the dietary segments by affecting ingestion and digestion. Epigenetic occasions can incite changes in DNA methylation example and along these lines impacting overall quality articulation that can be altered because of the food segments. Nutrition has played a recognizable and prevalent role in the management of health. Nutrigenetics is the science that recognizes and portrays the gene variations related with the reaction to supplements and relating this variety to variable diseases states particularly cancer, diabetes, obesity and other diseases. Numerous dietary constituents influence post interpretation occasions and numerous record for in any event part of the variety in light of the dietary segments (Ames, 1999).

3.1. Cancer: Various studies has considered that SNPs in a few Se-related qualities may influence weakness to disease. For instance, the Leu allele in the SNP at codon 198 in GPX1 was accounted for to be related with lung, bosom, and bladder malignancy (Villette et al., 2002), despite the fact that this was not affirmed for bosom disease (Ahn, 2005). Strangely, the relationship with bladder malignant growth might be affected by a SNP in the manganese superoxide dismutase (MnSOD) quality (Ichimura et al., 2004), demonstrating the expected significance of investigating different SNPs based on a metabolic pathway.

Basic variations in qualities controlling homocysteine digestion, for example, methylenetetrahydrofolate reductase (MTHFR), and methionine synthase (MTR), have been connected to expanded hazard for bosom malignant growth in people with low admissions of folate, nutrient B6, and nutrient B12 (Hill et al., 2004;Ahnet al., 2010). Likewise, it has been accounted for that notwithstanding daylight, nutrient D status can likewise be impacted by a few polymorphisms in nutrient D pathway qualities (Barry et al.,2014;Desmarchelier et.al., 2016), accordingly tweaking its natural capacities in the creature. Strikingly, SNPs in the nutrient D receptor (VDR) quality, which influence nutrient D accessibility (Heap et al., 2009; Stathopoulou et al.,2011), have been related with osteoporosis inclination in postmenopausal ladies with low calcium admissions (Hosseini-Esfahani et al., 2014).

The 15-kDa selenoprotein quality includes two varieties inside the 3′ UTR, at positions 811 and 112 selenocysteine insertion sequence or SECIS. Most of the findings showed that the two SNPs influence Se insertion, and the 2 polymorphisms were accounted for to influence the harmful effect of cancer. Fundamental information recommend that the T-C variation in the 3′ UTR of glutathione peroxidase-4 (GPX4) influences danger of colon malignant growth (Dumitrescu et.al, 2005). SNP affiliation considers have been completed on moderately little populaces and with single SNPs. Moreover, not many examinations have joined the SNP relationship with point by point investigation of Se status. Future investigations ought to examine bigger, rehash populace accomplices and consolidate genotyping with investigations of healthful admission or status to survey the significance on supplement quality collaborations in deciding defencelessness. A wide scope of SNPs ought to be examined, at first dependent on a pathway approach, to incorporate qualities encoding items engaged with Se consolidation instruments and Se transport.

As per our review is concerned the lipid is the key factor for this disease. The complete lipid based personalised food are found to be harmful for cancer as the cancer cells need more energy as they replicate and differentiate much faster than the normal cells. A personalised diet along with protein, Vitamin D, B12 and also vitamin C to boosting up our immune response and carbohydrate is always been recommended to be more effective than any other diet to stop these gene up regulations in cancer.

3.2. Obesity: Obesity has become one of the worldwide epidemics with over 35% of the total populace (2,100 million individuals) being assessed as either overweight or fat as indicated by weight list (BMI) (Kassebaum et al., 2015). Corpulence is related with an enormous number of medical issues including dyslipidemias, cardiovascular infections (CVD), type 2 diabetes mellitus (T2DM), non-alcoholic greasy liver sickness (NAFLD), and a few sorts of disease, with significant financial and social expenses (Seidell and Halberstadt, 2015). Deliberate investigations have uncovered that weight and overweight caused 3.4 million passing in 2010. The unbalanced eating regimens like, fat, fructose, the high substance of calories, and high omega-6/omega-3 unsaturated fat proportion and obvious combination of the inactivity in daily life, found to add the advancement of obesity and the diseases related to this. Additionally, it is currently perceived that associations of hereditary and epigenetic genes with ecological elements (dietary admission or physical action) assume a significant job in deciding individual phenotypes (Ramos-Lopez et al., 2017). In recent studies has shown after analyses of 240 SNPs, responsible for the genes which are nutrient-sensitive lipid metabolism among the people with obesity and overweigh, there is an interaction between the dietary protein and the LPIN1 rs4315495, which may lead the result of lowering the concentration TAG for minor allele which carriers on the high-protein weight maintenance diet (Braheet al., 2013).

Moreover, it was accounted for that revelation of hereditary data with respect to angiotensin I changing over chemical (ACE) genotype for customized nourishment brought about more notable changes in sodium admission contrasted with all-inclusive community based dietary exhortation (Nielsen and El-Sohemy, 2014). Moreover, people who consumed unsaturated fat desaturase 1 (FADS1) genotype were found progressively upregulation of omega-3, the unsaturated fats (Roke, 2017). These findings showed that the identification of good supplements of dietary products are dependent on hereditary diseases than general dietary is concerned (Nielsen and El-Sohemy, 2012). We have found that the unsaturated fatty acids are the key factor for developing the up regulation of the genes responsible for obesity and recent studies have shown that the most developing disease in the adults for changes in the food behaviour. The personalised food will be a great help for them. This may be designed as per the concerned age, BMI, food habit and the habitant. The personalised food may be composed of only proteins and necessary vitamins needed.

3.3. Thyroid: Thyroid hormone receptor-beta obstruction has been related with metabolic order. THRA quality sequencing of different genes has introduced as observational changes in the thyroid hormone receptor-α (THRA), may identified as a polymorphism (rs12939700) in the critical region of TRα processing . Genome-wide studies consider having the proof of numerous quality variations identified with thyroid and obesity. Another method of distinguishing the upregulation of the genes are found to be the hereditary approaches of different clinical cases. It has been observed that the treatment with higher percentage of l-thyroxine lead to the biochemically hyperthyroid, in this case the person may interface weight loss drastically within 6 months, and also raised thyroid hormones (Jiang et al., 2004).

Sequencing of the THRA locus uncovered a polymorphism in a basic district engaged with the guideline of grafting. In some case studies it has been found that the two polymorphisms of the THRA locus that had an important role with various aspect of body mass index (BMI).

Some of the reviews have mentioned, the polymorphism present in the record case (rs12939700) was related with obesity. Another polymorphism (rs1568400) that has a moderately high recurrence in the populace was likewise connected with BMI as per both cross-sectional (in two free overall communities of Spain and France) and follow-up examinations in the Spanish populace based associate (Fox, 2008; Reinehr, 2010; Fernández-Real et al.,2013).

It can be concluded from some of the findings that the co-operations of variations of the poly [ADP-ribose] polymerase 1 (PARP-1) expression play the key roles in sorting out the different changes that happen during the upregulation of thyroid. It has also been established that there is a connection between the PARP-1 polymorphisms (particularly rs1136410 TC) and the advancement of thyroid. Iodine and the unsaturated fats are found to be responsible for the up regulation of the genes responsible for the thyroid. The personalised diet excluded of unsaturated fat may be a possible way to stop these regulations of the genes.

3.4. Diabetes: In recent studies showed that the carbohydrate diet plays an important role in the pathway regulation of IFN- γ and IL-15 and both IFN-γ and IL-15 are proinflammatory cytokines that play role in advancement oftype 1 diabetes mellitus (T1DM) in non-stout diabetic (NOD) mice, while scurfin or Foxp3 is a transcription factor that coordinates the differentiation of the T cells (Patrick et al, 2013). In some case studies cereal diets has been given to the bio-breeding diabetes-prone (BBDP) rats and maintained a condition of specific pathogen-free, i.e., they have allowed the growth of gut microbes, thus these rats have showed an upregulation of the Lck gene or lymphocyte-specific protein tyrosine kinase (Sildorf et al., 2012).

Lck promotes the tyrosine kinase/p56 which is a lymphocyte-specific protein and plays an important role in the activation of the T cell (Knip et al., 2011). In fact the, BBDP rats has also showed the down regulation of the antimicrobial peptide, cathelicidin antimicrobial peptide (CAMP) gene. CAMP gene which may alter the gut microbes by immunomodulatory host defence factor (Hyppönen et al., 2011). Thus, for type 1 diabetes mellitus it has been found that diet can modify alone or through the changes in the gut microbes by the changes of the expression of genes which are found to be involved in the immune response (Wu et al., 2011).

More than 70 genes have been identified through different studies, which are involved and associated with the type 2 diabetes mellitus (T2DM). Through GWAS arrays, it has been reported that there is an association of 100 SNPs with T2DM. Previously identified 50 novel loci which are directly associated with T2DM, secondly with T2DM related traits there are more than 40 loci have been associated, including insulin and glucose and fasting proinsulin (Fu et al., 2010). However, the HOMA index known as the pancreatic β cell function for T2DM-related traits and studies has found that there is a profound relationship between these traits or the genotype and environment interactions. Some Clinical investigations on the loci have found that the genes of T2DM risk through the function of β cell.

Recent studies have shown that the function of the β cell may improve by the vitamin D by limiting the expression of the chemokinine and normalizing the partial expression of the class I molecules of the major histocompatibility complex (MHC) and decreasing the amount of the MHC I proteins on β cells (Gysemans et al., 2005; Wolden-Kirk et al., 2014). Some of the findings has also showed that the insulin secretion may increase with the help of the biotin (Vitamin B) which up regulates the genes of the islets (Lazo de la Vega-Monroy et al., 2013; Berná et al., 2014). The diet includes carbohydrates and some of the fatty acids are found to be responsible for the gene regulation in diabetes by influencing the β cells. Some of the Vitamins have also found to be involved in these regulations. Personalised food enriched in protein and certain amount of fats and also depending on the body weight, BMI may play a vital role in the management of this disease.

4. Personalised Diet and its relevance: Personalised diet is established in the idea that one size doesn't fit all; distinctions in organic chemistry, digestion, heredity, and microbiology contribute to the individual differences observed in response to nutrient status, nutrition, and timing of eating, dietary patterns, and environmental exposures. Personalised diet has been described in many possible ways, and various terms has also been used to describe such as "individualized nutrition," "precision nutrition," and "nutritional genomics". Biological systems like the immune system and the changes in immune cells due to age can propagate to the thymus to such a degree, that the capacity to react to new insusceptible difficulties is debilitated when an individual ranges midlife (Aspinall& Andrew 2000). But in most other organs like muscle, does not allow the changes due to different heavy training (Frischknecht 1998).

Long-term conditions that expansion in commonness with age can affect dietary needs. A person's portability will affect on vitality needs and poor versatility may add to the expanded pervasiveness of corpulence in individuals matured more than 50 years. Muscle versus fat deposition is a conspicuous and basic phenotypic measure that has significant ramifications for an individual's long-term wellbeing and requires a simple-to-administer test. Muscle to fat ratio synthesis information will feature expanded hazard for metabolic infection on one hand just as expanded hazard for feebleness on the other. While progresses in nourishment science have to a great extent annihilated supplement lack infections in the western populace, there are developing difficulties of stoutness with its comorbidities and of maturing.

Personalised diet, i. e., the daily intake of the energy may be advised to the people dependent on their present age group is one of the easiest nutriphenomic intercessions accessible and is as of now broadly applied

during the management of obesity. It has found that due to deposition of fat in the visceral organs, the risk of human health are being increased day by day (Direk et al., 2013).

CONCLUSION

Personalized nutrition came into limelight fifteen years ago through scientific publications, conferences and nutritional genomic analysis, though the term was first used by Dr. R.O Brennan in the year 1975 (Simopoulos, 2010). The outcome of Human Genome Project and knowledge of functional foods spurred research demonstrating the link between dietary habit, physiological disorders and genotype. Personalized nutrition deals with single nucleotide polymorphisms (SNPs), epigenetic modifications that dictates the diet plan for an individual to maintain optimum health condition. There are approximately ~20,000 genes in human body, having ten million SNPs present in one individual. On an average every five to fifty genes have at least one SNP per gene.

Thus, to analyse nutritional genetic tests, the gene-gene or gene-nutrient interactions have to be studied more extensively. In some cases nutritional genetic test data proved to be psychologically promising, though medical history, family history of genetic disorders, present physiological condition, regular dietary intake, and preference for certain types of foods, etc. had to be taken in account. The research on personalized nutrition needs robust computational approach to analyse individual data based on genetic setup, age, sex, and race and integrate them with "omics" data to actually recommend a personal diet. In the past ten years, no long-term study was carried out on personalized nutrition in a large population. Some investigators consider the change in lifestyle in a population and prefers a universal approach, rather than a targeted one, to lifestyle intervention (Langenberg, 2014) for prevention of diseases.

The progress of personalized nutrition can be facilitated by developing sound theoretical knowledge on the subject to identify the most prominent personal characteristic on the basis of which personal diet will be administered. The efficacy of the recommended diet and cost effectiveness data should be well documented from authentic intervention studies. Moreover, initiatives should be taken by the policy makers to introduce regulatory framework and proper guidelines for health professionals and dieticians to familiarize personal nutrition concept to public. The present state of knowledge regarding personalized nutrition warrants increase in scientific evidences.

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From Phytochemicals to Phytomedicines: Potential Roles of Plant-Based Biomolecules in the Covid Era

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ABSTRACT

The current pandemic of the Corona virus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has urged for the invention and implementation of effective drugs and vaccines to mitigate the adversity worldwide. Numerable studies are going on to identify and evaluate the efficacy of several synthetic drugs and vaccines. In this scenario, identification and use of plant-derived biomolecules against SARS-CoV-2 could be highly beneficial. Furthermore, upscale production of such plant metabolites and plant-based vaccines can help in controlling the pandemic. Several previous studies have reported the success of plant-based traditional medicines in immunity enhancement and decreasing viral loads. Thus, in depth researches involving the phytochemicals could reveal their roles and level of efficacy against SARS-CoV-2. Considering the present scenario, this review article presents the perspectives of using the phytochemicals in mitigating SARS-CoV-2, and the possible evolution of these phytochemicals into phytomedicines.

KEY WORDS: PHYTOCHEMICALS, SECONDARY METABOLITES, SARS-COV-2, COVID-19.

INTRODUCTION

The Coronaviruses (CoVs) family (Coronaviridae) consists of single-stranded RNA viruses and has been reported since the 1960s (Adhikari et al. 2020). These viruses can infect a wide array of hosts, including cattle, camels, bats, and humans (Boopati et al. 2019). The newly discovered severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) virus, previously known as novel corona virus, which cause the COVID-19 disease belong to the CoVs family (Prasad et al. 2020). As of present no vaccine has been officially available for the human consumption to curb or to treat the COVID-19 disease. Although, some vaccines in their trial stages are showing promising results, they are still to clear some more trial phases before getting available in the market.

In this scenario, a rampant search is going on to find chemicals or compounds of antiviral capacity, which is evident by the number of studies getting published recently. Apart from vaccines, development of effective drugs or the use of already existing drugs is the most practiced methods to treat COVID-19. For instance, different nucleoside analogs like ribavirin, favipiravir, and remdesivir, anti-HIV drugs like, ritonavir and lopinavir and reverse-transcriptase inhibitor drugs like azvudine are in use for treating COVID-19 patients (Wang et al. 2020; Chen et al. 2020; Harrison 2020). Although, much of the attention is given in discovering synthetic drugs and searching for a potent active pharmaceutical ingredient (API), use the plant-based biomolecules against COVID-19 is largely unexplored.

Plants are the producers of innumerable phytochemicals and bioactive metabolites having huge pharmacological attributes such as, antimicrobial, antioxidant, anti-hypertension, anti-diabetic, anti-inflammatory,

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anticancer, and immunomodulatory activity (Patel et al. 2020; Adnan et al. 2020; Patel et al. 2018). In addition, some phytochemicals are reported to have antiviral properties and are capable of inhibiting viral replications (Ben-Shabat et al. 2015; Arunkumar et al. 2019). Thus, finding out a potent phytochemical(s) by exploiting various technological interventions, which could be effective in controlling SARS CoV-2 and treating COVID-19 will be absolutely crucial. In this review, such plant-based biomolecules having antiviral properties and can thereof be extrapolated to use against SARS CoV-2 are highlighted. Also, the importance and the potential therapeutic applications of novel phytochemicals are discussed.

Plant-derived bioactive compounds and their effectiveness against SARS CoV-2:

The present scenario: Numerous plants are there with vital medicinal properties that can inhibit the viruses like, Middle East respiratory syndrome coronavirus (MERS-CoV), Human coronavirus 229E (HCoV-22E9), and SARS-Cov due to the presence of various bioactive compounds (Siddiqui et al. 2020). Previously, use of traditional plant-based medicines in China have successfully aided in treating the SARS-CoV during the pre-COVID-19 times (Lau et al. 2005). Although, like most of the traditional medicine practices, the exact mode or composition of the treatment is unknown, the role of phytochemicals and plant secondary metabolites of the used plant parts could be the best fit candidates to attain the results (O'Connor 2015). Studies involving plant secondary metabolites production and applications have revealed their efficacy in treating various diseases.

For instance, the alkaloid lycorine derived from *Lycoris radiate* possesses antiviral activity against different types of viruses, including Herpes simplex virus, Poliomyelitis virus, and SARS-CoV (Li et al. 2005). Similarly, the alkaloid quinine obtained from *Cinchona officinalis* plants is hugely effective in the treatment of malaria from many decades (Achan et al. 2011). Furthermore, the structural analog of quinine, hydroxychloroquine (HCQ) has been used to treat COVID-19 patients along with the antibiotic azithromycin (Gautreta et al. 2020). Cinatl et al. (2003) reported that glycyrrhizin, a saponin from *Glycyrrhiza glabra* can inhibit the viral replications and can be effective against SARS-CoV infection. Similarly, the water extract of the herb *Houttuynia cordata* was found to exhibit antiviral activity against SARS-CoV by inhibiting the viral RNA-dependent RNA polymerase (RdRp) and 3C-like protease (3CLP) (Lau et al. 2008). As SARS-CoV and SARS-Cov-2 share high similarities, the use of glycyrrhizin or *H. cordata* extracts could be a possible alternative to treat COVID-19.

Flavonoids like Myricetin from the Chinese bayberry plants *Myrica rubra* and Scutellarein from the herb *Scutettaria baicalensis* showed antiviral effects on the SARS-CoV virus (Yu et al. 2012). Likewise, flavones from the coniferous tree *Torreya nucifera*, including apigenin, quercetin, amentoflavone, and luteolin have been reported to have 3CLP inhibitory properties (Ryu et al. 2010). Additionally, bioactive compounds such as, hesperetin, emodin, and sinigrin obtained from the herb *Isatis tinctoria* exhibited 3CLP inhibitory properties (Lin et al. 2005).

Lectins are a class of plant secondary metabolites having crucial roles in plant immune responses, thus can be potential antiviral compounds. In the pre-COVID-19 times, 33 numbers of different plant-derived lectins showed antiviral activity against SARS-CoV virus (Keyaerts et al. 2007). Agglutinin, a lectin obtained from *Galanthus nivalis* was reported to show antiviral activity against the Feline coronavirus (Hsieh et al. 2010). Further, a natural occurring stilbene derivative resveratrol was reported to inhibit MERS-CoV infection (Lin et al. 2017).

Albeit, huge number of phytochemicals and the plant bioactive compounds have been studied for their antiviral properties (Table 1), no extensive research has been done to isolate the API and then exploit it into meaningful bio-therapeutics. Recently, the AYUSH ministry of India has recommended the people to consume a cocktail of medicinal plant parts/extracts containing as many as 15 plants, including ginger, clove, black pepper, and others (Ministry of Ayush PIB 2020). Moreover, Siddiqui et al. (2020) reviewed the potential role of about 40 medicinal plants in contributing towards the treatment of COVID-19. Thus, these available data suggest that there is a need of collaborating the researches to examine the synergistic effect of such plant-derived biomolecules and other synthetic drugs against SARS-CoV-2 to get the best solution in winning against COVID-19.

Plant biotechnology-based advancements and solutions:

Since last decade, plant biotechnology has taken a leap into the future by adopting new cutting-edge technologies and inventions. Processes like targeted metabolic pathway engineering, enhancement of phytopharma capacity and production of recombinant enzymes, hormones, and vaccines has been realized via biofarming (Rosales-Mendoza 2020). The advantage of producing these transgenic or transient recombinants is that they follow the required post-transcriptional and post-translational modifications, unlike in bacterial systems and they are devoid of the animal pathogens (Takeyama et al. 2015). In *Nicotiana benthamiana* plants, the human Type-I collagen was produced and commercialized. Furthermore, vaccines for diseases like influenza, rabies, and hepatitis-B were produced in plant systems and underwent clinical trials (Takeyama et al. 2015). Recombinant N-terminal of S-glycoprotein of swine-transmissible gastroenteritis coronavirus obtained from the leaf extracts of transgenic *Arabidopsis thaliana* and *Solanum tuberosum* lines exhibited antiviral activities against SARS-CoV virus (Gómez et al. 1998; Gómez et al. 2000).

Similarly, in *Lactuca sativa* and *N. benthamiana* the stable expression of S-glycoprotein of the SARS-CoV virus was achieved, which served as a potential oral vaccine (Li et al. 2006). Another study reported the consumption of

transgenic tomatoes expressing S-glycoprotein of SARS-CoV was successful in producing the virus-specific IgA in mice (Pogrebnyak et al. 2005). Many organizations related to manufacturing phytopharma based drugs have already commenced the development of plant-based vaccines against COVID-19 (Rosales-Mendoza 2020). On the other hand, the rapid sequencing of SARS-CoV-2 virus strains and availability of the data in public domains have triggered the epitome mapping studies that ultimately could result in developing new and potent vaccine against SARS-CoV-2. Interestingly, different efficient plant biomolecules and phytochemicals are often present in lower concentration. Thus, bio-firming of these chemicals via targeted metabolic engineering in the *in vitro* cultures could result in their boosted production and recovery (Kayser 2018). Moreover, plant biotechnology along with targeted metabolic pathway engineering leading to the upscale production of antiviral phytochemicals against SARS-CoV-2 could emerge as a big solution to the current COVID-19 pandemic.

Table 1. List of the bioactive compound from plants and their effect on viruses.

Plant Name	Product	Virus Type	Effect	Reference
Euphorbia jolkinin *Reseda luteola* *Aesculus hippocastanum*	Tetra-O-galloyl-β-d-glucose luteolin	SARS-CoV	Antiviral	Yi et al. 2004
Rauvolfia serpentina *Scrophularia scorodonia* *Heteromorpha* spp.	Aescin, reserpine	SARS-CoV	Viral replication inhibitor	Wu et al. 2004
Bupleurum spp.	Saikosaponins	HCoV 229E	Disruption of host penetration and surface attachment	Cheng et al. 2006
33 different plant spp.	Lectins	SARS-CoV, Feline coronavirus	Viral S-glycoprotein inhibition	Keyaerts et al. 2007
Rheum spp. *Polygonum* spp.	Emodin	SARS-CoV	Blocking of S-glycoprotein and ACE2 interaction	Ho et al. 2007
Toona sinensis *Malus* spp., *Camellia* spp.,	Leaf extract	SARS-CoV, HCoV 229E	Viral replication inhibitor	Chen et al. 2008
Allium spp.	Quercetin	SARS-CoV	Antiviral	Park et al. 2012
Euphorbia neriifolia	Ethanolic extract	SARS-CoV	Antiviral	Chang et al. 2012

CONCLUSION

The COVID-19 pandemic is unprecedented and has asked for the scientific solution in solidarity. Be it traditional or synthetic, any drug showing potential against the SARS-CoV-2 is highly welcomed in these trying times. Considering this fact, the plant-based biomolecules that exhibit antiviral properties should be explored and an exhaustive research on this could unearth new possibilities. To upscale the production and recovery of the valuable phytochemicals use of plant biotechnology and metabolic engineering can facilitate the initiative. Furthermore, production of plant-based vaccines and their successful clinical trials will not only help in controlling the global pandemic, but also can be hugely cost effective. Moreover, evaluating the synergistic effects of the phytochemicals and synthetic drugs could prove to be a successful strategy to curb COVID-19 and be a roadmap for future studies.

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Pitcher Irrigation Technology for Crop Cultivation in Arid Region

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ABSTRACT

Water is the primary input for crop production and increasingly becomes scarce due to its high demand in agricultural sector. Quality of water is assuming great importance with the increasing demand in industries, agriculture and rise in standard of living. Agriculture is the major user (89%) of India's water resources. Pitcher irrigation is an ancient and very effectual irrigation system employed in many arid and semiarid counties. Among traditional irrigation systems, pitcher irrigation is one among the foremost efficient and compatible for little farmers in many areas of the planet. Pitcher irrigation entails burying an unglazed, porous clay pot with in soil before seedling. Water poured into pot seeps slowly into the soil, feeding the seedling's roots with a gentle supply of moisture.

KEY WORDS: PITCHER POT, IRRIGATION, SALINITY, DRIP IRRIGATION.

INTRODUCTION

Pitcher irrigation is cost effective, farmer-friendly, and easy to install. Pitcher irrigation involves no high tech gadgets and does not require any maintenance. It is ideal for small holdings (1-2 acres) and suitable for growing vegetables, coconuts, and areca nuts. It consists of a clay pot with a cotton wick fixed at the bottom of the pot, and buried in the soil (up to its neck) and filled with water (Adhikary et al., 2020 and Pal et al., 2019). The natural pores in the pot allow the water to spread into the soil, creating moisture for crop growth. The water can be filled as and when required, thus maintaining a continuous supply of water to the plants (Umalaxmi et al., 2017 and Adhikary et al., 2020). While burying the pitcher in the soil, farmers should take care to see that the neck region of the pot is positioned in such a manner that rainwater runoff does not enter into the pitcher. Otherwise small sand particles will block the pores of the pitcher.

The main advantage of the wick which is attached at the bottom of the pot is to increase the water penetration into the soil and to deliver the water directly to the plant roots. The number of pitchers required per acre depends on the crop variety grown. For coconut seedlings about 170 pots per hectare (that is 70 pots per acre), and for areca nut about 1100 pots (440 pots per acre) will be required. A farmer can save 90 per cent of water as compared to flood irrigation. Fertilizers can also be mixed along with the water and poured into the pot. Weed growth has been found to be very minimal because water delivery is limited to the roots. Many farmers in the coastal districts are following this method. If you have a garden at home try this irrigation method (Adhikary et al., 2020 and Umalaxmi et al., 2017).

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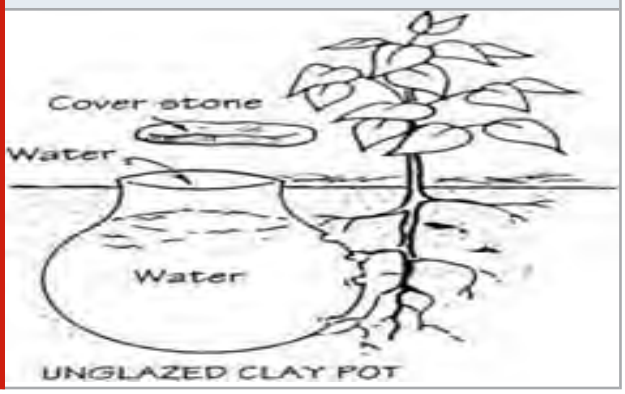
Figure 1: Pitcher irrigation using an unglazed clay pot (Vukasin et al. 1995)



History of pitcher irrigation: Pitcher irrigation is an ancient technique that has been practiced in many parts of the arid world including Iran, India, African and South American countries (Mondal, 1974; Stein, 1997). The technique is simple, cheap and could have blarge water-saving potential (Mondal, 1978; Bainbridge, 2001). Pitcher irrigation has been mentioned in a book written some 2000 years ago in China (Sheng, 1974). The method reportedly has been used to irrigate watermelons in India and Pakistan (Mondal, 1974; Soomro, 2002); horticultural crops in Brazil, Germany, and Indonesia (Stein, 1997; Setiawan et al., 1998); and corn, tomato, and okra in Zimbabwe (Batchelor et al., 1996). A few researchers have indicated that pitchers could have self-regulative capability in conditions where seepage is controlled by the soil water pressure head, which is, in turn, a function of the soil water content around the pitcher (Chigura, 1994).

Pitcher Irrigation:

An Overview: Pitcher Irrigation is an inexpensive small-scale irrigation method practiced in the semi-arid state of Karnal, India. The system consists of burying unglazed clay pots in the soil up to their neck. When the pot is filled with water, the natural pores in the pot's walls allow water to spread laterally in the soil, creating the moist conditions necessary for plant growth. Pitchers are filled as needed, maintaining a continuous supply of water directly to the plant root zone. One of the advantages of using pitchers for irrigation is the result of their water saving capacity. To compare pitcher irrigation to flood or sprinkler irrigation one must correct for the fact that the scales are radically different.

Pitcher irrigation is used on small-scale, while flood and sprinkler systems are for more extensive irrigation. Taking this into account, pitcher irrigation is still more efficient. Pitcher irrigation uses water more efficiently than other systems since it delivers water directly to plant root zones, instead of to broader areas of the field. With pitcher irrigation, deep percolation losses are negligible since water is released from smaller areas, and the rate of water loss can be controlled site to site by the amount of water put in each pitcher. Water requirements in a pitcher irrigated field can be even less than those of a drip irrigated system (of the same scale) due to the very low hydraulic conductivity of the pitchers, as well as reduced evaporation losses.

Research with pitcher irrigation at the Central Soil Salinity Research Institute (CSSRI) in Karnal India indicates that the amount of water which seeps out of the pots--and thus the number of plants which can be sustained by each pot--depends on the soil type, the porosity of the pot wall as well as the shape of the pot used. Pitchers are generally placed at distances so that wet areas do not overlap. Soil moisture and salt distribution in the plant root zone are much more favourable with pitcher irrigation than with any surface method of irrigation. Under pitcher irrigation salt accumulates at the soil surface, leaving the salt content of water in the root zone more favourable than the salinity of water used in the pitcher. Thus even saline water can be used for irrigation in the pitcher irrigation system. Scientists at the Central Soil Salinity Research Institute have found that seven to ten litre pots are sufficient to grow most vegetable crops. The number of pitchers needed per hectare varies with the crop. At least four plants of most vegetable crops could be grown around one pot. A creeping crop such as bitter gourd required 2,000-2500 pitchers per hectare. Upright crops or crops producing a canopy around the pot required more pots, up to 4,000-5000 pots per hectare.

The profitability of pitcher irrigation must consider the labour of acquiring, burying, and filling the pots, in addition to the labour involved in managing the crop. Researchers at CSSRI found that the most profitable crops for pitcher irrigation in that area were (in order) tomato> bottle-gourd> bitter-gourd> watermelon> cauliflower. The muskmelon was unprofitable, thus they do not recommend its cultivation with pitcher irrigation. The prospects of pitcher irrigation are reasonably high, especially in areas where water scarcity and salinity limit cultivation. The only difficulty with this method is the high labour demand which it places on the farmer. Pitcher irrigation may be an inappropriate solution where the labour needed to set up and run the system would fall on already overworked labourer's (CSSRI, Karnal).

Advantage of pitcher irrigation:

1. In this method, only the area near the pot gets irrigated and not the whole area.
2. Evaporation of water is minimum in this method.
3. Water seepage below the ground is also in minimum quantity.
4. It is the best method for horticulture crops and vegetables.

5. Once the pitchers are fixed, irrigation can be done for six years, which reduces expenditure.
6. It needs minimum technical knowledge.

Effect of Pitcher Irrigation on Crops Cultivation: Batchelor (1997) carried out irrigation trials and experiments in south-east Zimbabwe and northern Sri Lanka during 1985 to 1995 and found that subsurface irrigation using clay pipes was particularly effective in improving yields, crop quality and water use efficiency as well as being cheap, simple and easy to use. Comparing the field experiment conducted by Mondal (1974) and Scheuring (1983), it was found that yield of pitcher pot irrigated melon in India was 25 t ha-1 using only 2 cm water ha-1 (Mondal, 1974), whereas the yields of melon was 33 t ha -1using 26 cm of water with flood irrigation (Scheuring, 1983). Balakumaran et al., (1982) conducted a detailed study of cucumber production which showed that irrigation of 1.9 mm ha-1 with pitcher pots provided yields comparable to 7.3 mm ha-1 by hand irrigation.

Pachpute (2010) also concluded that the increase in total yield due to package of water management practices including pitcher irrigation method is 203 per cent and water use efficiency obtained is 12.06 kg m-3. Saha et al., (2005) conducted an experiment with pumpkin (C. moschata) involving three methods of irrigation (drip irrigation by direct pitcher, drip irrigation by pipe from pitcher and basin system of irrigation). The direct pitcher method recorded significantly higher values for vine length, number of nodes per vine, stem girth and significantly lower values for inter node length compared to the other two methods of irrigation at all stages of plant growth.

Effect of Pitcher Irrigation with Saline Water: The stable soil moisture maintained by pitcher pot irrigation enables crops to be grown in very basic or saline soil or with saline water under conditions in which conventional irrigation would fail (Rai, 1982). High tomato yields of 27 t ha-1, were obtained in India using saline irrigation water, EC 10.2 mmhos cm-1, while typical yields in this area with fresh water, EC 0.4, ranged from 15-25 t (Mondal, 1983). In Kenya 61% of normal crop yield was achieved with irrigation water of EC of 8 dS m-1, when typical irrigation failed at EC of 4 dS m-1 (Okalebo et al., 1995). Alemi (1980) stated that pitcher pot irrigation moved salt out of the plant root zone better than drip irrigation.

Very low-fired pots may break up in very saline soil as a result of chemical reactions with the salts. Mondal et al., (1992) showed a 20% decrease in brinjal yield at 12 dS m-1 compared with the control but was not adversely affected below this level of salinity. Pitcher irrigation is considered more efficient than surface, drip and sprinkler irrigation and produces yields even when saline water is used. Dubey et al., (1990) conducted experiment on ridge-gourd (Luffaacutangula) and found that increasing salinity resulted in increasing delay in germination; highest yield (4.45 kg/pitcher) was obtained with 0.4 dS m-1 irrigation

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Poly Tunnels: Advantages, Present Status and Future Prospects- Review Study

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ABSTRACT

Protected Cultivation practices are cropping technique to maximize the plant yield by controlling the micro environment around the plant either partially or fully during the time period of plant growth. Commonly used protected cultivation practices are greenhouse (forced and naturally ventilated), shade net house, polythene tunnel and mulching, raised beds and drip irrigation. Inside the greenhouses, micro controlled climate is maintained by changing the humidity, temperature and ventilation to facilitate healthy growth of the plant. These innovations needs careful planning, mindfulness and data about course of events of creation and also, gather time to correspond with high market costs, selection of assortments embraced for the slow time of year conditions. Nursery is a counterfeit structure takes a shot at the wonder which is known as nursery impact. Poly tunnel has all in all two essential parts, initial one is framework and second one is creation innovation of harvests. Foundation includes different designing parts of secured structure improvement. The second part creation innovation of harvests includes logical examinations to build up the assortments appropriate for ensured development, picking the kind of yields and normalizing the creation conventions. Polytunnels can be used to provide a higher temperature and/or humidity than that which is available in the environment but can also protect crops from intense heat, bright sunlight, winds, hailstones, and cold waves. This allows fruits and vegetables to be grown at times usually considered off season; market gardeners commonly use polytunnels for season extension. Legislature of India is additionally furnishing half appropriation on all out use with a most extreme slice off limit up to 4000 m^2 for each recipient for reception and introducing nurseries under National Horticulture Mission.

KEY WORDS: PROTECTED CULTIVATION, POLY TUNNEL.

INTRODUCTION

The impact of abiotic and biotic stresses under the present changing climate dictates the crop production and quality. The foremost constraints in horticultural crop production in North Indian condition are the extremes of temperature, sunlight, water, relative humidity, weeds, nutrient deficiency, wind velocity, carbon dioxide concentration and diseases and insect pest incidence. Protected cultivation means to grow with improved quality out of season under protected structures, thereby increasing the profitability for the farmer especially in hostile climatic conditions. This technology has a potential to cater for supply of high quality vegetables, flowers and fruits in the peri-urban areas by reducing the transportation time and delivering fresh produce. For Indian ranchers, this innovation can help in making worthwhile gets back from different high worth yields and will grant enough gauges to contend at International level (Nair et al 2014). The greenhouses had the second largest share in terms of the installation cost.

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A polytunnel is a passage normally produced using steel and canvassed in polyethylene, generally semi-round, square or extended fit as a fiddle. The inside warms up in light of the fact that approaching sunlight based radiation from the sun warms plants, soil, and different things inside the structure quicker than warmth can get away from the structure. Air warmed by the warmth from hot inside surfaces is held in the structure by the rooftop and divider. Temperature, mugginess and ventilation can be constrained by gear fixed in the polytunnel or by manual opening and shutting of vents. Polytunnels are for the most part utilized in calm areas in comparative manners to glass nurseries and column covers. Other than the latent sun based warming that each polytunnel gives, each variety of helper warming (from nursery warming through negligible warming to unheated houses) is spoken to in current practice. The settling of column covers and low passages inside high passages is additionally normal.

Polytunnels can be utilized to give a higher temperature as well as moistness than that which is accessible in nature however can likewise shield crops from serious heat, brilliant daylight, winds, hailstones, and cold waves. This permits products of the soil to be developed on occasion generally viewed as slow time of year; market planters regularly use polytunnels for season expansion. Past season expansion, polytunnels are likewise used to permit cold-solid yields to overwinter in areas where their strength isn't exactly sufficient for them to endure outside. Temperature increments of just 5 to 15 °C (9 to 27 °F) above outside surrounding, combined with assurance from the drying impact of wind, are sufficient to let chosen plant assortments develop gradually yet soundly as opposed to biting the dust (Del Amor 2007).

Plastic tunnels are small greenhouse-like structures, covering the plants along the row. These tunnels are 18" high by roughly 30" wide at the base and are erected with wire hoops and covered with clear plastic. The tunnels promote early growth by warming the air surrounding the plants, using heat from the sun. The tunnels also protect plants from frost that can destroy or damage them. Greater overall crop yields are obtained when the plants come into earlier production and continue to bear throughout the season. This combination of earliness and greater yields can significantly increase profits for the growers (Figure 1).

Crops should be grown under poly tunnel: The determination of the sorts of yields to develop under tunnels is a significant choice and ought to be made cautiously. The decision ought to incorporate the thought of the harvests most appropriate for burrow development, extended costs over the market time frame for the foreseen market, and expected yield on a for each section of land premise. Harel et al 2014 conducted a study on high value vegetables like tomato, cherry tomato, shaded capsicum, parthenocarpic cucumbers, french beans (pole type), winter watermelon, muskmelon and strawberries can be become effectively unavailable under poly-tunnels in Northern India. The innovation has likewise been demonstrated important to deliver joined natural product plants all year. Through experimentation, producers may discover different yields that might be developed effectively on their homestead under tunnels.

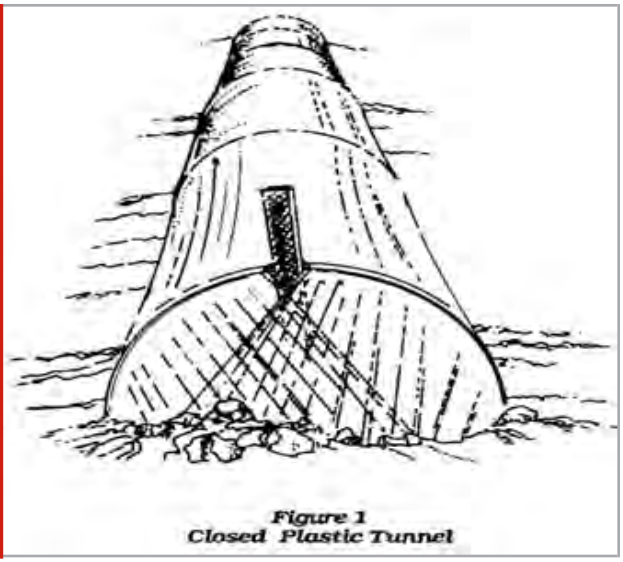

Figure 1
Closed Plastic Tunnel

1.2.1 Points to be considered while constructing poly tunnel:

- The poly tunnel should be constructed on the north and the west face to protect from winds, whereas it should remain open on east and south sides for better sunlight and ventilation.
- Before constructing the green house, a plan of required beds and paths on the ground are to be prepared.
- A proper selection of polyethylene film and shape of roof slope.
- Appropriate controlled climatic conditions to be provided.
- Area size and material of construction of greenhouse.
- Precautionary measures for plant protection.

1.2.2 Advantages of poly tunnels

- It supplies vegetables and flowers throughout the year.
- By adapting different protected cultivation practices, horticulture produce can withstand even unfavourable climatic conditions.
- Environmental factors such as water scarcity, arid land and irregular monsoon etc doesn't affect protected cultivation and thus it is lucrative to farmers.
- It is possible for cropping multiple times on the same piece of land.
- Growers yield better returns during off season
- High quality and healthy seedlings of horticultural crops can be grown.
- It improves the profitability per unit of land, better utilization of water, work and energy.
- It makes cultivation possible at high altitudes and deserts.

- It makes vertical development possible for horticultural crops using technologies like aeroponics, hydroponics etc and use of vertical beds for production.
- It facilitates the seed production of disease-free costly vegetables
- In this controlled environment, it is easy to manage and control of insect-pests, diseases and weeds.

1.2.3 Limitations of poly tunnels

- High cost of introductory framework (capital expense).
- Less-accessibility of talented skilled labour and their substitution locally.
- Lack of specialized information on developing yields under secured structures.
- It also needs a close supervision and observing.
- A couple of soil-borne microbes are troublesome to oversee.
- Repair and support are significant obstacles.
- Requires guaranteed showcasing, since the speculation of assets like time, exertion and funds, is expected to be high.

1.2.4 Crops grown under poly tunnel

Vegetable	Tomato, Cucumber, capsicum (coloured), red cabbage, raddish, spinach, leafy vegetable
Flower	Gerbera, Gladiolous, rose, Chrysanthemum, Carnation
Fruits	Sapota, Straberry

Secured development includes a complex arrangement of practices and innovations which require expound arranging, manufacture, the executives and value of horticultural crop yields (Adhikary et al 2020) to take bit of advantages of season, request and decision of market. It gives open doors for the development of horticultural crops in a pioneering structure for the up markets in metropolitan and semi-metropolitan territories, other than enabling youth, and innovation drove customary methods of yield development to such present day techniques.

1.3 Effect of low tunnel technology on yield of vegetables: Saini and Singh (2001) conducted a research study on growth and yield of chilly crop under low tunnel polyhouse, at research farm of Soil and Water Engineering Department at Punjab Agricultural University (PAU), Ludhiana–India. They found that there was no significant effect on the yield of chili due to variation in perforations on polythene cover. Drip irrigation system with IW/CPE ratio of 0.50 and 30 cm low tunnel polythene cover gave the best yield and water saving. Helbacka (2002) conducted a study on row covers for vegetable gardens. It was re-ported that many cucurbits (squash, cucumber, and melons) respond well under row covers with increased yield of as much as 25%. Joublan and Vergara (2003) conducted a study on vegetative and productive development of strawberry (Fragaria X ananassa Duch.), using row cover of spun-bonded polyester with different densities.

Row covers were placed directly over the plants as a tunnel without any support structure. Treatments were comprised of a control treatment (without row covers), row covers of 20 g/m2 and row covers of 30 g/m2. Fruit production started 4.8 and 2.2 days earlier under 20 and 30 g/m2 row covers, respectively, than under the control treatment. The use of row covers also increased the number of fruit and weight, yield per plant and sugar concentration compared to the control treatment. The best results were obtained with 30 g/m2 row covers. Henandez et al. (2004) was conducted studies on row covers for quality improvement of Chinese cabbage for three years in the area of Granada, Spain, under a Mediterranean continental temperate climate, on 55-day cycles with transplanting in mid-march.

The mean commercial yield for the 3-years was l 1.9 kg/m2 under row cover compared to only 2.1 kg/m2 in open air, owing primarily to important number of non-commercial cabbages. Vishnuvardhana et al. (2004) conducted a study on the economics on the propagation of cashew grafts in a mist chamber, naturally ventilated green house, low tunnel and shade net during the summer, monsoon and winter season. The initial investment for the establishment of the propagation structure (100 mi) reached Rs. 8,500 for the shade net, Rs. 300,000 for mist chamber, Rs. 36,400 for naturally ventilated green house and Rs. 21,000 for the low tunnel. The highest net profit was obtained with propagation in low tunnels, followed by propagation in a naturally ventilated green house, mist chamber and shade net.

2. Present Status of Protected Cultivation in India and in the world: In various parts of the world the viability of this innovation has been demonstrated. The zone under ensured development has increased exponentially in different parts of the globe by adopting practices like plastic mulching, polythene low tunnels, polythene walls, plastic-covered high tunnels, polythene-covered walk-in tunnels, temporary and permanent bug verification net houses and other different types of greenhouses etc. Protected Cultivation which has risen up from Northern India (Gyan et al 2010) invigorated its improvement in different pieces of the world including Northern America, Africa, Asia, Mediterranean region and Oceania with changing paces of progress.

Right now in secured development China is driving on the planet with a zone of around 3.5 million hectares out of which almost 96% is just being utilized for business development of vegetables and crossover seed creation of vegetables. A concurrent development has additionally been seen in different nations like India and African sub-mainland yet the achievement rate changed fundamentally. Singh et al 2019 suggested that this was a direct result of helpless arrangement between the predominant agro-climatic states of the

locales and plans of secured structures. Later on, it was understood that the ensured agriculture technological methods and strategies to be received and lined up with the nearby agro-climatic and financial conditions. This was accomplished through innovative work, expansion and preparing. In India secured cultivating of vegetables and other high-esteemed plant produce began through Indo-Israel venture on greenhouse development started at Indian Agricultural Research Institute (IARI) in 1998. In 2003 Israeli specialists left India and IARI kept on keeping up the office by calling it as Centre for Protected Cultivation Technology (CPCT).

Centre for Research in Rural and Industrial Development (CRRID) conducted a survey which shows vegetable or flower yields under protected cultivation are 5-10 times higher than the open cultivation depending on the crop. In India, agriculture plays a vital role in the country's economy with over 58% of the rural households depending on farming and contributing around 17-18% to India's GDP. In the last two and half decades, India having diversified agro-climatic conditions shown an overall growth of around 75,000 hectares area under protected cultivation. Depending upon the prevailing conditions of various regions and seasons in India, the success rate varied significantly. Protected cultivation technologies faced a tough challenge in North Indian plains due to its harsh climatic conditions whereas other regions with mild climatic conditions like Pune, Bangalore and some parts in North-Eastern states has achieved high success rate.

To advance development of secured development innovation in India, Government presents various strategies as far as appropriations and dispatches different plans with different State Governments like TM (Technology Mission), MIDH (recently known as NHM), RKVY, NHB and so on. Through noteworthy adjustments in specialized plans of different secured structures appropriate to the district's particular needs under winning climatic states of India, prompted development in the zone and creation under ensured development. This was accomplished through ideal work completed by different public sector in situations in innovative work territory as a team with created nations. In order to raise the farmer's income and improve their availability for extended period, National Horticulture Board (NHB),

Ministry of Agriculture, Government of India considered greenhouse technology potential in producing high quality of wide range horticultural products. Through various schemes, NHB extended support growing and processing of horticultural crops (vegetables, fruits, plantation crops, ornamental and spices etc) in the country (Singh 2015). Department of Agriculture and Cooperation has been actively encouraging capital investment in protected cultivation by extending capital investment subsidies extending from 25% to 50% of normative capital cost. Under other schemes of Central and State Governments, sizable financial assistance is also available for horticulture crops. Under various Government schemes, the norms for subsidy varies with the type of infrastructure such as cooling and heating purpose, naturally ventilated and fan and pad system; low, medium and high cost (Bamboo) poly houses etc;

3. Future prospects: The new age farming technique "Protected Cultivation" plays a critical role in the roadmap of actualizing Honourable Prime Minister's vision of 'Doubling Farmers income by 2022'.

Various opportunities exist for nursery men, business crop producers, seed makers and analysts in using greenhouse for their advantage. In mild and subtropical zones secured cultivating can without much of a stretch be utilized for bringing vegetable nursery up in late winters which could be relocated in late-winter. This can propel the editing by coordinated and half month and along these lines, may give profitable cost to the ranchers. In fields green house might be used for all year engendering of numerous tropical and subtropical natural products which could demonstrate an aid for nursery men.

All year round cultivation of vegetable crops, for example, tomato, capsicum and cucumber is conceivable under secured climate with single/multi crop in a year, which gets slow time of year more exorbitant cost with quality produce and furthermore with lower cost of development and longer length of yield (Issac 2015). There is appeal of hued capsicum, parthenocarpic cucumber and cherry tomato in the inn business and fare market all through year at exceptionally alluring business sector cost of the produce. India has an immense degree for trading cut - blossoms, for example, Gerbera, Carnation, Lilium and so on developed under secured climate.

CONCLUSION

The secured development of high value vegetables and flowers production has gotten indispensable both from economic and climate perspectives. It offers a few preferences to develop high value yields with improved quality considerably under ominous and negligible conditions. Though because of high preparing needs of the poly tunnel cultivators and some low quality produce with pesticide deposits has involved extraordinary concern. These issues can without much of a stretch be tended to by coordinating different creation what's more; security works on including area explicit planning and development of the polyhouses for effective info use. Making mindfulness among the greenhouse cultivators for sensible utilization of pesticides for safe creation can be instrumental in giving quality items without contaminating the climate.

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Assessment of Dietary Practices Among School-Going Children (7-12 Years) of Selected Private and Goverment. Schools

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ABSTRACT

The present study was undertaken to assess the nutritional status of 7-12 years of school-going children and to determine the nutrient intake in comparison with RDA. A total sample of 120 school-going children was selected from one Govt. and two private schools of Bhubaneswar city, Odisha. A structured questionnaire was used and anthropometric measurements were taken to collect the data. Data on dietary intake were collected by using 24 hours recall method. From the study, it was found that the prevalence of thinness was 58.33% in Govt. school children whereas 28.33% of private school children were overweight and 11.67% were obese. The school-going children of Govt. school were found to be stunted 6.67%. Consumption of all the nutrients by Govt. school children was a deficit from the recommended dietary allowances but in case of private school children, consumption of all the nutrients was excess from the Recommended Dietary Allowances except vitamin-A. Consumption of food items such as egg, fish, meat, milk and milk products, fruits and nuts were found to be lower in case of Govt. school children as compared to private school children. However, the study indicated that undernutrition in Govt. school children may be due to lower intake of food and nutrients than the recommended standard. Deficiency of vitamin-A in the diet was observed in both the schools due to negligible consumption of green leafy vegetables and minimal consumption of other vegetables and fruits.

KEY WORDS: SCHOOL GOING CHILDREN, ANTHROPOMETRIC MEASUREMENT, RDA AND DIETARY INTAKE.

INTRODUCTION

Children being the future wealth of the nation are considered an important segment of the population. Their survival, protection, and development are the prerequisite for the future development of society. The school-age is the active growing phase of childhood and dynamic period of growth when children undergo physical, mental, emotional and social changes. Nutritional intake has a special direct effect on children's health due to their physical and mental growth as well as cognitive development.During this age, children establish habits of their choice in eating, selecting hobbies, sports and performing an exercise that stick with them for their entire lives. The availability of quality food, affordability of family, choice of children etc. is the critical factors contributing to undernourishment and malnutrition.

The prevalence of malnutrition and obesity is significantly higher in India than many other developed and developing countries. Malnutrition is one of the principal public

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health problems affecting large sections of populations especially children in developing countries (Begum and Nessa, 2008). Healthy habits of children minimize the risks of many chronic diseases but, physically inactive children with poor eating habits are vulnerable to adverse health conditions during forthcoming adulthood. Good nutrition is an essential component of a healthy life which determines health, physical and mental growth. But, diet is one of the prime determinants of health and nutritional status. Assessment of food quality and quantity through dietary surveys are therefore essential for school children. To educate the school children and their parents for changing food habits, it is needed to collect information on nutritional habits involving the source of foods,associated factors of food consumption and feeding style of the children. Therefore, the present study has been designed to generate information on the assessment of dietary practices among school-going children (7-12 years) of selected private and Govt. schools in Bhubaneswar.

MATERIAL AND METHODS

2.1 Study Design: The study was undertaken in Bhubaneswar city. A total no.of 120 school going children (7-12 years) were selected randomly. Three schools were selected as the study areas namely Rajbhawan Project U.P school (Gopabandhu square), D.A.V Public school(unit-8) and Steward School(CRPF Square) in Bhubaneswar City by using a simple random sampling method.

2.2 Study Population: A total no. of 120 children of 7-12 years old school children (both boys and girls) were selected. Out of 120 samples, 60 childrenfrom Govt. School and another 60 from private school were taken.

2.3 Data Collection Procedure: A semi-structured questionnaire was used to collect the desired information regarding the socio-demographic profile ofthe child, anthropometric measurement and dietary information.

2.4 Anthropometric Measurement: Nutritional status of all the selected children was assessed by measuring body heights (cm) and weights (kg). The anthropometric assessment was conducted to identify children with moderate to severe undernutrition. Two indices were taken as a measure of chronic undernutrition i.e. BMI-for –age (thinness) and height -for -age (stunted) with reference to WHO standard,2007.

Weight: A bathroom scale weighing machine was used for taking weight measurement of girls and boys. Every time before taking measurement zero error was checked and data were recorded.

Height: The child was made to stand with feet flat together against the wall with legs straight, arms at sides, and shoulders at a level and removes their footwear. Mark was made where the bottom of the headpiece meets the wall. Then by using a measuring tape height was measured from the base on the floor to the wall to the nearest 0.1 centimetres.

Table 3.1. Socio-demographic profile of the school-going children N=120

Sl. No.	Variables	Categories	Govt. School (n=60)	Private School (n=60)
01	Age			
	7-9yr.	30 (50.00)	30 (50.00)	
		10-11 yr.	30 (50.00)	30 (50.00)
02	Type of family	Nuclear	21 (35.00)	42 (70.00)
		Joint	39 (65.00)	18 (30.00)
03	Caste General	7 (11.67)	53 (88.34)	
		OBC	39 (65.00)	2 (3.33)
		SC	10 (16.67)	5 (8.33)
		ST	4 (6.66)	-
04	Socio-economic status Upper	-	21 (35.00)	
		Upper middle	2 (3.33)	39 (65.00)
		Lower middle	21 (35.00)	-
		Upper lower	37 (61.67)	-

Figures in the parenthesis indicate per cent value

2.5 Diet survey: In recalling foods consumed by the child in the past 24-hours, asked to indicate the time and source of the food (whether purchased, home-made, or school-meal) for each eating event. Standardised cups were used to estimate the quantities of foods consumed. From the raw ingredients' amounts, the nutritive value of each food item was calculated by using the nutritive values given by C. Gopalan (1989). It was compared with Recommended Dietary Allowances (RDA) of nutrients for

those of specific age groups. It was recorded in terms of cereals & millets, pulses and legumes, green leafy vegetables, roots and tubers, other vegetables, fruits, milk and milk products, egg,fish, meat, sugar and jaggery, nuts and oilseeds. The nutrient value of calorie, protein, CHO, fat, calcium, iron and vitamin-A were computed by using the nutritive value of Indian foods (C.Gopalan, 1989). The RDA value(2010) was also taken into consideration for comparing the values.

Statistical Analysis: Data entry and analysis was done by using SPSS software version 20.0. Anthropometric indices were calculated using the 2007 WHO Anthro 3.2.1 Software. Descriptive analysis was used to describe the percentages and frequency of sociodemographic characteristics and other relevant variables in the study. t-test was used to test the difference between two population means based on two sample means.

RESULTS AND DISCUSSION

The data obtained were analyzed for the stated objectives. A total sample of 120 children aged 7-12years was selected for the present study. Out of total children, 50.00% were taken from Govt. school and 50.00% from the private school.

The socio-demographic profile of children between the age group of 7-12 years was presented in Table3.1. The majority of children (70.00%) from private school belonged to a nuclear family whereas 65.00 %of children from Govt. school were from joint family. Out of total children from the private school, the majority were from general caste category (88.34%) followed by SC (8.33%) and OBC (3.33%). In Govt. school, the major category of children was from OBC (65.00%) followed by SC (16.67%), general (11.67%) and ST (6.66%). The study revealed that all children of private school belonged from upper (35.00%) and upper-middle class (65.00%) category whereas Govt. school children were from upper-middle (3.33%), lower-middle (35.00%) and upper-lower (61.67%).

Anthropometric measurement of children:

Table 3.2. Nutritional status according to BMI-for-age N=120

Nutritional Status	WHO Indicator	Govt. School (n=60)	Private School (n=60)
Thinness	≤ -2S.D	35 (58.33)	-
Normal	≥ 1S.D to -2S.D	25 (41.67)	36 (60.00)
Overweight	≥ +1S.D	-	17 (28.33)
Obesity	≥ +2S.D	-	7 (11.67)

Table 3.3. Nutritional status according to height-for-age N=120

Nutritional Status	WHO Indicator	Govt. School (n=60)	Private School (n=60)
Stunting	≤ -2S.D	4 (6.67)	-
Normal	≥ -2S.D	56 (93.33)	60 (100.00)

Table 3.4. Age-wise anthropometric measurement of Govt. and private school children N=120

Age group	Parameters	Govt. School (n=60)	Private School (n=60)	t-value
7-9 yrs.	Height (cm.)	121.53±6.68	125.5±8.54	2.002**
	Weight (kg.)	24.8±3.75	35.93±5.68	8.946**
10-12yrs.	Height (cm.)	137.43 ±9.40	138..63 ±12.10	0.428 (NS)
	Weight (kg.)	33.33±6.11	42.4±7.93	4.955**

The nutritional status of school children according to BMI-for-age was presented in Table-3.2. The school-going children of Govt. school were found to be thinness and normal category i.e. 58.33% and 41.67% respectively. No children were found in the overweight and obese category in Govt. school, but in private school, 60.00% children were in the normal group followed by overweight (28.33%) and obese (11.67%). Thinness was not observed among children belonged from the private school. A similar study was also conducted in the Western Region of Nepal. The study revealed that out of 786 students, 26.00% of the students were found to be undernourished (Joshi. et al., 2011). In the other hand, the private school children were an estimated 19.9% for under-nourished, 10.2% for overweight and 5.7% for obese categories (Ganganahalli. et al., 2016).

The nutritional status of school children according to height-for-age was presented in Table-3.3. The school-going children of Govt. school were found to be stunted and normal group i.e. 6.67% and 93.33% respectively. But in private school, all children (100.00%) were in the normal group and stunting was not found in children from the private school. Out of 253 children, 28.8% were in underweight, 19.4% stunting and 17.8% in wasting in Panchakula city, Haryana (Talwar. et al., 2015). Further, a study was carried out amongst 558 school children aged 3-16 years in Ghaziabad city reported that 59(10.5%) were stunted (Garg.et al., 2015). In the present study, it was observed that the children from Govt. school were compelled to avail low-quality nutrition and remain undernourished due to their lower family income.

The mean anthropometric measurement of Govt. and private school children were presented in Table-3.4. Under the age group of 7-9 years, the mean height (121.53±6.68) and (125.5±8.54) and weight (24.8±3.75and 35.93±5.68) of Govt. and private school children varied significantly ($p<0.01$) where higher values were measured for private school children than those for Govt. school. Similar results with significant variation ($p<0.01$) were observed for weight (33.33±6.11 and 42.4±7.93) in Govt. and private school children leaving the variation of height non-significant under the age group of 10-12 years.

The study was also in line with the mean height and weight of 7-10 years of school-going children of Allahabad district were significantly ($p<0.05\%$) less than the National Centre for Health Statistics standards (Handa. et al., 2008). The findings of the present study resemble with the aforesaid reports. Observation of high values for height and weight in private school children may be attributed due to higher nutritional status of the family based on the socio-economic background of private school parents. On the other hand, the general lower economic standard of the parents of Govt. school, may not maintain the recommended nutritional standard for their children for which the growth factors are adversely affected.

Diet and nutrient intake: Table-3.5 revealed that all the children (100%) of both schools consumed cereals on daily basis. Govt. school children consumed pulses daily (31.67%) and 4-6 times in a week (23.33%) whereas 61.67% and 38.33% children from Govt. school consumed pulses on daily basis and 4-6 times in a week respectively. No children of Govt. and private school consumed green leafy vegetables, egg, fish and meat on daily basis. Intake of pulses, root and tuber, other vegetables are consumed more daily by private school children as compared to Govt. school children. Majority of private school children consumed milk and milk products daily but very few children of Govt. school consumed milk and milk products daily. The low consumption of costly food items such as egg, fish, meat, fruits and nuts for 4-6 times in a week by Govt. school children as compared to private school children. The present study was also in line with (David. et al., 2012) which revealed that low consumption of high-quality protein from meat, eggs, margarine, fish and oils and African leafy vegetables by the school-going children in Machakos district was the reason for poor anthropometric and nutritional status.

Table 3. 5. Food consumption pattern by school children N=120

	Govt. school F(%)	Private school F(%)	Govt. school F (%)	Private school F (%)	Govt. school F (%)	Private school F (%)	Govt. school F (%)	Private school F (%)
Cereals	60 (100.0)	60 (100.0)	-	-	-	-	-	-
Pulses	19 (31.67)	37 (61.67)	41 (23.33)	23 (38.33)	-	-	-	-
Green leafy vegetables	-	-	7 (11.67)	12 (20.00)	30 (50.00)	26 (43.33)	23 (38.33)	22 (36.67)
Root and tubers	30 (50.00)	49 (81.67)	27 (45.00)	7 (11.67)	27 (45.00)	7 (11.67)	-	-
Other vegetables	18 (30.00)	44 (73.33)	23 (38.33)	12 (20.00)	16 (26.67)	4 (6.67)	3 (5.00)	-
Fruits	-	23 (38.33)	4 (6.66)	18 (30.00)	18 (30.00)	28 (46.67)	28 (46.67)	-
Milk and milk product	10 (16.67)	50 (83.33)	-	-	18 (30.00)	10 (16.67)	32 (53.33)	-
Egg	-	-	4 (6.67)	38 (63.33)	48 (80.00)	22 (36.67)	8 (13.33)	-
Fish	-	-	25 (41.66)	37 (61.67)	28 (46.67)	21 (35.00)	7 (11.67)	2 (3.33)
Meat	-	-	6 (10.00)	21 (35.00)	36 (60.00)	33 (55.00)	18 (30.00)	6 (10.00)
Sugar &jiggery	30 (50.00)	33 (55.00)	23 (38.33)	22 (36.67)	7 (11.67)	5 (8.33)	-	-
Nuts & oil seeds	-	16 (26.67)	-	17 (28.33)	15 (25.00)	24 (40.00)	45 (75.00)	3 (5.00)

Around half of the children from both Govt. and private school consumed sugar and jaggery on daily basis. The consumption of cereals, pulses and fat-based foods in excess and vegetables and fruits in a deficient manner were the reason for obesity in school going children of Western Maharashtra (Kamble. et al., 2016). However, all the school going children belonging to Trans Yamuna Region of Allahabad consumed cereals, pulses, sugar, fats and oils daily (Perween., 2018). Among private school children (7-12 yrs), the nutrient intake was significantly higher ($p<0.01$) than those of Govt. school children but, in case of Govt. school children, it was less than the RDA value.

Table 3.6. Average daily nutrient intake* of children (7-9 yr.) boys N=30

Nutrients intake	RDA value	Children from Govt. School (n=15)	Children from Private School (n=15)	t-value
Energy (Kcl)	1690	1543.23±114.44	1906.73±301.36	4.367**
Protein (gm)	29.5	26.70±3.0	33.70±3.05	6.333**
CHO (gm)	253	238.79±47.22	286.93±18.26	3.394**
Fat (gm)	30	27.16±3.14	36.30±3.37	7.669**
Calcium (mg)	600	510.36±85.78	630.36±43.08	4.841**
Iron (mg)	16	14.17±2.22	16.50±2.15	3.373**
Vitamin-A (µg)	4800	2556.79±615.09	4195.34±595.78	7.410**

Figures were presented as (Mean± SD)
'**'denotes significant variation (p<0.01) between columns
'*' denotes Nutritive value of Indian foods

Table 3.7. Average daily nutrient intake* of children (7-9 yr.) girls N=30

Nutrients intake	RDA value	Children from Govt. School (n=15)	Children from Private School (n=15)	t-value
Energy (Kcl)	1690	1536.56±86.02	1883.23±141.88	8.091**
Protein (gm)	29.5	26.71±2.23	33.37±1.90	9.365**
CHO (gm)	253	240.13±45.72	284.61±27.39	3.232**
Fat (gm)	30	27.09±3.04	35.97±2.34	8.943**
Calcium (mg)	600	512.79±87.12	627.02±68.11	4.177**
Iron (mg)	16	14.25±1.95	16.19±2.02	2.601**
Vitamin-A (µg)	4800	2541.74±651.52	4128.48±682.19	6.676**

Figures were presented as (Mean± SD)
'**'denotes significant variation (p<0.01) between columns
'*' denotes Nutritive value of Indian foods

Table 3. 8. Average daily nutrient intake* of children (10-12 yr.) boys N=30

Nutrients intake	RDA value	Children from Govt. School (n=15)	Children from Private School (n=15)	t-value
Energy (Kcl)	2190	1992.90±134.67	2420.40±119.16	9.207**
Protein (gm)	39.9	36.29±2.57	45.85±2.57	10.165**
CHO (gm)	328.5	286.11±53.78	370.83±17.74	5.793**
Fat (gm)	35	31.87±11.41	41.61±3.37	3.169**
Calcium (mg)	800	683.83±119.51	824.30±121.01	3.198**
Iron (mg)	21	18.65±2.40	21.71±1.23	4.384**
Vitamin-A (µg)	4800	2510.12±657.42	3963.45±669.53	5.998**

Figures were presented as (Mean± SD)
'**'denotes significant variation (p<0.01) between columns'*'
denotes Nutritive value of Indian foods

The intake of energy, protein, CHO, fat, calcium, iron and vitamin-A by the children of Govt. school was a deficit for RDA as per the recommendation suggested by ICMR. On the other hand, the consumption of energy, protein, CHO, fat, calcium and iron by private school children were adequate and Vitamin-A was deficit basing on RDA of ICMR. The mean nutrient intake per day as well as how much this varies from the RDA can be seen in Table -3.6, 3.7,3.8 and 3.9. The intake of protein, energy, calcium, iron, carotene, thiamine, riboflavin and niacin was lower and fat and ascorbic acid was more than the RDA in 7-9 years of school-going children of Himachal Pradesh (Soni. et al., 2014).

Table 3.9. Average daily nutrient intake* of children (10-12 yr.) girls N=30

Nutrients intake	RDA value	Children from Govt. School (n=15)	Children from Private School (n=15)	t-value
Energy (Kcl)	2010	1828.65±80.19	2268.48±123.69	11.555**
Protein (gm)	40.4	36.11±3.33	45.04±3.65	6.996**
CHO (gm)	301.5	285.16±37.96	340.76±31.84	4.345**
Fat (gm)	35	31.96±5.23	41.96±4.24	5.746**
Calcium (mg)	800	685.00±124.44	821.70±100.59	3.308**
Iron (mg)	27	23.49±3.42	27.84±4.78	2.868**
Vitamin-A (µg)	4800	2508.57±969.02	4071.14±716.68	5.021**

Figures were presented as (Mean± SD)
'**'denotes significant variation (p<0.01) between columns
'*' denotes Nutritive value of Indian foods

The obese school-going children in Western Maharashtra were observed for excess energy and protein and deficit vitamins and minerals consumption (Kamble. et.al, 2016). Consumption of all the nutrients by the majority of the students (10-12 years) was comparatively less than the recommended dietary allowances (Handa. et al., 2008). The children of private school take comparatively better quality food than those in Govt. school. Therefore, in the present study adequate rate of energy, protein, CHO, fat, calcium, and iron consumption was observed in private school children. But, the deficit of vitamin-A may be due to less incorporation of fruits and vegetables rich in vitamin-A in the diet.

CONCLUSION AND RECOMMENDATION

The present study revealed that out of total children, the prevalence of thinness was 58.33% in Govt. school children whereas 28.33% of private school children were overweight and 11.67% were obese. The school-going children of Govt. school were found to be stunted (6.67%). Under the age group of 7-9 years, the mean height and weight of Govt. and private school children varied significantly (p<0.01) whereas higher values were measured for private school children than those for Govt. school. Similar results with significant variation (p<0.01) were observed for weight in Govt. and private school children leaving the variation of height non-significant under the age group of 10-12 years.Govt. school children were found undernourished (thinness and stunting) than the children of private school because the children studying in Govt. school belong to lower socio-economic condition whereas private school children belong to upper socio-economic condition, they consumed fatty foods and were found normal to obese.In general negligible consumption of green leafy vegetables and minimal consumption of other vegetables and fruits due to which their diet deficient in vitamin-A were observed in the children of both schools.

However, more studies on large population are needed to understand the alarming threats of child obesity, overweight, underweight, malnutrition anddifferent types of nutrient deficiency diseases. To enhance the quality of eating of children from high and low socioeconomic strata by imparting nutrition education at school course curriculum. Especially, mothers should be educated about the importance of balanced diet, the right choice of foods, limiting junk foods consumption, nutrient conservation by proper cooking methods, utilization of seasonal foods to ensure foodand nutritional security.

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Management of Blight in Chickpea Caused by *Ascochyta rabiei*: A Review

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ABSTRACT

Chickpea is the world's second-largest pulse crop and ascochyta blight caused by Ascochyta rabiei is the most destructive disease which occurs in all its growing areas. Particularly during the flowering and podding stages, it causes enormous economic losses. Infected seed and crop residue play an important role in the survival of pathogen from time to time. All the above-ground plant parts get affected in form of necrotic lesions, which girdle the stem in susceptible cultivars and lead to reduced yield even under favourable conditions. A. rabiei pathogen is highly variable in its genotype making it very difficult to control. The available resistance sources are not enough and it is important to explore new sources since from time to time there has been a breakdown of resistance in existing chickpea varieties. This majorly occurs due to the ongoing evolution of new pathotypes. Thus, we have attempted to cover loss, disease distribution, symptoms, epidemiology, and disease control in this review.

KEY WORDS: EPIDEMIOLOGY, IPM, BIOLOGICAL CONTROL, FUNGICIDE, TRICHODERMA, DISEASE CYCLE.

INTRODUCTION

The causative agent of Ascochyta blight is *Ascochyta rabiei* Labrousse (Teleomorph: *Didymella rabiei* (Kovachevski) Arx.). It is one of the important foliar phytopathogens of chickpea. The main incident of this disease occurs in the region where the chickpea growing season coincides with cool and humid weather. This disease is regarded as calamitous and ubiquitous diseases in the chickpea growing regions causing severe crop losses. Due to the persistent incidence of this disease, the area of chickpea production in Western Canada has decreased from over 500,000 ha in 2001 to less than 130,000 ha in 2006 (Statistics Canada, 2001, 2007). When environmental conditions are ideal, the yield loss may exceed up to 100 per cent. This disease is a seed and stubble-borne disease which develops both airborne ascospores and water-splashed conidia during the cropping season (Armstrong et al., 2001; Gossen and Miller, 2004).

Development of chickpea may be restricted by Ascochyta blight worldwide as it is documented from all over the world (Nene and Reddy, 1987; ICARDA, 1996; Akem, 1999; Khan et al., 1999; Kaiser et al., 2000; Chongo et al., 2003). Some techniques, such as crop rotation, can contribute to the epidemic management of this disease. Many areas of the world, including Australia (Ackland et al., 1998; Knights and Siddique, 2002), Canada (Chongo and Gossen, 2001), Latin America (Kaiser et al., 2000), Southern Europe (Trapero-Casas and Jime'nez-Dı'az, 1986), the United States (Kaiser and Muehlbauer, 1984), are under a serious effect of this disease and suffer significant economic losses. The early symptoms include epinasty and loss of turgor, accompanied by water-

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soaked lesions followed by infection on petioles, leaflets, and young branches of chickpea. The phytopathogen additionally secretes a symptom-causing toxin. The disease spreads primarily by infected seeds developed from infected crops (Weltzien and Kaark, 1981).

The use of contaminated crop seeds was responsible for the introduction of this disease in Australia (Cother, 1977), Canada (Morrall and McKenzie, 1974), Iran (Kaiser, 1972), and the USA (Kaiser and Muehlbauer, 1984). Chickpea seeds with mild *A. rabiei* infections appear to be discoloured and have a very low seed weight. However, the pathogen occurs on the seed coat, cotyledons, and embryo axis during serious infection (Dey and Singh, 1994). The mycelium of *A. rabiei* develops on the seed coat of the infected seed (Luthra and Bedi, 1932), on cotyledons, (Lukashevich, 1958) and the emerging seedlings (Kaiser and Hannan, 1988). Pycnidium is the dormant survival structure of *A. rabiei* on soil surface debris (Galloway and MacLeod, 2003). It also can colonize naturally infested debris as a teleomorph and can form viable pseudothecia on the uninfected portions of the debris. Under favourable conditions, pycnidium develops conidia and serves as the primary inoculum for the initiation of the disease in the succeeding crop. The teleomorph, however, grows on chickpea debris present in the field and eventually produces ascospores for the long-distance spread of disease under favourable conditions (Navas-Corte's et al., 1998).

Geographical distribution: The following countries have been reported with the presence of this blight causing fungus: Algeria, Australia, Bangladesh, Bulgaria, Canada, Cyprus, Ethiopia, France, Greece, Hungary, India, Iran, Iraq, Israel, Italy, Jordan, Lebanon, Mexico, Morocco, Pakistan, Romania, Spain, Syria, Tanzania, Turkey, and Tunisia (Nene, 1980; Kaiser et al., 1998). In Iraq, Cyprus, Greece, Algeria, Bulgaria, Israel, Lebanon, Jordan, Morocco, Pakistan, Romania, Spain, Syria, Tunisia, Turkey and the USSR, the disease has been seen more to occur frequently and significantly (Nene, 1982).

Symptoms: The symptoms of this disease in different countries have been identified by several researchers which showed remarkable similarities. All the above-ground components of the plant are under attack by this phytopathogen. The lesions are circular or elongated on the leaflets, holding irregularly depressed brown dots and surrounded by a red or brownish border. The lesions are typically circular with dark margins on the green pods and have pycnidia formed in concentric circles. The affected seeds also bear lesions. The lesions on the stem and petiole are brown, elongated (3-4 cm), bear black dots, and sometimes girdle the affected portion. The section above the point of attack easily dies as lesions girdle the stem. The entire plant dies if the primary stem is girdled in the collar area. As the disease progresses, diseased plant patches become prevalent in the field and expand gradually, covering the entire area. (Atanasoff and Kovacevski, 1929; Benlloch and Del Canizo, 1931; Labrousse, 1930; Luthra and Bedi, 1932).

Disease Cycle and Epidemiology: The pathogen overwinters in the residue and seed of infected chickpea crop. As the pathogen is easily transferred from the seed to seedlings, infected seeds play an important role, both in the introduction of *A. rabiei* to newer areas and also in the early development of the disease. The residue of crop may also serve as a source for the production of both asexual spores (conidia, spread by rain-splash) and sexual spores (ascospores, spread by wind). Sexual reproduction yields pseudothecia that house the ascospores in late fall and early spring. Under sufficient moisture and moderate temperatures (near 10°C), production of pseudothecia takes place during five to seven weeks. Mature pseudothecia release ascospores into the air in spring and early summer, that can move to several miles. The initial cause of infections in the spring is thought to be airborne ascospores, although the rain-splashed conidia are also considered to be involved (Bogdan, 2018). Infection and development of Ascochyta blight disease occur at a temperature frame of 5-30°C with an optimal of 20°C, which produce serious infections after 17 hrs of wetness. Dry phase (6-48 hrs) directly after inoculation often maximizes severity of the disease, but dry periods >12 h after an early wetting duration of 6 h typically provide a detrimental impact on the development of disease (Trapero-Casas and Kaiser, 1992a).

Management: Ascochyta blight management includes fungicide application, cultural management, and host resistance development. While, some chickpea cultivars have been reported to have genetic resistance to this pathogen, but the resistance is only partial and begins to break down during the plant's flowering period (Nene and Reddy, 1987; Chongo and Gossen, 2001). The successful control of this pathogen has been documented by a variety of fungicides, but the residual effects of these fungicides lead to contamination of the environment thus, affecting the natural world. The main key for effective chickpea cultivation and development against this disease is integrated disease management (IDM). One of the methods of minimizing the loss caused by Ascochyta blight is the treatment of chickpea seeds with effective chemical fungicides. Numerous chemical and physical methods for seed treatment and disease minimization, such as copper sulphate (Sattar, 1933), malachite green (Zachos, 1951), pimaricin (Zachos et al., 1963) and, hot water (Sattar, 1933) were applied from 1930 to early 1960. These seed treatments, however, were found to be generally less effective in controlling the seed-borne disease transmission.

The microbial community residing in the plants also gets badly affected due to the intensive use of fungicides. This

is well known because legumes are capable of fixing nitrogen (N) in the atmosphere (Kyei-Boahen et al., 2001; Gan et al., 2005). Symbiotic N-fixing microorganisms such as rhizobium live in close contact with the plants in the field. The fungicide application decreases the survival of rhizobium and affects the symbiosis between the plant root and the microorganism (Revellin et al., 1993; Kutcher et al., 2002). The rate of application of fungicides to the seed is the main factor affecting the viability of rhizobium as a biofertilizer added to the seed coat. The major cause of the decrease in the rhizobial population may be excessive use of fungicides which are recommended for seed treatment (Kyei- Boahen et al., 2001).

The length of incubation with fungicide can also affect the survival of rhizobium (Matus et al., 2003; Gan et al., 2005). The use of chemical pesticides for IDM should be limited, for the development of a technique with a combined use of pesticides and rhizobium (Welty et al., 1988). Before rhizobium inoculation, fungicides should be applied. Rhizobium and fungicides should be applied in a manner that helps to enhance the survival of seed coat rhizobium. The use of granular rhizobial inoculants may decrease the risk of fungicide physical contact, thereby raising symbiosis (Kyei-Boahen et al., 2002; Gan et al., 2005).

The use of biocontrol agents has become a safer choice for the management of this disease. Several researchers are currently working in this field to discover environmental friendly biocontrol agents having stronger capabilities against this phytopathogen. Few fungal antagonists such as *Chaetomium globosum, Trichoderma viride*, and *Acremonium implicatum* have been studied for their biological control activity, under in-vitro and in-vivo conditions. The mycelium was inundated by *A. implicatum* isolate-1 and caused its breakdown. A clear zone was formed by *A. implicatum* isolate-2. However, the mycelium of *A. rabiei* is covered by *C. globosum* and *T. viride*. A noticeable impediment to pycnidiospore germination and colony expansion of *A. rabiei* was observed while applying culture filtrates of antagonist microorganisms. Additionally, the culture filtrates of all three antagonists were found to be efficient in bringing a reduction in the production of disease under glasshouse condition. *C. Globosum* was found to be the most successful one with a disease index deduction of around 73.12 per cent (Rajakumar et al., 2005). Dugan et al. (2009) confirmed the natural occurrence of *Aureobasidium pullulans* in the post-harvest debris of chickpea. *A. pullulans* spore suspension spray onto chickpea debris contributes to a 38 per cent lower incidence of Ascochyta blight.

CONCLUSION

Ascochyta management is an important component of chickpea to grow successfully. A mixture of cultivar tolerance, seed and crop hygiene, seed and foliar fungicides, and suitable sowing dates are used for integrated disease management. Due to the complexity of the pathosystems and the inter-relationship with resistance and the environment, choosing the most successful strategies can be challenging. Therefore, a greater understanding of the factors that affect pathogen population survival and fitness and their study of diversity can help in the development of proper management practices. Further investigations are needed for a thorough study of the climatic factors responsible for the incidence and severity of this disease. The information presented in this review on the condition of ascochyta blight will be helpful for growers to prepare and implement strategies for management for reducing the blight below threshold levels.

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Review on Antibiotic Potential Microbes

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ABSTRACT
The advent of biotechnology has prompted researchers to attempt the synthesis of pharmaceutical compounds. Some success has been achieved with synthetic combinatorial chemistry and biosynthetic gene cluster manipulations. However, natural product discovery still proves to be one of the best sources for new bioactive chemicals. Nature's superiority in bioactive chemical production and the unknown number of undiscovered compounds provide poignant motivation to protect the aquatic environment from human alteration. The alteration of the delicate aquatic environment due to human pollution has unknown consequences for the undiscovered life forms. The vast diversity of undiscovered life in the aquatic environment affords many opportunities for increasing the arsenal of therapeutic chemicals used to treat human disease. With each new finding we can continue to marvel at nature's ability to produce such complex and beneficial structures.

KEY WORDS: BIOACTIVE CHEMICALS, BIOTECHNOLOGY, THERAPEUTIC CHEMICALS.

INTRODUCTION

French bacteriologist Vuillemin used the term antibiosis for the first time that means "against the life," in the year 1877, later Louis Pasteur and Robert Koch observations came in to light and the word antibiosis renamed as antibiotics by an American microbiologist Selman Waksman, in the year 1942. The idea of microorganisms as a new source of novel pharmaceuticals spurred an extensive search for microbial metabolites of medicinal value. Early efforts led to the discovery and development of several diverse classes of antibiotics which are believed to have added over a decade to man's life span. A majority of the antimicrobials in clinical use today are microbial products, products of microbial origin or are their synthetic/semi-synthetic analogs. However the last three decades have been disappointing as new classes of microbial metabolites worthy of commercialization as antibiotics have not been found. The main focus has been on the semi-synthetic, "me-too" compounds and on synthetic classes the fluoroquinolones and oxazolidinones.

The widespread use of these various antimicrobial agents has resulted in the gradual emergence of multi-resistant pathogens. In response to the threat that these organisms now pose a search for novel agents lacking cross-resistance with the older compounds and perhaps possessing new modes-of-action has begun. Microorganisms from unique ecological niches are being explored, libraries of historical compounds are being reevaluated in the light of current needs and new sub-cellular targets discovered through bacterial genomics are being used for screening. These efforts may result to the discovery of new, more effective antibiotics that will meet current formidable challenges in the concerned field.

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The effect of microbes in the environment has been recognized for centuries but until the accidental discovery of penicillin by Alexander Fleming in 1928 their true beneficial potential was not recognized. This fortuitous event seemingly provided the first scientific clue that microorganisms could be an enormous source of novel pharmaceuticals.

Since then microbial products have been proven to be a rich source of novel compounds with diverse biological activities, Davies(1999); Demain(1998 and 1999), Imada and Hotta,(1992), Bernan et al.,(1997). Although synthetic compounds have continued to play important role in our fight against various diseases (Table.1) The antibacterial and anticancer therapeutic agents currently in clinical use are dominated by either microbial products or one of their analogs, Singh and Greenstein, 2000, Cragg et al.,(1997). The Physicians' Desk Reference of 1999 lists a total of 403 antimicrobial formulations of which 211 are listed as antibacterial (Physicians' desk reference, 1999). This list includes 19 penicillins, 26 cephalosporins, 1 monobactam, 2 carbapenems, 6 aminoglycosides, 5 tetracyclines, 7 macrolides, 6 fluoroquinolones, etc.

Most of these new antibiotics were discovered through extensive screening of microbial fermentations from the year 1940 - 1960 which is also referred to as the 'golden' period of antibiotics. There after conventional screening became less productive and an upsurge in semi-synthetic and synthetic chemistry efforts expanded the number and quality of antibiotics derived from the known classes Lawrence et al.,(1999); Lee et al.,(1999). In the year 1970's the discovery of the monobactams, carbapenems and clavulanic acid by three different microbial screening efforts re established the importance of microorganisms in the discovery of new antibacterial compounds Christopher et al.,(1991). Continued chemical and microbiological efforts provided several superior analogs of various antibiotic classes for the antibacterial market and semi-synthetic approaches are still being pursued to further improve the activities of old compounds.

Table1. List of common antibiotics and their origin.(M.P.Singh and M. Greenstein,Wyeth-Ayerst Research,Pearl River, New York, USA 2000)

	Antibiotics of microbial origin			(Majority of these are produced by *Streptomyces* sp.)
Year	Beta-lactams			
1928		Penicillins: Antibacterial activity of *Penicillium notatum* discovered.		
1940			Natural	Penicillin G, Penicillin V
1960's			Pen'ase resistant	Methicillin, Nafcillin, Oxacillin, Cloxacillin, Flucloxacillin
1960's			Aminopenicillins	Ampicillin, Amoxycillin, Bacampicillin
1970's			Antipseudomonal	Carbenicillin, Ticarcillin, Mezlocillin, Pipracillin
1945		Cephalosporins: Cephalosporin C was the first 6-membered b-lactam isolated from a *Cephalosporium* sp.		
1960's			First Generation	Cephalothin, Cefazolin, Cephapirin, Cephradine, Cephalexin, Cephadroxil
1960's			Second Generation	Cefachlor, Cefamandol, Cefuroxime, Cefonicid, Cefmetazole, Cefotetan, Cefprozil, Ceftibuten
1965			Cephamycin: first cephalosporin isolated from a *Streptomyces* sp., led to the development of Cefoxitin	
1970's			Third Generation	Cefetamet pivoxil, Cefperazone, Cefotaxime, Ceftizoxime, Ceftriaxone, Ceftazidime, Cefixime, Cefpodoxime proxetil
1980's			Fourth Generation	Cefpirome, Cefepime
1973		Monobactam		Aztreonam (synthetic version used)
1976		Carbapenems		Imipenem, Meropenem
1978		Oxacephem		Moxalactam
1980		Carbacephem		Loracarbef
		Lactamase inhibitors		
1973			Clavulanic acid	Amoxycillin+Clavulanate (Augnetin), Ticarcillin+Clavulanate (Timentin)
1980			Sulbactam	Ampicillin+Sulbactam (Unasyn)
1984			Tazobactam	Pipracillin+Tazobactam (Zosyn)
1939	Polypeptides			Polymyxin B (produced by *Bacillus polymyxa*)
1944	Aminoglycosides			Streptomycin, Amikacin, Gentamicin, Isepamicin, Kanamicin, Netilmicin, Sisomicin, Tobramycin, Neomycin

Table 1 Continue

1947	Phenylpropanoid	Chlorampohenicol produced by *Streptomyces venezuelae*
1948	Tetracyclins	Oxyteracycline, Doxycycline, Chlortetracycline, Minocycline
1950	Cyclic peptide	Bacitracin produced by *Bacillus licheniformis* and *Bacillus subtilis*
1950	Macrolides	Erythromycin (various esters derivatives), Azithromycin,
		Clarithromycin, Dirithromycin
1955	Glycopeptides	Vancomycin, Teicoplanin
1955	Lincosamide	Clindamycin, Limcomycin
1955	Lipopeptides/Glycolipopeptide	Daptomycin, Ramoplanin
1955	Streptogramins	Virginiamycin, Synercid (Quinupristin + Dalfopristin)
1959	Ansamycins	Rifampin, Rifabutin
1962	Steroidal antibiotic	Fusidic acid produced by *Fusidium coccineum*
1969	Phosphonate	Fosfomycin (the first C-phosphonate containing microbial metabolite)
1971	Mupirocin	Pseudomonic acid A from *Pseudomonas fluorescens*
	Synthetic antibacterial agents	
1932	Sulfonamides	Sulfadiazine, Sulfixazole, Sulfamethoxazole, Trimethoprim
1959	Nitroimidazoles	Metronidazole
1960's	Anti-TB drugs	Isoniazid, Ethambutol, Pyrazinamide, Thiacetazone, Dapsones
1962	Quinolone	Nalidixic acid, Cinoxacin, Oxolinic acid
1980's	Fluoroquinolones	Norfloxacin, Ciprofloxacin, Ofloxacin, Enoxacin, Lomefloxacin, Pefloxacin, Sparfloxacin, Trovafloxacin, Grepafloxacin
1980's	Oxazolidinones	Linezolid (approved in 2000)

In nature all organisms need to compete in order to survive in their habitats and this Biological task can only be achieved by the development of competitive mechanisms such as the production of toxins, enzymes and antimicrobial agents like antibiotics. The search for antibiotics began in the late 18th century with the growing acceptance of the germ theory of disease, a theory which linked bacteria and other microbes to the causation of a variety of ailments as a result scientists started to devote more time to search for drugs that would kill these disease-causing bacteria without causing any side or adverse effect to the host. An antibiotic is a drug that kills or slows down the growth of bacteria. Antibiotics are one class of "antimicrobials" a larger group which also includes anti-viral, anti-fungal, and anti-parasitic drugs. They are relatively harmless to the host and therefore can be used to treat infection. The term originally described only those formulations derived from living organisms but now applied also to synthetic antimicrobials such as the sulfonamides.

Antibiotics are labeled (magic bullets) drugs which target disease without harming the host. Antibiotics are not effective in viral, fungal and other non-bacterial infections and individual antibiotics vary widely in their effectiveness on various types of bacteria. Some specific antibiotics target either gram-negative or gram-positive bacteria and others are more wide-spectrum antibiotics. The effectiveness of individual antibiotics varies with the location of the infection and the ability of the antibiotic to reach the site. Oral antibiotics are the simplest approach when effective with intravenous antibiotics reserved for more serious cases. Antibiotics may sometime be administered topically as with eye drops or ointments.

The first widely used antibiotic compounds used in modern medicine were produced and isolated from living organisms such as the penicillin class produced by fungi Penicillium, streptomycin from *Streptomyces* but the actinomycetes are the group of prokaryotic filamentous soil microorganims which are known as the top producers of antimicrobial agents especially *Streptomyces* Osborne et al.,(2000), Rondon M., et al(2000). Some of the antibiotics produced by *Streptomyces* are erythromycin, amphotericin, neomycin, streptomycin and rifamycin. Advances in organic chemistry led to contribute many synthetic antibiotics. Many antibiotics are relatively small molecules with a molecular weight less than 2000 Dalton, with different modes of action. Generally they interfere with biological processes such as replication, translation and cell wall synthesis. Some antibiotics like tetracycline interfere with protein synthesis by associating with the 30S ribosomal sub-unit penicillin, produced by Penicillium notatum prevent transpeptidation of N-acetyl-muramic acid resulting in a weakened peptidoglycan structure.

The search for new natural products from aquatic microorganisms has already shown promising results by discovering compounds with possibly useful as anticancer and cardiovascular agents Lei and Zhou (2002). With the worldwide sale of 45 billion dollars, anti-infective compounds represent the third largest therapeutics on commercial sale and is expected to increase by folds Bush(2004). During the last three decades pharmaceutical

companies have been searching for new antibiotics to counter the problem of increasing bacterial resistance. During this period use of new chemical derivatives of pre-existing antibiotics has been the only method available because no novel chemical class of antibiotics has been discovered Burgess(1999).The oceans covers over the 70% of the earth's surface and roughly half of the biodiversity found on this planet is by the aquatic environment with 34 of the 36 phyla of life as represented by Donia and Hamann(2003).

Over the last 40 years aquatic natural product research has become a multi-disciplinary field touching on subjects within biology, chemistry, chemical ecology and pharmacology. During this brief period bacterial secondary metabolites with pharmacodynamic properties encompassing such diverse biological activities as antibiotics, antivirals, antimitotic and antineoplastics have already been documented Kelecom(2002). Other aquatic organisms such as blue green algae, seaweeds, horse shoe crabs, marine fungi and sponge metabolites have also yielded substantial natural products such as steroids, cytotoxic and antimicrobial agents as well as novel and biologically active peptides Blunt et al., (2004).

The bacteria have long been the subject of scientific study due to their ability to cause disease in humans Lederberg(2000). One of the major advances in the health and well-being of human civilizations was the development of antibiotics. Although the introduction of antibiotics has had an enormous impact on the ability to treat bacterial infections bacteria continues to be the leading cause of deaths worldwide. Moreover the effectiveness of antibiotics has been eroded by the appearance of pathogenic strains that are resistant to nearly all classes of antibiotics coupled with the fact that only one new class of antibiotics has been introduced by the pharmaceutical industry since 1970 Binder et al.,(1999).

Soil microbial communities are among the most complex, diverse and important assemblages of organisms in the biosphere. They participate in various biological activities such as mineralization and decomposition of organic matter, biocontrol and antagonism Hackl et al., 2004. It is said that bacteria that are found colonizing soil are ubiquitous since the chemical, physical and biotic characteristics present in such medium vary immensely. Recently Horner et al., 2003 postulated that microbial population can be various in a particle of soil due to oxygen concentration. Sprusansky et al.,(2005) postulated that soil bacteria display amazing versatility in their ability to use relatively poor sources of carbon, nitrogen and thrive on a mixture of complex carbohydrates and proteins that result from the degradation of organic material which demonstrates that soil microorganisms are an important source for the search of novel antimicrobial agents and molecules with various biotechnological importance. Some of the cultivable microbes that most commonly are isolated from soil samples belong to the genera of *Bacillus, Streptomyces* and *Pseudomonas*, Belma et al .,(2002), Stabb et al .,(1994).*Streptomyces* are responsible for the production of over 70% of the antibiotics that have been isolated and reported Dairi (1999), Lo C.(2002). The genus *Pseudomonas* is comprised of a gram-negative bacteria and is vastly involved in biological control of many plant pathogens.

Soil is a diverse medium composed of many minerals and substrates essential for metabolic pathways of prokaryotic and eukaryotic inhabitants Dakora et al., 2002. The abiotic and biotic diversity present in this medium makes it difficult for the isolation of all the microbial community present therefore not even 1% of the entire soil microbial community has been identified Courtis et al.,(2003) , Hackl et al.,(2004), Rondon et al.,(2000). There is great opportunity for discovering new microorganisms of industrial and clinical importance in soil. It is not possible to recreate all of the specific requirements that every soil microorganism needs. That is why standard microbiological techniques and innovative molecular and genetic technologies are being designed Satoshi et al.,(2004), Sprusansky et al.,(2005), Zhou et al., (1996).

Applying these techniques to a given environment one can obtain large quantities of genomic material and study a vast part of a given microbial community. Scientists have developed a new molecular strategies in the field of functional genomics which involves the construction of soil metagenomic libraries for the better understanding of microbial diversity and its possible applications in medical research . Satoshi et al.,(2004). The irrational use of antibiotics has caused an increase in number of multiple drug resistant strains (MDR) of bacteria, fungi and many MDR strains are being reported from the genera *Pseudomonas, Streptococcus* and *Staphylococcus* Chitnis et al .,(2000). Some of these strains are resistant to most used antibiotics including methicillin, cephalosporins, and other beta-lactams that target peptidoglycan synthesis. Others have gained resistance toward neomycin and streptomycin which attack the bacterial ribosome.

Antibiotic resistance got lot of attention in many forms including the recent developments in which the superbug NDM made lot of noise in news at global level there fore the hunt for the novel antibiotics from nature is the need of the day. NDM-1 is a newly-identified enzyme that makes bacteria resistant to a broad range of beta-lactam antibiotics. United Kingdom Health Protection Agency has stated that "most isolates with NDM-1 enzyme are resistant to all standard intravenous antibiotics for treatment of severe infections NDM-1 is known as New Delhi Metallo-Beta-Lactamase. This bacterium which was found recently is called the Superbug because of its efficiency to resist almost all antibiotics invented. NDM 1 has high resistance to survive against antibiotics such as carbapenems and beta-lactams which is its dangerous side. Though the investigations are on it has already effected India's medical tourism. Infact US President Obama has issued an advisory to US nationals

not to go to the cheaper treatment in India to avoid the MDR. It is well understood that antibiotic resistance is an evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal; the underlying molecular mechanisms leading to antibiotic resistance can vary. Intrinsic resistance may naturally occur as a result of the bacterial genetic makeup.

At the molecular level it is assumed that the bacterial chromosome may fail to encode a protein that the antibiotic targets or an acquired resistance may result from a mutation in the bacterial chromosome or the acquisition of extra-chromosomal DNA. The spread of antibiotic resistance mechanisms occurs through vertical transmission of inherited mutations from previous generations and genetic recombination of DNA by horizontal genetic exchange.

Antagonism: Antagonism may occur for space or for a common resource. Nair and Simidu 1987 hypothesized that antagonistic properties are linked to the tropic status of the habitats. Studies by the authors Burgess et al.,(1991) show that in shallow areas such as Tokyo Bay, organic nutrients derived from metabolic processes or death and decay of massive phytoplankton population and constant nutritional inputs from external sources assuage the necessity for bacterial populations to produce antibacterial substances to survive competition. Auto-inhibition acts as a controlling factor in maintaining species diversity by allowing the population to partially limit itself and co-exist with competitors. Many marine free-living and sediment-inhabiting marine bacteria have been shown to produce secondary metabolites that display antibacterial properties Burgess et al., (1991).

Antibacterial activity has been widely exploited for the past 50 years and antibiotics have revolutionized Medical science by providing cure for formerly life-threatening diseases. Microbial populations have a resilient dynamic stability produced by biological buffering from competition. Microorganisms compete for nutrients, oxygen and favorable ecological niches and are selective for their tolerance towards ambient conditions i.e. pH, carbon dioxide, water and microbial toxins Baker(1980). Microorganisms secrete metabolites some of which inhibit other microorganisms (antibiotics) while others stimulate other microorganisms to form essential stages of their life cycle. A negative interaction can therefore directly inhibit a pathogen or inhibit a stimulatory microorganism there by indirectly inhibiting the pathogen. The aquatic environment harbors a wide range of microbes capable of exhibiting bacteriolytic and antibiotic activity. Bacteriolytic activities were found to be higher in the zooplankton than in sea water and the major group isolated were the gram negative bacteria in particularly the *Vibrio parahaemolyticus* followed by the gram positive strain, *Staphylococcus aureus* Nair et al., 1985. There are reports on such bacteria from nutrient-rich algal surfaces Jensen and Fenical, (1994); Bernan et al.,(1997).

The bacteriolytic bacteria are mainly inhabitants of the places where the organic matter is high and contribute to its decomposition Nair et al.,(1985).A number of surface-associated marine bacteria have been found to produce antibiotics. Trischman et al.(1994) isolated a species of *Streptomyces* from the surface of a jellyfish. Though the property of production of antimirobial compounds is constitutive, Patterson and Bolis(1997) observed that chemical signals received from potential competitor strains elicit an antagonistic response. However, this aspect still remains a little studied phenomenon Mearns-Spragg et al.,(1997); Mearns-Spragg et al.,(1998). In contrast to antibiotics which promote interspecies antagonism, bacteriocins are responsible for intra specific antagonism. Colicin produced by Eschereshia coli has been studied extensively and similarly there are brevicin, nisin, pediocin produced by various groups.

Bacteriocin produced by *Halobacterium mediterranei* ATCC 33500 has been shown active against many other halobacteria Meseguer et al., 1985. Bacteriocin -producing bacteria can change their strategy from anti- to pro-biotic depending on the environment. Therefore a brevicin producer could be skillfully used as probiotic to ward off unwanted microbes or to mitigate pathogenesis. A deep sea pigmented *Brevibacterium* sp has been shown to produce linocin-like compound that can be used as probiotic in aquaculture feeds. The extracts of this bacteria have not only been suggested to be useful in prolonging shelf-life of dairy products but the culture per se could be used as probiotics and also as feed additives in aquaculture Loka Bharathi et al., (2003).

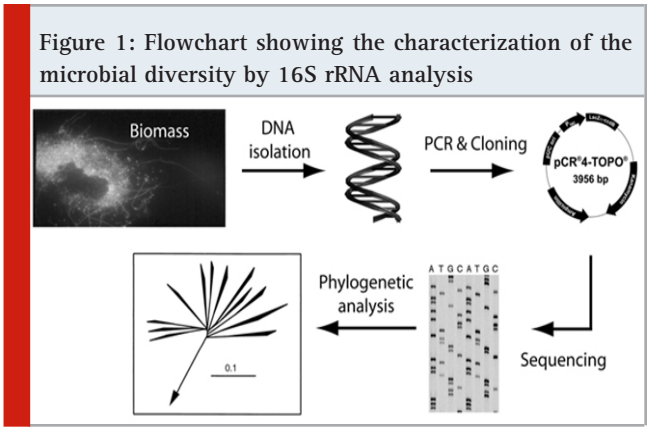
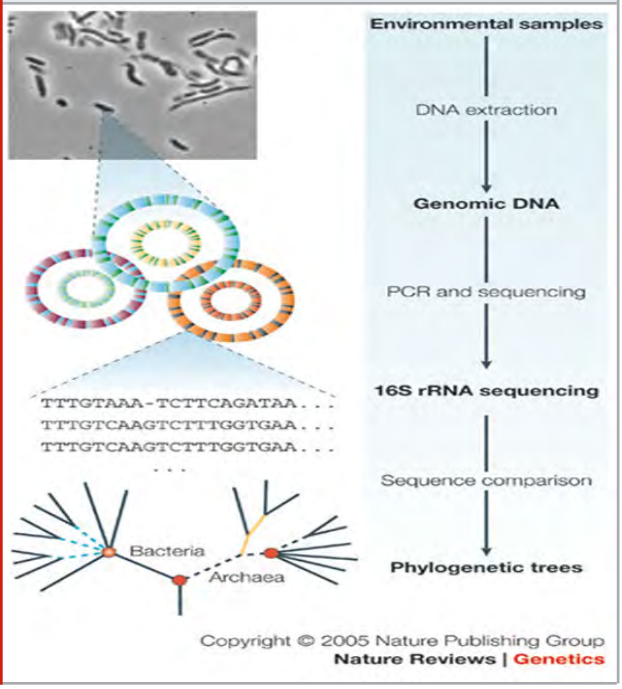


Figure 1: Flowchart showing the characterization of the microbial diversity by 16S rRNA analysis

16s rRNA sequencing & Metagenomics: There are various methods to identify, characterize and exploit the microbes and the most accepted and popular technique of bacterial identification and classification is 16s rRNA gene sequencing in bacterial systematics. The rRNA is the most conserved (least variable) gene in all cells. Portions of the rDNA sequence from distantly-related organisms are remarkably similar. This means that the sequences from the distantly related organisms can be precisely aligned making the true differences required for easy measurements, therefore the genes that encode the rRNA (rDNA) have been used extensively to determine taxonomy, phylogeny and to estimate rates of species divergence amongst the bacteria. The comparison of

16s rDNA sequence can show evolutionary relatedness amongst microorganisms. This work was pioneered by Carl Woese 1977 who proposed the three Domain system of classification - Archaea, Bacteria and Eucarya - based on such sequence information.

Figure 2



Prokaryotic microorganisms comprise the largest part of the earth's total biomass. This group contains a vast array of species, with enormous genetic, metabolic, physiological and behavioral diversity. However less than 1% of them have been cultured. Despite their ubiquity very little is known about their fundamental properties, range of diversity, interaction with the environment, evolution and the role they play in global biogeochemical cycles Rodrìguez-Valera,(2004). It is believed that progress towards filling these knowledge gaps will advance significantly when more whole genome sequences become available for the investigations.

The current availability of bacterial genome information originated from molecular biology accomplishments and made available hundreds of protein-protein interactions based solely on sequence comparisons. Moreover genome sequence information can now be coupled with other experimental data (structures, domain shuffling, expression patterns, and gene adjacency in genomes) to allow new approaches to determine gene function. Nowadays genomics and especially metagenomic approaches contributing advancement in knowledge and understanding of microbiology, since it is not possible to transform a bacterial strain, delete gene information or manipulate any level of protein expression of non-culturable bacteria using traditional classical genetics techniques. The information derived from whole-genome sequences following their comparative analysis can be used to study the novel aspects of biochemistry, physiology and metabolism of these organisms to investigate the role of microorganisms played in complex ecosystems and in global geochemical cycles, study their diversity and to predict the impact of microorganisms on the productivity and sustainability of agriculture, forestry and safety and quality of food supply. Simultaneously new genome sequences can be used to infer phylogenetic relationships among prokaryotes that deal with the organization and evolution of microbial genomes, mechanisms of transmission, exchange and reshuffling of genetic information Koonin(1997).

Figure 3: Rooted universal phylogenetic tree as determined by comparative analysis of ribosomal genes sequences. The data supports the discrimination of three domains, two of which contain prokaryotic representatives (Bacteria and Archaea). The root represents the position of a suspected universal ancestor of all cells. In dashed lines are indicated phylogenetic groups which are exclusively thermophilic or contain few thermophilic representatives (modified from Madigan et al 1997).

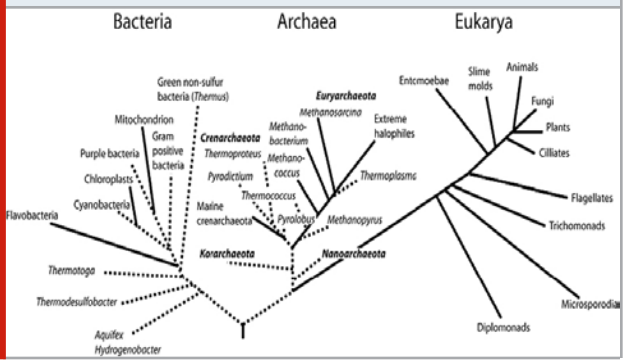


Figure 4: 16S rRNA-based tree showing the major groups of Archaea and Bacteria.

The ability to culture a microbe certainly assists the sequencing of genomes. Some laboratories have already developed techniques to sequence organisms without ever culturing them Kemmer and Fraser(2002). This technique is important for those organisms that are not well understood or those live in very complex environments like extreme habitats, deep sea, rocks etc. Nelson(2003). This technique allows scientists to discover new enzymes, antibiotics and other microbial products useful in various biotechnological applications

including medicine and industry. Another application of sequencing directly from environment gives better understanding of the soil metagenome, metagenome of a healthy vs. diseased individuals. The gene pools present in a prokaryotic species can be order of magnitude larger that that of the genome of a single strain. Contrasting with eukaryotic genomes the repertoire of genes present in a prokaryotic cell does not correlate stringently with its taxonomic identity. Therefore the gene catalogues from a particular environment may provide more meaningful information than the classical species catalogues.

The industrial sector and researchers have employed great efforts searching for novel antimicrobial agents. They have screened many types of soils in order to culture antimicrobial agent producing microbes. One of the biggest problem that groups encountered is the rediscovery of the same antimicrobial agents. Zachner and Fiedler(1995) stated that there is 99% of redundancy when searching for antimicrobial agents. The problem observed can be vastly related with the fact that 99% of the entire soil microbial population cannot be cultivated by conventional microbiological techniques.

Different approaches such as culture modification techniques in which culture media are prepared with ecological extracts and the use of density gradients for the separation of microbes based on cellular density are being used in order to culture the uncultivable, as mentioned above a limitation is that most of the time these methods render the same group of cultivable microbes resulting in the isolation of similar antimicrobial agents. In order to fulfill the need for novel antimicrobial screening methods significant contributions had been made by DeLong(2002), Gillespie et al.,(2002), Handelsman and Wackett (2004) using metagenomic tools and techniques leading to the construction of many metagenomic libraries.

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Soil and Water Quality for Healthy Crop: A Review

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ABSTRACT

It is expected that ninety- five present of our food is directly or indirectly made on our soil. Healthy soil is the base of the food system. Soil is the main basis for agriculture and consequently the medium during which nearly all food-producing plants grow. Healthy soils produce healthy crops that successively feed people and animals. Indeed, soil quality is directly connected to food quality and quantity. Soil is that the resource of essential nutrients, water, oxygen and root sustenance that our food-producing plants got to grow and embellishment. They also function a buffer to guard delicate plant roots against drastic fluctuations in temperature. Irrigation water quality may be a critical aspect of crop production. There are several factors that regulate water quality. Among the foremost important are alkalinity, pH and soluble salts. But there are several other factors to think about, like whether water salts like calcium and magnesium or heavy metals which will clog irrigation systems or individual toxic ions are present. so as to work out this, water must be tested at a laboratory that's equipped to check water for agricultural irrigation purposes

KEY WORDS: SOIL QUALITY, WATER QUALITY, RECLAMATION AND CROP PRODUCTION.

INTRODUCTION

Soil, water, air, and plants are vital natural resources that help to supply food and fiber for humans. They additionally maintain the ecosystems there on all life on Earth ultimately depend. Soil may be medium for plant growth; a sink for heat, water, and chemicals; a filter for water; and a biological medium for the breakdown of wastes. Soil interacts intimately with water, air, and plants and acts as a damper to variations inside atmosphere. Soil mediates several of the ecological processes those manage water and air quality that promote plant growth.

Poor quality water usually in change of slow growth, poor aesthetic quality of the crop and, in some cases, could result inside the gradual death of the plants. High soluble salts will directly injure roots, meddling with water and nutrient uptake. Salts will accumulate in plant leaf margins, initiating burning of the sides. Water with high pH will adversely have an impact on the hydrogen ion concentration of the growing medium, meddling with nutrient uptake and inflicting nutrient deficiencies that compromise plant health (Pal et al., 2019).

Reclaimed water, runoff water, or recycled water may need reconditioning before use for irrigation since infection organisms; soluble salts and traces of organic chemicals may even be present. Soil quality is best outlined in reference to the functions that soils perform in natural and agro ecosystems. The slandered of soil resources has traditionally been closely associated to soil productivity

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(Bennett and Chapline, 1928., and Lowdermilk, 1953., Hillel., 1991). Indeed, in several cases the terms soil quality and soil productivity nearly similar (Soil Science Society of America, 1984). Additional recently, however, there's growing recognition that the functions soils perform in natural and agro ecosystems go well on the far side promoting the development of plants. The need to broaden the conception of soil quality on the far side ancient considerations for soil productivity highlighted at a series of recent conferences and symposia.

1. Importance of soil quality: Soils have vital direct and indirect impacts on agricultural productivity, water quality, and global climate (Pal et al., 2020 & Pine et al., 2013). Soils make it potential for plants to grow by mediating the biological, chemical, and physical processes that fund plants with nutrients, water, and other different essentials. Microorganisms in soils convert nutrients into forms that may be utilized by growing plants. Soils are the stores for water and nutrients. Plants draw on these stores as grow roots, stems, leaves, and, eventually, food and fiber for human consumption (Adhikary et al., 2020). In Soils the biological, chemical, and physical processes they make possible an essential resource on that productivities of agricultural and natural ecosystems rely.

The soil interacts with landscape features and plant cover, is a key element in regulating and partitioning water flow through the environment (Jury et al., 1991). The biological, chemical, and physical processes that occur in soils buffer environmental changes in air quality, water quality, and global climate (Lal and Pierce., 1991). The soil matrix is that the major incubation chamber for the decomposition of organic wastes, as an example, pesticides, sewage, and solid wastes. Soil is the important sources which sink of greenhouse gases that contribute greenhouse effect in environment. Soils store, degrade, or immobilize nitrates, phosphorus, pesticides, and different substances that can become air or water pollutants.

Soil degradation through erosion, compaction, loss of biological activity, acidification, salinization, processes reduce soil fertility & quality. These processes decrease soil quality by changing the soil attributes, such as nutrient status, organic carbon content, texture, available water-holding capacity, structure, maximum rooting depth, and. Some changes in these soil attributes may be reversed by external inputs. Nutrient losses, as on example, may be replaced by adding fertilizers (Adhikary et al., 2020 & Adhikary et al., 2016).

1.1 Soil quality and agricultural productivity: Damage to agricultural productivity has traditionally been the key concern concerning soil degradation. Agricultural technology has, in some cases, improved the standard of soils. In different cases, improved technology has covered considerable of the yield loss that might be attributed to declining soil quality, except on those soils that are liable to quick and irreversible degradation. A healthy soil is a living, dynamic eco-system, swarming with microscopic and higher organisms that perform several important functions as well as transforming dead and decaying matter likewise as minerals to plant nutrients (nutrient cycling); monitoring plant disease, insect and weed pests; improving soil structure with positive effects for soil water and nutrient holding capacity, and ultimately improving crop production. A healthy soil also contributes to mitigating global climate change by maintaining or increasing its carbon content (Pal et al 2020 & Pal et al 2019).

1.2 Soil degradation on productivity: The result come from different experiment, yield losses resulting from soil erosion would be less than 10 percent over the next 100 years (Crosson and Stout., 1983; Hagen and Dyke., 1980; Pierce et al., 1984; Putnam et al., 1988). Such projections of low-yield losses, in addition to increasing concern over off-site water quality damages from agricultural production, have begun to shift the emphasis of federal policy to the off-site damages caused by erosion. More vital, estimates of productivity losses ensuring from erosion haven't accounted for damages caused by gully and ephemeral erosion, sedimentation (Pierce 1991), or reduced water accessibility attributable to belittled infiltration of precipitation. The cultivated land also being damaged by compaction, salinization, acidification. These damages will reduce yield losses resulting from erosion. Erosion accelerates the processes of compaction, salinization, and acidification. Yield losses are larger than those projected within the past if all degradation processes and their interactions are considered.

2. Improve Soil Quality: Given the multiple processes of soil degradation and therefore the probable underestimation of the full cost of erosion on the cost of production, it can be determined that soil degradation may have major effects on the ability of the India to bear a productive agricultural system. The costs of reversing multiple causes of soil degradation to keep up yields may be large sufficient to affect the costs of production, even if total yields are not affected. To date, enhancements in agricultural technologies kept the costs of return for losses in soil quality low enough or will increases the yields large enough to offset the price of soil degradation on most croplands.

2.1 Soil Management: Finally, though attention has clearly been targeted on soil degradation, soil management to enhance soil quality holds the promise of producing gains in productivity. Soil management to enhance soil quality is a chance to concurrently improve profit and environmental performance.

Soil quality change by the following steps: Identification of the soil attributes that can serve as indicators of change in soil quality, Standard field and laboratory methodologies that can be used to measure changes in indicators of soil quality, A coordinated monitoring program that can quantify changes in soil quality indicators, and A coordinated research program designed

to support, test, and confirm models that can be used to predict the impact of management practices on soil quality.

2.2 Soil Nutrient Availability: Nutrient availability is an essential soil aspect for plant productivity and water quality and is expressively altered by soil management practices. Nutrient availableness is often calculated by extracting nutrients from the various components in the soil with chemicals and measurement of nutrient content within the extract. Nitrogen, phosphorus, and potassium are the major nutrients in the soil that are dignified by extraction.

2.3 Soil Organic Carbon: Soil organic carbon or soil organic matter is the most important indicator of soil quality and productivity. Depletion of soil organic carbon is followed by depletion of plant nutrients, deterioration of soil structure, diminished soil workability (Frye, 1987), and lower water-holding capacity of the soil. The availability of organic carbon in the soil affects soil permeability, water retention, and hydraulic conductivity, which all define the way rainfall is portioned and potential pollutants transported. It changes the efficacies and fates of applied pesticides

2.4 Soil Texture: The soil texture in the surface soil layer may be changed as a result of removal fine particles during the erosion, as a result of mixing subsoil into the surface layer during tillage and as a result of deposition of eroded sediments on the soil surface. Changing the surface soil texture can have important effects on crop productivity (Frye, 1987 and Lal, 1987), for example, by reducing the amount of nutrients or water holding capacity or by restricting the growth of plant roots. Texture also effects the segregating of rainfall in surface and the movement of water and potential pollutants through the soil.

2.5 Structure: The term soil structure, as broadly defined by Kay (1989), has three components. The first is structural form, which refers to the geometry of the soil pore space (porosity, pore size distribution, and pore continuity). The second is aggregate stability, which refers to the size distribution and resistance of aggregates to degradation. The third is structural resiliency, which refers to the ability of the soil structure to re-form once it has been degraded.

2.6 Acidity and Alkalinity: Soil pH is a measure from which many general interpretations about the chemical properties of a soil can be made. The acidity, neutrality, or alkalinity of a soil suggests the solubility of various compounds in the soil, the relative bonding of ions to exchange sites, and the activities of microorganisms (McLean, 1982 and Pal et al 2020). pH value is less than 4 indicates the presence of free acids in soil and from oxidation of sulphides; pH of less than 5.5 indicates that presence of exchangeable aluminium; and a pH from 7.8 to 8.2 indicates the presence of calcium carbonate (Thomas, 1967). Acidity and alkalinity in soil can be managed, by management of proper agricultural practices, fertilizer and lime applications.

2.7 Erosion: Soil erosion is a natural marvel that has happened since Earth was formed. Erosion by water and wind has helped shape the landscapes that people know today. Quantitative studies of the amount of erosion that occurred during periods of geologic and historic time show that the rate is highly variable in space and time. This variability can be caused by external factors, such as changes in climate and vegetation, or by internal factors that result in episodic erosion. The rates of erosion under current farming systems are much higher than before farming began and are greater than those in uncultivated areas.

2.8 Water-Holding Capacity: An important attribute of a soil is its ability to store and release available water to plants. The importance of water available to plants and its measurement were discussed by Ritchie (1981). Plant-available water is required to absorbed nutrients from soil of all crop simulation models. The plant-available water capacities should be determined to the depth of rooting, and temporal changes in plant-available water capacities those that are either natural or induced by management or erosion should be determined in the surface layers.

3. Irrigation Water: Irrigation water is used to grow fresh produce and sustain livestock. It is using of makes it possible to produce fruits and vegetables and raise livestock, which are consumed in everyday our diet. Agricultural water is used for irrigation, pesticide and fertilizer application, crop cooling etc. When irrigation water is used efficiently and manages properly, production and crop yield is affected. Reducing of water can cause reduce production and yield. Management practices is the main important way to improve the irrigation water use and maintain optimal production and yield.

3.1 Concerned about the agricultural water quality: Water quality can be pretentious by poor planning of industrial sites, animal farms, and barnyards and feedlots. Water source are continue indicative and potential risks of contamination due to improper management. Poor water quality will have an effect on the standard quality of food crops and cause to illness in people who consume them. Groundwater is one of the safest sources of water but depending on field location and field size, it may not be possible to use water from these sources for irrigation. Water quality should be tested to ensure it is acceptable for plant growth and to minimize the risk of discharging pollutants to surface or ground water.

3.1.1 Salinity Hazard: The most important water quality guideline on crop productivity is that the water salinity hazard as restrained by electrical conductivity (EC). The key effect of high EC water on crop productivity is that the inability of the plant to vie with ions within the soil solution for water (physiological drought). The greater the EC, the less water is accessible to plants, although though

the soil might seem wet. As a result of plants will solely transpire "pure" water, usable plant water within the soil solution decreases dramatically as EC increases (Adhikary et al 2020). Actual yield reductions from irrigating with high EC water varies considerably. Factors influencing yield reductions embody soil type, drainage, salt type, irrigation system and management. On the far side effects on the immediate crop is that the long period impact of salt loading through the irrigation water.

3.1.2 Sodium Hazard: Water infiltrations will reduce when irrigation water contains high sodium as well as present of calcium and magnesium contents. It is called "sodicity," results from excessive soil accumulation of sodium. Sodic water is not the similar as saline water. Sodicity reasons swelling and dispersion of soil clays, surface crusting and pore plugging. This degraded soil structure condition blocks infiltration and may rise runoff. Sodicity causes a decrease in the downward movement of water through the soil, and plants roots may not get adequate water, in spite of pooling of water on the soil surface after irrigation. The most common measure to measure sodicity in water and soil is called the Sodium Adsorption Ratio (SAR).

3.1.3 pH and Alkalinity: The acidity or alkalinity of irrigation water is expressed as pH. In normal range of pH in irrigation water is varies from 6.5 to 8.4. Abnormally low pH's are not suitable, but may cause accelerated irrigation system corrosion where they occur. High pH's above 8.5 are often caused by high bicarbonate and carbonate concentrations, known as alkalinity. Extreme bicarbonate concentrates can also be difficult for drip or micro-spray irrigation systems when calcite or scale formed, causes reduced flow rates through holes or emitters. In this stage to control the situations, injecting sulphuric or other acidic materials into the system is required.

3.1.4 Chloride: Chloride is a common in irrigation waters. It is essential to plants in very low quantities; because of it can cause toxicity to sensitive crops when it is high concentrations. Similarly sodium, with high chloride concentrations causes creates more problems, when applied through sprinkler irrigation. Leaf burn symptoms with sprinkler irrigation from presence of both sodium and chloride which reduced by night time irrigation or application on cool, cloudy days.

3.1.5 Boron: Boron is an element that is essential in low amounts, but toxic at higher concentrations. Toxicity may occur on sensitive crops at concentrations less than 1.0 ppm. Indian soils and irrigation waters contain enough boron that additional of boron fertilizer is not required in most of the situations. Boron toxicity can occur at low concentrations; in irrigation water examination is recommended for ground water before applying any additional boron to irrigated crops.

3.1.6 Sulphate: The sulphate ion is a major contributor to salinity in irrigation waters. As with boron, sulphate in irrigation water has fertility benefits, and irrigation water in India often has enough sulphate for maximum production for most crops. Exceptions are sandy fields with <1 percent organic matter and <10 ppm SO4-S in irrigation water.

3.1.7 Nitrogen: Nitrogen in irrigation water (N) is largely a fertility issue, and nitrate-nitrogen (NO3-N) can be a significant N source in different part of India. The nitrate ion often occurs at higher concentrations than ammonium in irrigation water (Pal et al 2020). Waters high in N can cause quality problems in crops such as barley and sugar beets and excessive vegetative growth in some vegetables. However, these problems can usually be overcome by good fertilizer and irrigation management. Regardless of the crop, nitrate should be credited toward the fertilizer rate especially when the concentration exceeds 10 ppm NO3-N (45 ppm $NO3^-$).

CONCLUSION

The global population that can predicate to exceed 9 billion by 2050, compounded by the struggle for land and water resources and the effect of climate change continue coming to our current and future food security pivots the ability to increase the yields and food quality. Complete production management systems that encourage and improve agro-ecosystem health that is socially, ecologically, and economically sustainable are essential in order to protect our soils where preserving high production capacities. Farmers play an essential role in this aspect. Numerous and diverse farming methods encourage the sustainable management of soils with the goal of improving productivity, for example, agro ecology, conservation agriculture, organic farming, zero tillage farming, and agroforestry. A better understanding in between soil life and ecosystem function and the influence of human interferences will enable the reduction of negative influences and allow to capture the benefits of soil biological activity more effectively for a more sustainable.

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Effect of Different Levels of Fertilisers on Nutrient Uptake of Indian Mustard (*Brassica juncea* L.)

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ABSTRACT

The present study was carried out with the objectives to study the growth the effect of different levels of fertilisers on yield, nutrient uptake and economics of the Indian mustard variety NRCHB-101 under graded doses of fertilizer. The field experiment was conducted during 2016-17 at the Agronomy Main Research Station, OUAT, Bhubaneswar laid out in a Factorial Randomized Block Design with three replications and twelve treatments. The total N uptake by Indian mustard var. NRCHB-101 was highest at N3 (120 Kg ha^{-1}) and lowest at P1 (20 kg ha^{-1}) i.e., 64.08 kg ha^{-1} and 60.95 kg ha^{-1}, respectively. The total P uptake was highest at N3 (120 Kg ha^{-1}) and lowest at N1 (80 kg ha^{-1}) i.e., 27.32 kg ha^{-1} and 21.51 kg ha^{-1}, respectively. The total K uptake by Indian mustard var. NRCHB-101 was highest at N3 (120 Kg ha^{-1}) and lowest at N1 (80 kg ha^{-1}) i.e., 51.79 kg ha^{-1} and 45.60 kg ha^{-1}, respectively.

KEY WORDS: INDIAN MUSTARD, NUTRIENT INTERACTION, NUTRIENT UPTAKE

INTRODUCTION

Role of oilseeds in Indian agriculture needs hardly any emphasis. Oilseeds constitute an important group of crops next to cereals. India is a premier oilseed growing country. India is the fourth largest oilseed economy in the world. Among the seven edible oilseeds cultivated in India, rapeseed-mustard contributes 28.6 per cent of the total area. Presently, rapeseed-mustard is the third most important oilseed crop in India after groundnut and soybean. India is one of the largest producer, consumer and importer of oilseeds in the world. Out of nine major oilseeds grown in India, Indian mustard (Brassica juncea) is an important winter season Rabi crop. The gap between production and demand of rapeseed-mustard is progressively widening and therefore, the production is to be increased for self sufficiency. Indian mustard requires relatively larger amount of nutrients for realization of higher yield potential. Moreover, with increase in irrigated area and introduction of high yielding varieties, it becomes imperative to work out the response of Indian mustard to nitrogen, phosphorus and potassium in Odisha condition. The mustard growing areas in India are experiencing the vast diversity in the agro climatic conditions and different species of rapeseed-mustard are grown in some or other part of the country. Under marginal resource situation, cultivation of rapeseed-mustard becomes less remunerative to the farmers. This results in a big gap between requirement and production of mustard in India.

Therefore site-specific nutrient management through soil-test recommendation based should be adopted to improve upon the existing yield levels obtained at farmers field. Effective management of natural resources, integrated approach to plant-water, nutrient and pest management and extension of rapeseed-mustard cultivation to newer areas under different cropping systems will play a key role in further increasing and stabilizing the productivity and production of rapeseed-mustard. With this backdrop,

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the present paper on Indian mustard high yielding variety NRCHB-101 entitled "Effect of different levels of fertilisers on nutrient uptake of Indian mustard (Brassica juncea L.)" has been presented with the objectives to study the interaction effect of nutrients on the variety under different fertility levels.

MATERIAL AND METHODS

The field experiment was conducted during Rabi 2016-17 at the Agronomy Main Research Station, Odisha University of Agriculture and Technology, Bhubaneswar (20026'N, 85081'E, 25.9m above MSL), Odisha.The soil of the experimental sandy loam acidic (pH-5.4) medium in organic carbon (0.628%) and available nitrogen (1673.3kg/ha), phosphorus (64.5kg/ha) and potassium (123.4 kg/ha). The experiment was laid out in a factorial randomized block design with three replications. Twelve treatment combinations comprising 3 nitrogen levels (80, 100, 120 kg N/ha), two (20, 40 kg P205/ha) and two potassium levels (0, 30 kg K_2O/ha) were tested in the experiment. Indian mustard variety 'NRCHB-101' was sown 30 cm row distance.

Table 1. Nitrogen uptake by Indian mustard as influenced by nitrogen, phosphorus and potassium levels

Treatment	Nitrogen Uptake		
	Seed Yield (kg ha^{-1})	Stover Yield (kg ha^{-1})	Total (kg ha^{-1})
N-levels (kg ha^{-1})			
80	50.17	10.90	61.07
100	49.33	12.05	61.38
120	51.44	12.64	64.08
SE(m)±	2.26	0.56	2.82
CD(P=0.05)	NS	1.36	NS
P2O5 levels (kg ha^{-1})			
20	49.51	11.44	60.95
40	51.11	12.28	63.39
SE(m)±	1.84	0.46	2.30
CD(P=0.05)	NS	NS	NS
K2O levels (kg ha^{-1})			
0	49.82	11.62	61.44
30	52.12	11.80	63.92
SE(m)±	1.84	0.46	2.30
CD((P=0.05)	NS	NS	NS

Thinning was done as 15 DAS to maintain plant to plant distance of 10 cm. All the recommended agronomic practices are done throughout the crop season. The crop was sown on 20th November and harvesting was done manually during last week of February. The N,P and K uptake in seed and stover was estimated by following standard procedure described by Jackson(1973) and all the data were analysed as per standard statistical procedures.(Gomez and Gomez,1984).

RESULT AND DISCUSSION

The N uptake was significant for different levels of N, P and K. The N uptake increases as the doses of N, P and K increases. The seed uptake was highest at K_2 (30 Kg ha^{-1}) and lowest at N2 (100 kg ha^{-1}) i.e., 52.12 kg ha^{-1} and 49.33 kg ha-1, respectively. The stover N uptake was highest at N3 (120 Kg ha^{-1}) and lowest at N1 (80 kg ha^{-1}) i.e., 12.64 kg ha^{-1} and 10.90 kg ha^{-1}, respectively. The total N uptake in seed by Indian mustard var. NRCHB-101 is highest at N_3 (120 Kg ha^{-1}) and lowest at P_1 (20 kg ha^{-1}) i.e., 64.08 kg ha^{-1} and 60.95 kg ha^{-1}, respectively. The N uptake by both, seed and stover, was significant for all the interactions. The higher uptake at the increased doses of N was due to higher efficiency of the crop to make use of the increased levels of N because of increased growth and vigour. Similar results of N uptake with increasing levels of N have been reported by Kumawat et al. (2014) and Dabi et al. (2015).

Table 2 represents the interaction effect of NP, PK and NK levels on total uptake of N by seed and stover of Indian mustard var. NRCHB-101. As evident from the table, the highest N uptake by seed was at N_3K_2 i.e., 51.73 kg ha $^{-1}$ and the lowest was at N1K1 i.e., 49.58 kg ha^{-1}. The table revealed that the highest N uptake by stover was at N_3P_2 i.e., 12.90 kg ha^{-1} and the lowest was at N1P1 i.e., 10.30 kg ha^{-1}. The N uptake by both, seed and stover, was significant for all the interactions. The N-P-K interaction effect on total N uptake by seed and stover has been shown in Table 3. As depicted in the table, the total N uptake by both, seed and stover, is highest at N3 P_2 K_2 i.e., 52.10 kg ha^{-1} and 13.10 kg ha^{-1}, respectively. The N uptake by both, seed and stover, was significant for all the interactions.

The seed uptake was highest at P2 (40 Kg ha^{-1}) and lowest at N_1 (80 kg ha^{-1}). The stover P uptake was highest at N_3 (120 Kg ha^{-1}) i.e., 8.32 kg ha-1. The lowest stover P uptake was observed at N1 (80 kg ha^{-1}) and P_1 (20 kg ha^{-1}) i.e., 5.71 kg ha-1. The total P uptake in seed by Indian mustard var. NRCHB-101 is highest at N3 (120 Kg ha^{-1}) and lowest at N1 (80 kg ha^{-1}) i.e., 27.32 kg ha^{-1} and 21.51 kg ha-1, respectively. The P uptake was significant for different levels of N, P and K. The P uptake increases as the doses of N, P and K increases. The total P uptake by both, seed and stover, is highest at N3 P2 K2 i.e., 19.82 kg ha-1 and 8.83 kg ha^{-1}, respectively. The P uptake by both, seed and stover, was significant for all the interactions.These results for P uptake corroborated with the findings of Ghimire and Bana (2011). The higher removal of N and P might be due to synergistic effect chlorophyll content, cell division, photosynthetic rate and root activities of plants which has been reported.

Table 5 represents the interaction effect of NP, PK and NK levels on total uptake of P by seed and stover of Indian mustard var. NRCHB-101. As evident from the table, the highest N uptake by seed was at N3P2 i.e., 19.47 kg ha^{-1} and the lowest was at N_1P_1 i.e., 15.45 kg ha^{-1}. The table revealed that the highest P uptake by stover was at N_3P_2 i.e., 8.59 kg ha^{-1} and the lowest was

at 5.33 i.e., 10.30 kg ha^{-1}. The P uptake by both, seed and stover, was significant for all the interactions. The N-P-K interaction effect on total P uptake by seed and stover has been shown in Table 6. As depicted in the table, the total P uptake by both, seed and stover, was the highest at the interaction level of N3 P2 K2 i.e., 19.82 kg ha^{-1}and 8.83 kg ha^{-1}, respectively. The P uptake by both seed and stover was significant for all the interactions.

Table 2. Interaction effect of NP, PK and NK on total uptake of N by Indian mustard

	Seed (kg ha^{-1})		Stover (kg ha^{-1})	
N-levels (kg ha^{-1})	P-levels (kg ha^{-1})		P-levels (kg ha^{-1})	
	20	40	20	**40**
80	50.17	50.17	10.30	11.50
100	49.33	49.33	11.65	12.45
120	51.44	51.44	12.38	12.90
SE(m)±	3.19		0.79	
CD(P=0.05)	NS		1.92	
P_2O_5 levels (kg ha^{-1})	K-levels (kg ha^{-1})		K-levels (kg ha^{-1})	
	0	30	0	**30**
20	48.94	50.08	11.23	11.66
40	50.70	51.52	12.01	12.56
SE(m)±	2.60		0.64	
CD(P=0.05)	NS		NS	
N-levels (kg ha^{-1})	K-levels (kg ha^{-1})		K-levels (kg ha^{-1})	
	0	30	0	**30**
80	49.58	50.75	10.53	11.27
100	48.73	49.92	11.87	12.23
120	51.15	51.73	12.47	12.82
SE(m)±	3.19		0.79	
CD(P=0.05)	NS		1.92	

Table 3. Interaction effect of nitrogen, phosphorus and potassium on total uptake of N by mustard seed and stover

Total uptake of N by mustard seed				
Levels of N	**Levels of P & K**			
	P_1		**P_2**	
	K_1	K_2	K_1	K_2
N_1	48.23	50.30	50.93	51.20
N_2	47.93	48.57	49.53	51.27
N_3	50.67	51.37	51.63	52.10
	N×P	P×K	N×K	N×P×K
SE(m)±	3.19	2.60	3.19	4.51
CD(P=0.05)	NS	NS	NS	NS
Total uptake of N by mustard stover				
Levels of N	**Levels of P & K**			
	P_1		**P_2**	
	K_1	K_2	K_1	K_2
N_1	9.93	10.67	11.13	11.87
N_2	11.53	11.77	12.20	12.70
N_3	12.23	12.53	12.70	13.10
	N×P	P×K	N×K	N×P×K
SE(m)±	0.79	0.64	0.79	1.12
CD(P=0.05)	1.92	1.56	NS	NS

Table 3. Interaction effect of nitrogen, phosphorus and potassium on total uptake of N by mustard seed and stover

Phosphorus Uptake Treatment	Seed (kg ha^{-1})	Stover (kg ha^{-1})	Total (kg ha^{-1})
N-levels (kg ha^{-1})			
80	15.80	5.71	21.51
100	17.13	7.13	24.26
120	19.00	8.32	27.32
SE(m)±	0.49	0.35	0.84
CD(P=0.05)	1.19	0.86	2.05
P_2O_5 levels (kg ha^{-1})			
20	17.19	5.71	22.9
40	19.26	7.13	26.39
SE(m)±	0.40	0.29	0.69
CD(P=0.05)	0.97	0.70	1.67
K_2O levels (kg ha^{-1})			
0	16.92	6.73	23.65
30	17.69	7.38	25.07
SE(m)±	0.40	0.29	0.69
CD((P=0.05)	NS	NS	NS

Table 4. Phosphorus uptake by Indian mustard as influenced by nitrogen, phosphorus and potassium levels

Phosphorus Uptake Treatment	Seed (kg ha^{-1})	Stover (kg ha^{-1})	Total (kg ha^{-1})
N-levels (kg ha^{-1})			
80	15.80	5.71	21.51
100	17.13	7.13	24.26
120	19.00	8.32	27.32
SE(m)±	0.49	0.35	0.84
CD(P=0.05)	1.19	0.86	2.05
P_2O_5 levels (kg ha^{-1})			
20	17.19	5.71	22.9
40	19.26	7.13	26.39
SE(m)±	0.40	0.29	0.69
CD(P=0.05)	0.97	0.70	1.67
K_2O levels (kg ha^{-1})			
0	16.92	6.73	23.65
30	17.69	7.38	25.07
SE(m)±	0.40	0.29	0.69
CD((P=0.05)	NS	NS	NS

Table 5. Interaction effect of NP, PK and NK on total uptake of P by mustard seed and stover

	Seed (kg ha^{-1})		Stover (kg ha^{-1})	
N-levels (kg ha^{-1})	P-levels (kg ha^{-1})		P-levels (kg ha^{-1})	
	20	40	20	40
80	15.45	16.15	5.33	6.10
100	16.79	17.47	6.79	7.47
120	18.54	19.47	8.06	8.59
SE(m)±	0.69		0.50	
CD(P=0.05)	1.68		1.21	
P_2O_5 levels (kg ha^{-1})	K-levels (kg ha^{-1})		K-levels (kg ha^{-1})	
	0	30	0	30
20	16.75	17.10	6.60	6.85
40	17.40	17.98	7.11	7.65
SE(m)±	0.57		0.41	
CD(P=0.05)	NS		0.99	
N-levels (kg ha^{-1})	K-levels (kg ha^{-1})		K-levels (kg ha^{-1})	
	0	30	0	30
80	15.66	15.94	5.56	5.87
100	16.86	17.39	6.86	7.39
120	18.71	19.30	8.14	8.50
SE(m)±	0.69		0.50	
CD(P=0.05)	1.68		1.21	

Table 6. Interaction effect of N, P and K on total uptake of P by mustard seed and stover

Total uptake of P by mustard seed				
Levels of N	Levels of P & K			
	P_1		P_2	
	K_1	K_2	K_1	K_2
N_1	15.25	15.64	16.08	16.23
N_2	16.70	16.88	17.02	17.91
N_3	18.30	18.77	19.11	19.82
	N×P	P×K	N×K	N×P×K
SE(m)±	0.69	0.57	0.69	0.98
CD(P=0.05)	1.68	1.37	1.68	2.38
Total uptake of P by mustard stover				
Levels of N	Levels of P & K			
	P_1		P_2	
	K_1	K_2	K_1	K_2
N_1	5.15	5.51	5.97	6.23
N_2	6.70	6.88	7.02	7.91
N_3	7.94	8.18	8.34	8.83
	N×P	P×K	N×K	N×P×K
SE(m)±	0.50	0.41	0.50	0.71
CD(P=0.05)	1.21	0.99	1.21	1.72

Table 7. Potassium uptake by Indian mustard as influenced by nitrogen, phosphorus and potassium levels

Potassium Uptake	Seed (kg ha^{-1})	Stover (kg ha^{-1})	Total (kg ha^{-1})
N-levels (kg ha^{-1})			
80	10.24	35.26	45.60
100	11.51	36.13	47.64
120	13.42	38.37	51.79
SE(m)±	0.35	0.83	1.18
CD(P=0.05)	0.85	2.02	2.87
P_2O_5 levels (kg ha^{-1})			
20	11.11	36.22	47.33
40	13.02	38.75	51.77
SE(m)±	0.22	0.68	0.9
CD(P=0.05)	0.70	1.65	2.35
K_2O levels (kg ha^{-1})			
0	11.25	35.78	47.03
30	11.93	37.45	49.38
SE(m)±	0.29	0.68	0.97
CD((P=0.05)	0.69	1.65	2.45

The seed uptake was highest at N_3 (120 Kg ha^{-1}) and lowest at N1 (80 kg ha^{-1}) i.e., 13.42 kg ha^{-1} and 10.24 kg ha-1, respectively. The stover K uptake was highest at P_2 (40 Kg ha^{-1}) and lowest at K1 (0 kg ha^{-1}) i.e., 38.75 kg ha^{-1} and 35.26 kg ha^{-1}, respectively. The total K uptake in seed by Indian mustard var. NRCHB-101 is highest at N3 (120 Kg ha^{-1}) and lowest at N1 (80 kg ha^{-1}). The K uptake was significant for different levels of N, P and K. The K uptake increases as the doses of N, P and K increases. Similar results have been reported by Grewal et al. (2009).The highest K uptake by seed was at N3P2 i.e., 13.35 kg ha^{-1}. The highest K uptake by stover was at N3P2 i.e., 38.87 kg ha^{-1}.

The total K uptake by both, seed and stover, is highest at N_3 P_2 K_2 followed by N_3 P_2 K_1 in seed and stover, respectively. The K uptake by both, seed and stover, was significant for all the interactions.The stover yields of Indian mustard were significantly obtained under application of higher levels of N and P could be ascribed to better transformation of growth and yield attributes into yield which corroborated with findings of Dabi et al. (2015).

Table 8. Interaction effect of NP, PK and NK on total uptake of P by mustard seed and stover

	Seed (kg ha^{-1})		Stover (kg ha^{-1})	
N-levels (kg ha^{-1})	P-levels (kg ha^{-1})		P-levels (kg ha^{-1})	
	20	40	20	40
80	9.89	10.59	33.86	36.66
100	11.17	11.85	35.62	36.83
120	12.68	13.35	37.86	38.87
SE(m)±	0.50		1.18	
CD(P=0.05)	1.21		2.86	
P_2O_5 levels (kg ha^{-1})	K-levels (kg ha^{-1})		K-levels (kg ha^{-1})	
	0	30	0	30
20	11.10	11.40	35.47	36.08
40	11.75	12.12	37.20	37.71
SE(m)±	0.41		0.96	
CD(P=0.05)	0.98		2.32	
N-levels (kg ha^{-1})	K-levels (kg ha^{-1})		K-levels (kg ha^{-1})	
	0	30	0	30
80	10.08	10.40	34.83	35.69
100	11.38	11.64	36.05	36.39
120	12.81	13.22	38.13	38.60
SE(m)±	0.50		1.18	
CD(P=0.05)	1.21		2.86	

Table 9. Interaction effect of nitrogen, phosphorus and potassium on total uptake of K by mustard seed and stover

Uptake of K by mustard seed				
Levels of N	Levels of P & K			
	P_1		P_2	
	K_1	K_2	K_1	K_2
N_1	9.72	10.06	10.44	10.75
N_2	11.09	11.26	11.67	12.03
N_3	12.48	12.88	13.14	13.57
	N×P	P×K	N×K	N×P×K
SE(m)±	0.50	0.41	0.50	0.70
CD(P=0.05)	1.21	0.98	1.21	1.71
Uptake of K by mustard stover				
Levels of N	Levels of P & K			
	P_1		P_2	
	K_1	K_2	K_1	K_2
N_1	33.27	34.45	36.39	36.93
N_2	35.51	35.72	36.59	37.07
N_3	37.64	38.08	38.63	39.12
	N×P	P×K	N×K	N×P×K
SE(m)±	1.18	0.96	1.18	1.66
CD(P=0.05)	2.86	2.32	NS	NS

Table 8 represents the interaction effect of NP, PK and NK on total uptake of K by seed and stover of Indian mustard var. NRCHB-101. As evident from the table, the highest K uptake by seed was at N3P2 i.e., 13.35 kg ha $^{-1}$ and the lowest was at N1P1 i.e., 9.89 kg ha^{-1}. The table also showed that the highest K uptake by stover was at N3P2 i.e., 38.87 kg ha -1 and the lowest was at N_1P_1 i.e., 33.86 kg ha^{-1}.

The N uptake by both, seed and stover, was significant for all the interactions. The N-P-K interaction effect on total K uptake by seed and stover has been shown in Table 9. As depicted in the table, the total K uptake by both, seed and stover, was highest at N_3 P_2 K_2 i.e., 13.57 kg ha^{-1} and 39.12 kg ha^{-1}, respectively followed by N_3 P_2 K1 i.e., 13.14 kg ha^{-1} and 38.63 kg ha^{-1} in seed and stover, respectively. The K uptake by both, seed and stover, was significant for all the interactions.

CONCLUSION

The total N uptake by Indian mustard var. NRCHB-101 was highest at N3 (120 Kg ha^{-1}) and lowest at P1 (20 kg ha^{-1}) i.e., 64.08 kg ha^{-1} and 60.95 kg ha^{-1}, respectively. The total P uptake was highest at N3 (120 Kg ha^{-1}) and lowest at N1 (80 kg ha^{-1}) i.e., 27.32 kg ha-1 and 21.51 kg ha^{-1}, respectively. The total K uptake by Indian mustard var. NRCHB-101 was highest at N3 (120 Kg ha^{-1}) and lowest at N1 (80 kg ha^{-1}) i.e., 51.79 kg ha^{-1} and 45.60 kg ha^{-1}, respectively. The total N, P and K uptake by both, seed and stover, was highest at interaction N3 P2 K_2. The N, P and K uptake increased as the doses of N, P and K increased.

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CRISPR/CAS 9: A Novel Technique for Genome Editing

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ABSTRACT

Genome editing is an approach in which a specific target DNA sequence of the genome is altered by adding, removing, or replacing DNA bases. Recently, genome editing based on the bacterial defence mechanism.CRISPR/Cas9 has emerged as an easily applicable and reliable laboratory tool. Combining organoids and CRISPR/Cas9 creates exciting new opportunities to study organ development and human disease in vitro. The potential applications of CRISPR in organoids are only beginning to be explored. The development of antibiotic resistance in bacteria is a major public health threat. Infection rates of resistant pathogens continue to rise against nearly all antimicrobials, which have led to development of different strategies to combat the antimicrobial resistance. In this review, we discuss how the newly popular CRISPR-cas system has been applied to combat antibiotic resistance in both extracellular and intracellular pathogens. Recent investigations have revealed the implications of the CRISPR–Cas system as a promising tool for targeted genetic modifications in plants. This technology is likely to be more commonly adopted in plant functional genomics studies and crop improvement in the near future.

KEY WORDS: CRISPR, CAS9, GENOME EDITING, SGRNA.

INTRODUCTION

Due to recent advances in genome engineering technologies are evolving a new revolution in biological research. Researchers can now directly edit or modulate the function of DNA sequences in their endogenous context in virtually any organism of choice, permitting them to elucidate the functional organization of the genome at the systems level, as well as identify causal genetic variations. Genome editing is also referred to as genome engineering or gene editing. It is a technique that is used precisely and efficiently to modify DNA within a cell. So, genome engineering refers to the process of making targeted modifications to the genome, its context, or its outputs (e.g. transcripts). The development of effective ways to make accurate targeted modifications to the genome of organisms is an established objective in biological research. In recent times, a new method for genome editing based on a bacterial CRISPR-associated protein-9 nuclease (Cas9) from Streptococcus pyogenes was developed which created a buzz in the scientific world.

The main purpose of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and cas9 in some bacteria and archaea is to develop a defence mechanism towards an invasion of plasmids and viruses through adaptive immunity. It was proved that Streptococcus thermophilus acquired resistance towards a bacteriophage through incorporating a genomic part of a virus into its CRISPR locus. This type of technology enables the researchers to manipulate the genome by adding, removing, or varying the DNA sequence. It can be used to control gene expression in plants, animals, and even humans. It has the potential to delete detrimental traits and add desirable traits with more accuracy, effortlessly

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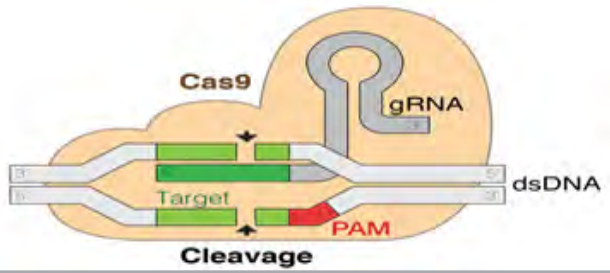
than before. Not only CRISPR can be used to "silence" genes by removing them but also to substitute desired genes by harnessing repair enzymes. So, for this, Cas9 enzyme can be modified to snip out disease-causing genes and insert a "good" gene to replace it.

What is the meaning of CRISPR?: CRISPR which stands for 'Clustered Regularly Interspaced Short Palindromic Repeats'. Early in 1987. It was originally discovered in E. coli and later in many other bacterial species. This molecule is made up of short palindromic DNA sequences that are repeated along the molecule and are regularly-spaced. Between these types of sequences, there are "spacers", foreign DNA sequences from organisms that have previously attacked the bacteria. The CRISPR molecule also includes CRISPR-associated genes or Cas genes. These can encode proteins that unwind DNA, and cut DNA, called helicases and nucleases, respectively.

Generally, the CRISPR-Cas systems are divided into two different classes based on their organization style and structural variation of the Cas genes: Class 1: Multiprotein effector complexes Class 2: Single effector protein The most frequently used subtype of CRISPR systems is the type II CRISPR/ Cas9 system, which mainly depends on a single Cas protein from Streptococcus pyogenes (SpCas9) targeting particular DNA sequences. CRISPR-Cas9 is the most common, cheap, and efficient system used for genome editing. This Cas9 stands for CRISPR-associated protein 9 and is the nuclease part that cuts the DNA / chops the DNA. CRISPR is the DNA-targeting part of the system which consists of an RNA molecule, or 'guide', designed to bind to specific DNA bases through complementary base-pairing.

Figure 1: CRISPER-Cas9

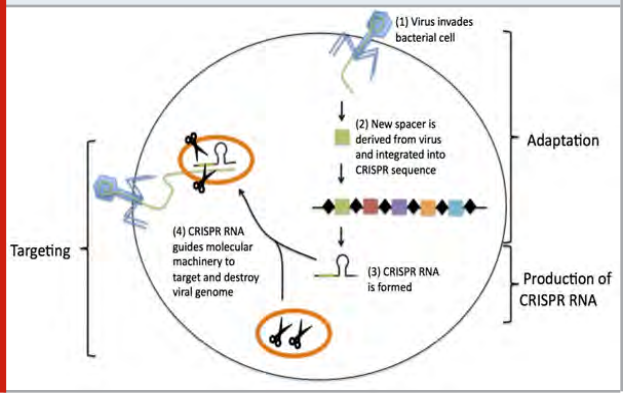


General Principle of CRISPER/Cas9: Genome editing can be achieved in vitro or in vivo by delivering the editing mechanism in situ, which powerfully adds, deletes, and "corrects" genes as well as performs other highly targeted genomic modifications. These can be used by cells to repair harmful breaks that occur on both strands of DNA, known as double-strand breaks. Targeted DNA alterations begin from the generation of nuclease-induced double-stranded breaks (DSBs), which leads to the stimulation of highly efficient recombination mechanisms of cellular DNA in mammalian cells. Nuclease-induced DNA DSBs can be repaired by one of the two major mechanisms that occur in almost all cell types and organisms: homology-directed repair (HDR) and non-homologous end-joining (NHEJ), resulting in targeted integration or gene disruptions, respectively.

Genome editing mainly relies on this concept of DNA double stranded break (DSB) repair mechanics. First report of CRISPR clusters by Ishino et al. in E. Coli bacteria in 1987. Among these, CRISPR/Cas9 is the most popular and efficient technique. It has two components such as Cas9 protein and a guide RNA (cr-RNA). RNA (cr-RNA) guides the Cas9 protein to the complementary sequence on target DNA which is subsequently cleaved by Cas9 proteins. The mechanisms of the CRISPR/Cas9 system can be easily understood by three different stages. The first stage is an adaptation, which leads to the insertion of new spacers in the CRISPR locus. In the second stage, expression, the system gets ready for action by expressing the cas genes (cas operon) and transcribing the CRISPR into a long precursor CRISPR RNA (pre-crRNA).

The pre-crRNA is subsequently processed into mature crRNA by Cas proteins (endonuclease activity) and accessory factors. In the third and last stage, interference, the combined action of cr-RNA and tracr-RNA(transactivating cr-RNA) fused into single sg-RNA(small guide RNA) which interact with their target DNA through complementary base pairing and Cas proteins associated with it, recognized the PAM (Protospacer Adjacent Motif) region in target DNA and ultimately degrade/destroy the target DNA and make them inactive. The application of CRISPR/Cas9 is being used at the molecular level like gene deletion/ insertion/ replacement, base editing, transcription modulation, and DNA labeling, etc and as a practical application level, it is being used in biological research, human medicine, biotechnology, and agriculture.

Figure 2: The steps of CRISPR-mediated immunity.



Types of CRISPR/ Cas Systems: Three different types of CRISPR/Cas systems are present: A) type I, B) type II and C) type III. Types I and III are found in both bacteria and archaea but, type II is unique in bacteria. The type II CRISPR/Cas system is the most studied and best characterized in which Cas9 protein is the critical component. The Cas9 endonuclease is a four-component system that includes two small RNA molecules named

CRISPR RNA (crRNA) that is specific to the targeted DNA, and trans-activating CRISPR RNA (tracrRNA) sequence that interacts with the Cas9 protein, that has DNA endonuclease activity. This complex will affect target-specific double-stranded DNA cleavage and will be repaired by the DNA repair mechanism by non-homologous end joining (NHEJ) or homology-directed repair (HDR). Cas9 endonuclease was re-engineered into a more controllable two-component system by joining the two RNA molecules into a "single-guide RNA"(sgRNA) that, when united with Cas9, could find and cut the DNA target specified by the guide RNA (Jinek, et al., 2012).

Thus, by manipulating the nucleotide sequence of the single-guide RNA, the synthetic Cas9 system could be programmed to target any DNA sequence for cleavage. A recent improvement is the use of the modified version of CRISPR/Cas9 system i.e., Cas9 to target protein domains for transcriptional regulation and epigenetic modifications. CRISPR/Cas9 expertise has altered the outlook of genome editing, allowing an earlier unachievable stage of genome targeting, high efficacy, easiness, and flexibility of the system. Many laboratories around the world are using the technology for variety of applications including use as a basic biology research tool, development of biotechnology products, and potentially to treat diseases in plants, animals and humans.

Following initial demonstration of genome editing by CRISPR-Cas9 by Feng Zhang's and George Church's groups simultaneously, in human cell cultures for the first time. It has since already been successfully adopted to target important genes in many cell lines and organisms, including yeast, Xenopus tropicalis, Candida albicans, zebrafish, fruit flies, ants, mosquitoes, nematodes, mice, rabbits, monkeys, pigs, human embryos and plants (Arabidopsis, rice, wheat, sorghum, tobacco). Though, initial success was achieved by the use of CRISPR/ Cas9, it takes time for routine use of this technology in humans, plants and animals. To a large extent, research is still focusing on its use in plant and animal models or isolated human cells to treat diseases. The CRISPR/ Cas9 system is widely used in biomedical research to edit the human genome and try to knock out genetic diseases like Huntington's disease or cystic fibrosis, breast and ovarian cancers (BRCA-1 and 2 mutations), hypertrophic cardiomyopathy, Down Syndrome, and HIV infections out of T cells.

Three Common Strategies have been developed for Genome Editing with the CRISPR/Cas9 Platform

1. Plasmid-based CRISPR/Cas9 strategy, where a plasmid is used to encode Cas9 protein and sgRNA, assembles Cas9 gene as well as sgRNA into the same plasmid in vitro. this strategy is longer lasting in the expression of Cas9 and sgRNA, and it prevents multiple transfections. The Key challenge in this system is the introduction.
2. Encoded plasmid needs to be introduced inside the nucleus of target cells, which is a key challenge in this system.
3. Direct intracellular delivery of Cas9 messenger RNA (mRNA) and sgRNA, the greatest drawback of which lies in the poor stability of mRNA, which results in transient expression of mRNA and a short duration of gene modification. 3. Direct delivery of Cas9 protein and sgRNA which has several advantages, including rapid action, great stability, and limited antigenicity.

How CRISPER/Cas9 system works: The CRISPR immune system is a mechanism which protects the bacteria from repeated virus attacks through three different steps:

1. Adaptation: When DNA from a different viruses invade the bacteria, the viral DNA is processed into short segments and is made into a new spacer between the repeats. These will serve as a genetic memory of previous infections.
2. Production of CRISPR RNA: The CRISPR sequence undergoes transcription, including spacers and Cas genes, creating a single-stranded RNA. The resulting single-stranded RNA is called CRISPR RNA, which contains copies of the invading viral DNA sequence in its spacers.
3. Targeting: This CRISPR RNAs will identify viral DNA and guide the CRISPR-associated proteins to them. The protein then cleaves and destroys the targeted viral material.

Uses of CRISPR system

1. Gene Knock-Out: Gene silencing using CRISPR starts with the use of a single guide RNA (sgRNA) to target genes and initiate a double-stranded break using the Cas9 endonuclease. These type of breaks are then repaired by an repair mechanism, the non-homologous end-joining (NHEJ). However, NHEJ is error-prone and results in genomic deletions or insertions, which then translates into permanent silencing of the target gene.
2. DNA-Free Gene Editing: CRISPR can be used for DNA-free gene editing without the use of DNA vectors, requiring only RNA or protein components. This system can be a good choice to avoid the possibility of undesirable type of genetic alterations due to the plasmid DNA integrating at the cut site or random vector integrations.
3. Gene Insertions or "Knock-ins": The CRISPR-induced double-strand break can also be used to create a gene "knock-ins" by exploiting the cells' homology-directed repair. The precise insertion of a donor template can alter the coding region of a gene. Previous studies which have demonstrated that single-stranded DNA can be used to create precise insertions using the CRISPR-Cas9 system.
4. Transient Gene Silencing: By modifying the Cas9 protein so it cannot cut DNA, transient gene silencing or transcriptional repression can also be done. The modified Cas9, led by a guide RNA, targets the promoter region of a gene and reduces transcriptional activity and gene expression. Transient activation or upregulation of specific genes can be effectively done.

Development of Disease Resistance in Plant Using a Novel Technique CRISPR / CAS9: The challenge for all disciplines of agriculture is to increases production and improves the quality of produce. As a global scenario, plant diseases are major challenge and biotic constraint that leads to significant crop yield losses in terms of both quantity and quality of the produce. Over the past few decades, the excessive & unnecessary use of chemical pesticides was the dominant form of disease control and subsequently created many problems such as the frequent emergence of fungicide resistance in pathogens and the harmful effects of fungicides to human health and negative impact on plants and environment. To overcome all these problems, adopting an integrated disease management strategy as an alternative tool for disease management. Integrated disease management (IDM) is a sustainable approach that combines all the suitable techniques such as biological, cultural, physical, and chemical control strategies holistically rather than using a single component strategy proved to be more effective and sustainable and minimizes economic, health and environmental risks.

But in the current scenario, due to the changing climatic conditions, the plant pathogenic organisms have developed different types of resistance mechanisms against pesticides and also the emergence of the new race of the pathogens in the environment through which diseases caused by the pathogens has become resistant which is a very difficult task to manage it effectively by adopting the traditional approaches including IDM. So, for this, scientists have evolved a novel, emerging, and a latest and most popular technique known as CRISPR/ CAS9 based genome editing technology through which plant disease can be managed by developing disease resistance in plants at the genetic level. CRISPR/CAS9 is an important tool for genome editing in an organism. Genome editing is a technology that can produce modifications such as insertion/deletion/substitution at specific sites in the genome of an organism. CRISPR stands for clustered regularly interspaced palindromic repeats. It is an array of short repeated sequences separated/ interspaced by spacers sequence derived from foreign DNA with unique sequences. First report of CRISPR clusters by Ishino et al. in E. Coli bacteria in 1987.

CRISPR is a defense system in bacteria which fight against the phage infection and provide sequence-specific adaptive immunity acts by integrating short virus sequences in the cell's CRISPR locus, allowing the cell to remember, recognize and clear infections. There are different tools for the genome editing process which includes Meganucleases, Zinc Finger Nucleases (ZFNs), Transcription Activator Like Effector Nucleases (TALENs), CRISPR/Cas9, and CRISPR/Cas12a (also called CRISPR/ Cpf1).In agricultural application, being used as improved yield, pest and disease resistance, herbicide tolerance, and improved nutritional quality. It is simple and cost-effective and has got broad spectrum applications in agriculture including plant disease management in the future. As a future perspective point of view, to improve the specificity of the CRISPR/Cas9 system to prevent off-target mutations and identifying smaller and more efficient Cas9 variants with distinct specificities. Scientists should be focused on more detailed studies on Homology Directed Repair mechanisms and also the development of more safe and efficient delivery mechanisms for Cas9/ sg RNA into organisms and exploring more potential applications of CRISPR/Cas System. There is a need to overcome the ethical and political barriers through proper awareness of society.

Advantages of the CRISPR/Cas9 System

1. Unlike ZFNs and TALENs, the CRISPR/Cas9 system can cleave methylated DNA in human cells, allowing genomic modifications that are beyond the reach of the other nucleases.
2. The main practical advantage of CRISPR/Cas9 compared to ZFNs and TALENs is the ease of multiplexing. The simultaneous introduction of DSBs at multiple sites can be used to edit several genes at the same time and can be particularly useful to knock out redundant genes or parallel pathways.
3. Applications of Cas9 as a Genome Engineering Platform

1. The Cas9 nuclease cleaves DNA via its RuvC and HNH nuclease domains, each of which nicks a DNA strand to generate blunt-end DSBs. Either catalytic domain can be inactivated to generate nickase mutants that cause single-strand DNA breaks.
2. Two Cas9 nickase complexes with spaced target sites can mimic targeted DSBs via cooperative nicks, doubling the length of target recognition.
3. Expression plasmids encoding the Cas9 gene and a short sgRNA cassette driven by the U6 RNA polymerase III promoters can be directly transfected into cell lines of interest.
4. Purified Cas9 protein and in vitro transcribed sgRNA can be microinjected into fertilized zygotes for rapid generation of transgenic animal models.
5. For somatic genetic modification, high-titer viral vectors encoding CRISPR reagents can be transduced into tissues or cells of interest.
6. Genome-scale functional screening can be facilitated by mass synthesis and delivery of guide RNA libraries.
7. Catalytically dead Cas9 (dCas9) can be converted into a general DNA-binding domain and it can be fused to functional effectors such as transcriptional activators. The modularity of targeting and flexible choice of functional domains enables rapid expansion of the Cas9 toolbox.
8. Cas9 coupled to fluorescent reporters facilitates live imaging of DNA loci for illuminating the dynamics of genome architecture.
9. Reconstituting split fragments of Cas9 via chemical or optical induction of heterodimer domains, such as the cib1/cry2 system from Arabidopsis, confers temporal control of dynamic cellular processes.

Applications and Implications in Plant Breeding

1. Genome editing can accelerate plant breeding by allowing the introduction of predictable modifications directly in an elite background, and the CRISPR/Cas9 system is particularly beneficial because multiple traits can be modified .
2. NHEJ-mediated gene knockouts are the simplest form of targeted modification, and these could be used e.g., to eliminate genes that negatively affect food quality, to confer susceptibility to pathogens or to divert metabolic flux away from valuable end-products. Both TALEN and CRISPR/Cas9 technologies have been used to target the genes of the mildew resistance locus (MLO) in wheat and successfully knocked out all three MLO homoeoalleles, generating plants resistant to powdery mildew disease.
3. Site-specific nucleases also allow targeted molecular trait stacking, i.e., the addition of several genes in close vicinity to an existing transgenic locus. This makes it feasible to introduce multiple traits into crops with a low risk of segregation, which is difficult to achieve by classical breeding or even conventional genetic engineering. Once stacking has been achieved, the entire array of transgenes can be mobilized into another germplasm by crossing because it behaves as a single locus.

Drawbacks of CRISPR: CRISPR-mediated genome editing has drawbacks, though. The PAM requirement limits target sequences. Cas9 is large, so its gene is difficult to deliver to cells via vectors used in gene therapy. Scientists worry about off-target effects, although experts note that concerns about unintended mutations are often based on calculations from studies on improving editing. These studies may deliberately use low-specificity conditions to facilitate monitoring progress.

CONCLUSION

CRISPR/Cas9 based genome editing is a fundamental breakthrough technique. Application of genome editing tools in crop improvement to enhance yield, nutritional value, disease resistance and other traits will be a prominent area of work in the future. CRISPR has played a huge part in the increase in genome editing studies in recent years. This type of system has broad applications in plant and animal improvement, as well as in the medical field. Even though modifications to CRISPR have been made to minimize the possibility of off-target effects, it has yet to be proven. There is even a chance of genome vandalism viz., the system cuts on target without a precise edit. Once the precise and deeper mechanism underlying how the CRISPR/ Cas9 system works was understood, it could be harnessed for applications in molecular biology and genetics. Still there are many ethical issues raised by genome editing have to be addressed by researchers. Regulatory agencies will also need to regulate how best CRISPR/Cas9 technology can be exploited without hindering applicable research and development.

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BBRC
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An Overview of Bio-Rational Approaches for Brinjal Insect Pest Management

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ABSTRACT

Bio-rational insecticides have been introduced to control insect pests since chemical insecticides are highly toxic to living organisms and to the environment. Bio-rational insecticides have low toxic effect or no toxic effect to plant,as it is the prerequisite to control insect pest by different bio-rational approaches rather than conventional insecticide and to give emphasize on bio-rational approaches. Again, the chemical ones are adversely affected the natural enemies as wellas human beings. The resistance developed against the conventional chemicals surge for the approach towards the bio-rational insecticides. A diversity of new botanical insecticides with special activity on insect pests is in the process of development as well as their importance are very much high to control effectivelyinsect pests of Brinjal.

KEY WORDS: BIO-RATIONAL, ENVIRONMENT, RESISTANCE DEVELOPED.

INTRODUCTION

Egg plant or Brinjal (*Solanum melongena*) has a considerable economic importance in our country in regard to agriculture. Particularly,Brinjal plant is affected by various insect species like shoot and fruit borer, leaf eating insects like Epilachna beetle and sucking insects like thrips, whiteflies, aphids etc very easily.It is estimated that every year around 70-92 percent yield loss is happening due to the major pestsof Brinjal(Reddy and Srinivasa, 2004; Chakraborti and Kanti, 2011; Jagginavar et al., 2009). Most of the farmers depend on synthetic chemical insecticides for the management of these pests. Butusage of chemical pesticidesis undesirable due to high cost, high toxicity, possibility to develop resistance among pest species, resurgence of certain pest populations and adverse effect on beneficial organisms such as pollinators and natural enemies etc. So,it is necessary to adopt such bio-rational approaches for brinjal insect pests, which match into IPM strategy and will be much safe, economical as well as selective. The primary objective is to evaluate the efficiency of microbial preparations, bio-rational and neem-based insecticides against major pests of brinjal.

RESULTS AND DISCUSSION

Bio-rational pesticide may be referred as"any type of insecticide that act against target insect pests but less deleterious to non target organism like pollinators and natural enemies. They have systemic action and less residual toxicity also. The different types of Bio-rational Pesticides Botanicals, Microbial, Insect Growth Regulators (IGR), Bacterial Fermentation Products.

Brinjal shoot and fruit borer Leucinodes orbonalis (Pyralidae; Lepidoptera): It is one of the devasting pest on eggplant. Varma et al., (2009) observed that the fruit damage and weight loss of fruit varied from 3.76 to 45.45 % and 3.00 to 67.71 % in Ist year and 5.71 to 44.26 per cent and 3.00 to 51.33 per cent in IInd year due to Brinjal shoot and fruit borer.

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Management by parasitoids: The releasing of egg parasitoid, *Trichogramma chilonis*@1g parasitized eggs/ha/week and larval parasitoid, Bracon habetor @ 800-1000 adults/ha/week could be followed (Alam,et.al, 2006).The bio-rational pesticides reduce its population on brinjal. The lowest shoot and fruit infestation were obtained from the parasitoid applied plot (23.75 & 20.45%), whereas the highest in chemical applied plot (36.72 and 29.65%). The infestation status of bio-rational pesticide and non treated plots reflects in the yield. Comparative higher yield was obtained from treated plots (20.24 t/ha) than non treated (14.76t/ha)which was 37.12% higher compared to non treated.

Management by Microbial: Mostly,B. thuringiensis(Bt) is utilized as a bio-pesticide. The proteins Cry and Cyt generated by the bacteria are highly toxic to insect pest but not to mammals or for the environment. To combat the infestation of Brinjal shoot and fruit borer, scientist gave efforts to develop the natural resistance or tolerance against the pest. They insert Cry1 Ac gene from soil bacterium, *Bacillus thuringiensis* into brinjal. Hence,two local brinjal cultivars namely,ISD006 and Uttara were transformed under ABSP-II program (BARI, 2014).

Leaf eating insect pest of brinjal: Epilachna beetles *E. vigintioctopunctata* (Coccinellidae, Coleoptera): *Epilachna* beetles are polyphagous pest and mostly distributed around Asia and Australia.

Management: Saxena and Sharma (2005) studies on insecticidal activity of Neem leaves extract againstfirst instars of *E. vigintioctopunctata*. However,Satpathi and Ghatak (1990) have observed 90 percent mortality of the beetle with same concentration of *T. nerifolia* root extract.It was recorded 1.0 percent concentration of *N. indicum* seed and *E. globulus* flower extractsgive most significant mortality. However, the extracts of lower concentrations did not see any significant effect on larval survivalist exhibiting mortality.

Sucking insect pests of brinjal: Leaf hopper Amrasca devastans Distant (Cicadellidae; Hemiptera): Both nymphs and adults damage the plants by sucking the sap by their piercing and sucking mouthparts from the lower leaf surfaces. The symptom of damages iscrinkling, bronzing, and drying, or "hopper burn"(Srinivasan,2009).

Management: The nymphs and adults are predated by ladybird beetles and green lacewings generally. Parasitoids such as *Anagrus flaveolus* and *Stethynium triclavatum* are efficacious against leafhopper. Neem-based bio pesticideslike Neem seed kernel extract (NSKE) @ 5%can be sprayed.

Aphid Aphis gossypii (Aphididae; Hemiptera): It is a polyphagous pest attacking feeding on cotton, cucurbits, brinjal, and okra.

Management: The predators like ladybird beetles (Coccinella sp.) and green lacewings are mostly effective against aphids. Augmentive release of ladybird beetles @ 200 pairs per ha at 15days interval canreduce the aphid population.

Red spider mite Tetranychus urticae (Tetranychidae; Acarina): It is considered as a polyphagous pest attackingsolanaceous as well as field crops. They use their long, needle- like mouthparts and extract the cell sap from the leaves.

Management; Under protected and humid condition predatory mites are effective to control spider mites. The third instars grubGreen lacewings (*Chrysoperla carnea*) can effectively consume25–30 spider mite adults per day; however, it needs supplemental food for long-term survival (Hazarika et al., 2001).

CONCLUSION

Vegetable production is one of the more dynamic sectors of agriculture in view of the economic value of the production.And, Brinjal crop is attacked with various types of insect pest throughout the year. The adverse effect of chemicals such as insect resurgence and secondary out breaks make bio-rational approaches more prominent. The use of botanicals, microbial as well as natural enemies can be the novel approaches to manage the pest intensively. From different review findings, it is observed that combine approach of different bio rational management are more successful against brinjal shoot and fruit borer infestation. For management of leaf eating insects like *Epilachna* as well as sucking pests like aphids, leaf hopper, whitefly the microbial agents and botanical extracts can give most suitable result without causing any adverse secondary effects. So, it can be recommended based on this study that a combine bio rational approach is effective against the pest complex of brinjal crop.

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Evaluation for Nutrient Utilization Pattern of *Bacillus* Spp. Isolates in Response To Water Quality Parameters In vitro

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ABSTRACT

In the current investigation efforts have been made to study the effect of Bacillus spp. on different water quality parameter in in vitro. A total of 16 (RJ1 to RJ16) bacterial strains comprising of B. subtilis (n=08), B. cereus (06) and, B. pumilis (02) were subjected to grow in synthetic pond water to evaluate the nutrient cycling and utilization pattern with respect to ammonia, nitrite, nitrate and phosphorus verses their growth rate for a 16 hrs study individually. After incubation the strains showed luxuriant growth in the media with turbidity increasing upto12 hrs and after 14hrs stationary growth was observed in most of the isolates. All the strains showed increase in ammonia concentration (NH_4-N). Highest ammonia concentration up to 50% level was observed with RJ15 and remaining isolates also increased ammonium ion concentration with the lowest range producer was RJ3 with 4%. All the strains showed nitrate reduction activity. Strain RJ12 reduced nitrate (NO_3-N) concentration (30%) from 12.04 to 8.37 mg/L. RJ5 was the lowest nitrate reducer with activity of 2%. Six strains showed reduction in nitrite concentration including RJ1 and RJ5 where the highest nitrite reduction with 38% activity was observed. All other isolates showed increased in nitrite concentration and highest was observed with RJ11 (47%). In phosphorus metabolism RJ2 was found to be utilize highest phosphorus in synthetic pond water with 32% decrease in phosphorus concentration whereas most of the strains showed no change in activity. This study showed that Bacillus spp. are ammonia producers whereas they have the potential to reduce nitrate, nitrite and utilize phosphorous in water environment.

KEY WORDS: AMMONIA, BACILLUS SPP., NITRATE, NITRITE, PHOSPHOROUS.

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INTRODUCTION

The genus *Bacillus* represents a major microflora in many natural biotopes, where they play an important role in ecosystem development and transform many nutrients that supports the quality of water (Lalloo et al., 2007). Water environment is connected with the accumulation of organic and nitrogenous wastes viz. ammonia, nitrite and a heavy amount of organic matter (Hlordzi et al., 2020). Increasing concentration of these wastes can be toxic to aquaculture leading to stress and mortality of fish health (Loh, 2017). Total ammonia nitrogen ($NH4^+$-N), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N) and phosphorous are utilized by many microorganisms for their metabolism, leading to nitrogen removal from the water column (Martínez Córdova et al., 2015). Nitrogenous substances mainly ammonia, nitrites and nitrates are typical water pollutants connected with aquatic environments and have drawn attention of many investigations (Boopathy et al., 2015; Liang et al., 2015).

Nevertheless, fish culture follows in a heavy load of these wastes; that require careful measures to improve the water quality. Nitrification, denitrification, ammonification, and phosphate utilization are the major steps involved in the nutrient cycling of water ecosystem. The different initial forms of nitrogen include dead plants materials, animals or their released waste organic products. This organic nitrogen present are finally converted to ammonium (NH_4+) and ammonia (NH_3) by different fungal and bacterial species counting, *Bacillus* species in ammonification process (Hlordzi et al., 2020). The biologically available nitrogen and returning it to the atmosphere from nitrification and denitrification from ammonia by *Nitrosomonas* and *Nitrobacter* species mostly (Bernhard, 2010). However, *Bacillus* species present in aquaculture ecosystems (Mohanty et al., 2011) play a unique role in the nitrogen cycle through ammonification (Hui et al., 2019), nitrification (Rout et al., 2017), and denitrification (Verbaendert et al., 2011) as well as nitrogen fixation (Yousuf et al., 2017).

This activity is dissimilar to the processes govern by Nitrosomonas and Nitrobacter that are primarily associated in nitrification and denitrification (Liu et al., 2020). As far as *Bacillus* species is concerned B. amyloliquefaciens DT has observed to be able to covert organic nitrogen into ammonium (Hui et al., 2019) and *Bacillus* cereus PB8 removed NO_2-N from wastewater as reported by Barman et al., 2018. This signifies that *Bacillus* species have the potential to eliminate the different forms of toxic nitrogen waste from aquaculture ecosystem. Furthermore, *Bacillus* sp. proved to be a budding probiotic applicant which can improve aquaculture water quality and digestibility of feed (Dat et al., 2019). Keeping in view towards the improvement of water quality and application of these bacteria as probiotic candidate in aquaculture, the study was undertaken to observe the utilization pattern of soluble nutrients in freshwater ecosystem by application of *Bacillus* spp. *in vitro*.

MATERIALS AND METHODS

Procurement of *Bacillus* spp. isolates: A total of 16 isolates designated as RJ1 to RJ16b were taken into the investigation, already isolated from the same study conducted at Fish Health management Division, ICAR-Central Institute of Freshwater Aquaculture, Odisha, India. All the isolates were revived from 10% glycerol stock and maintained at Tryptone Soya Broth (TSB, Himedia, Mumbai) as broth culture for further use in the experiment. The purity and activity were checked microscopically as per standard microbiological procedures.

Preparation of standard curves: Standard curve for ammonia ($NH4^+$-N): For the preparation of standard curve for ammonia, ammonium chloride was taken as a standard curve reagent. Ammonium chloride was oven dried at 100 overnight and 382.07mg (100 mg $NH4^+$-N/L) was taken and dissolved in 1L of double distilled water (DDW). From this solution 20 mL was taken and added to 480 mL of DDW to make final conc. of 4 mg/L. This ammonium solution was used for preparation of different conc. of $NH4^+$-N mg/L. Different concentrations of the solution 0.00, 0.04, 0.08, 0.2, 0.4, 0.6, 0.8, 1, 1.4, 1.8, 2 mg/L were made by diluting ammonium chloride solution with DDW to make volume of 1 ml in eppendorf tubes in triplicates.

In eppendorf tubes to every dilution 40 µL of phosphate buffer (5% Na_3PO_4 solution), was added using a micropipette and then mixed well. Then 100 µL of ammonia reagent A [40 mg sodium-nitroprusside in 30 mL stock phenol solution (11.1 mL of liquefied phenol with 95% of v/v ethyl alcohol/ 100 mL] followed by 50 µL of ammonia reagent B (Equal volume of) sodium hypochlorite and 27% sodium hydroxide) was added and mixed properly. Then the tubes were transferred to dark place for 1 hr. After 1hr the absorbance was measured against blank (DDW) at 635 nm in an UV visible spectrophotometer (6050 UV/VICS spectrophotometer, Jenway). Then standard curve was plotted for the absorbance of each of the standard solutions against its concentration of NH4- N mg/L.

Standard curve for nitrate (NO_3-N): For the preparation of standard curve for nitrate, potassium nitrate was taken as a standard curve reagent. Potassium nitrate was oven dried at 100 overnight and 722.21mg (100 mg NO3-N/L) was taken and dissolved in 1L of DDW. From this solution 20 mL was taken and added to 480 mL of DDW to make final conc. of 4 mg/L. This nitrate solution was used for preparation of different conc. of NO3-N mg/L. Different concentrations of the solution 0.00, 0.04, 0.08, 0.2, 0.4, 0.6, 0.8, 1, 1.4, 1.8, 2 mg/L were made by diluting potassium nitrate solution with DDW to make volume of 1 mL in eppendorf tubes in triplicates. In eppendorf

tubes to every dilution 40 µL of 0.5 N HCl was added using a micropipette and then mixed well. After 30 min the absorbance was measured against blank (DDW) at 220 nm in an UV visible spectrophotometer (6050 UV/VICS spectrophotometer, Jenway). Then standard curve was plotted for the absorbance of each of the standard solutions against its concentration of NO3-N mg/L.

Standard curve for nitrite (NO_2– N): For the preparation of standard curve for nitrite, sodium nitrite was taken as a standard curve reagent. Sodium nitrite was oven dried at 100 overnight and 492.85 mg (100mg NO2-N/L) was taken and dissolved in 1L of DDW. From this solution 20 mL was taken and added to 480 mL of DDW to make final conc. of 4 mg/L. This nitrate solution was used for preparation of different conc. of NO2-N mg/L. Different concentrations of the solution 0.00, 0.04, 0.08, 0.2, 0.4, 0.6, 0.8, 1, 1.4, 1.8, 2 mg/L were made by diluting sodium nitrite solution with DDW to make volume of 1 mL in eppendorf tubes in triplicates. In eppendorf tubes to every dilution 40 µL of colour reagent (160 mL of distilled water was taken and 20 mL of 85% H3PO4 was added and mixed. Then 2 gm of sulphanilamide and 0.2 gm of N (1-napthyl) ethylene diamine dihydrochloride (NEDD) was added and final volume was made up to 200 mL with distilled water) was added using a micropipette and then mixed well. After 10 min the absorbance was measured against blank (DDW) at 543 nm in an UV visible spectrophotometer (6050 UV/VICS spectrophotometer, Jenway). Then standard curve was plotted for the absorbance of each of the standard solutions against its concentration of NO2-N mg/L

Standard curve for phosphorus (PO4-P): For preparation of standard curve for phosphorus, potassium dihydrogen phosphate was taken as a standard curve reagent. Potassium dihydrogen phosphate was oven dried at 100 overnight and 439.42 mg (100 mg PO4 -P/L) was taken and dissolved in 1L of DDW. From this solution 20 mL was taken and added to 480 mL of DDW to make final conc. of 4 mg/L. This phosphorus solution was used for preparation of different conc. of PO4-P mg/L. 2. Different concentrations of the solution 0.00, 0.04, 0.08, 0.2, 0.4, 0.6, 0.8, 1, 1.4, 1.8, 2 mg/L were made by diluting potassium dihydrogen phosphate solution with D. D.W to make volume 1 mL in eppendorf tubes in triplicates. In eppendorf tubes to every dilution 40 µL ammonium molybdate reagent (6.5 gm ammonium molybdate was mixed with 43 mL of distilled water and 62.5 mL of conc.

H_2SO_4 was mixed with 100 mL distilled water and, final volume was made up to 250 mL) was added using a micropipette and then 40 µL of SnCl2 (5gm $SnCl_2$ was mixed with 200 mL of glycerol) was added and mixed well. After 10 min but before 12 min the absorbance was measured against blank (DDW) at 590 nm in an UV visible spectrophotometer (6050 UV/VICS spectrophotometer, Jenway). Then standard curve was plotted for the absorbance of each of the standard solutions against its concentration of phosphorus mg/L.

Nutrient cycling parameters assay: The study was conducted according to Lalloo et al., 2007. The isolated *Bacillus* sp. were pre-incubated in TSB at 30 for 24 hrs for luxuriant growth. On next day the cultures were centrifuged at 10,000 rpm for 10 min and the supernatant was decanted. The pellets formed were suspended in 10 mL sterile NSS and washed twice and were resuspended in 10 mL sterile NSS. Synthetic pond water [KNO_3-0.0085, $NaNO_2$-0.006, $(NH_4)_2SO_4$-0.0093, H_3PO_4-0.0038, Yeast extract- 0.1, Glucose-0.1 and final pH was adjusted to 7.0 ± 0.2, suspend the constituents in 1L distilled water and adjust the pH with liquor ammonia (25%), then filter the media using 0.22 µm membrane filters] was prepared and 300 mL was filtered to sterile flasks through 0.22 µm membrane filter (Millipore). About 300 µL (1% v/v) of the culture was inoculated in the filtered media and incubated in water bath at 100 rpm/min maintaining the temperature at 30.

Initial sample was collected, and after in every 2 hr the culture medium was taken out and the supernatant was used to see specific growth rate and determine the respective ions concentrations in the synthetic pond water up to the entry of stationary phase, when no change in optical density of cell biomass was observed. The rate of increase or decrease in ion concentrations (ammonia, nitrate, nitrite and, phosphorus) by each *Bacillus* sp. in synthetic pond water was determined by using the standard curve methods mentioned above and calculated using the straight-line curve equation. Data Analysis: The data of the experiments were represented in terms of concentration of ammonia (NH4-N), nitrate (NO3-N), nitrite (NO2-N) and phosphorous (PO4-P) in mg/L verses incubation time. The growth (biomass production) optical density was presented in tertiary axis in relation to hrs of incubation for all the 16 *Bacillus* sp. isolates.

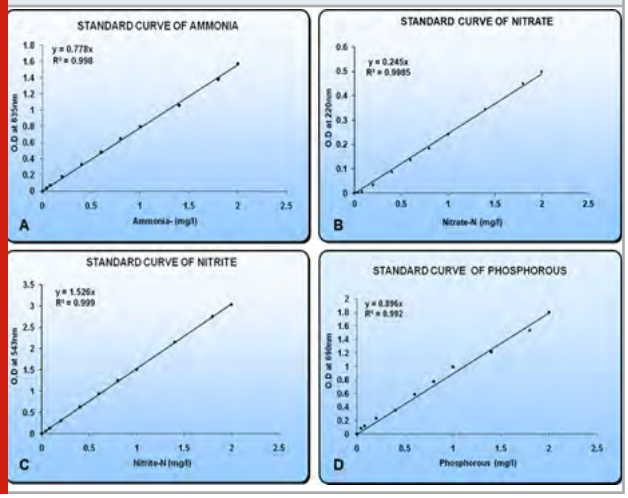
RESULTS AND DISCUSSION

Microbial culture and propagation: All the procured culture isolates were observed to be Gram positive, rod shaped either in single or in a chain as observed microscopically. All are able to grow aerobically and showed endospore formation. The growth propagation showed luxurious growth in TSB media, which are further used for nutrient utilization assay in synthetic pond water.

Standard curve preparation: The standard curve for NH4+-N, NO_3-N, NO_2-N and PO_4-P prepared were depicted in Fig. 1 [A], [B], [C] and [D] respectively. All

the graphs showed R2 value of more than 0.99, showing a potential procedure of preparation of standard curve that can be applied for estimation these water quality parameters. Standard curve of NH4+-N showed y=0.778x (Fig. 1A), NO3-N showed 0.245x (Fig. 1B), NO2-N showed y=1.526x (Fig. 1C) and PO4-P showed y=0.896x (Fig. 1D) respectively. The amount of these nutrients present in the synthetic pond water were after incubation of *Bacillus* spp. isolates were estimated using the graph equations.

Figure 1: Photograph showing different standard curves for Ammonia nitrogen (mg/L) [A], Nitrate nitrogen (mg/L) [B], Nitrite nitrogen (mg/L) [C] and Phosphorous (mg/L) respectively.



Nutrient cycling parameters: The nutrient cycling parameters of NH4+-N, NO_3-N, NO_2-N and PO4-P with respect to incubation period were depicted in Fig. 2[A], [B], [C] and [D]; Fig. 3[A], [B], [C] and [D]; Fig. 4[A], [B], [C] and [D]; fig. 5[A], [B], [C] and [D] for all the 16 *Bacillus* sp. isolates. The results showed that all the isolates attended stationary phase after 14 hrs of growth in synthetic pond water as observed in plateau in biomass production curve with the supplemented 1% inoculum presented in X axis of all the figures. All the isolates were observed to be ammonifiers as increase in NH4+-N, was found after incubation of 8 hrs in most of the isolates. Highest ammonia concentration up to 50% level was observed with RJ15 and remaining isolates also increased ammonium ion concentration with the lowest range producer was RJ3 with 4%. All the strains showed nitrate reduction activity. Strain RJ12 reduced nitrate (NO3-N) concentration (30%) from 12.04 to 8.37 mg/L whereas RJ5 was the lowest nitrate reducer with activity of 2%. Six of all the strains showed reduction in nitrite concentration including RJ1 and RJ5 where the highest nitrite reduction with 38% activity was observed. All other isolates showed increased in nitrite concentration and highest was observed with RJ11 (47%).

This study was supported with the findings of Lalloo et al. (2007, but a number of studies stated that B. amyloliquefaciens (Xie et al., 2013); B. subtilis (Cha et al., 2013; Zokaeifar et al., 2014) and B. megaterium (Hura et al., 2018), to reduce ammonia level in aquaculture practices. A study in carp rearing water after the addition of *Bacillus* sp. total ammonia nitrogen compared to the control was found to be reduced although nitrate level as found to increased (Naderi Samani et al., 2016). In connection to these findings Reddy et al. (2018) also recorded decreased ammonia nitrogen and illustrates that *Bacillus* spp. have the capacity to mineralize nitrogenous wastes through nitrification and denitrification (Nimrat et al., 2012).

Figure 2: Nutrient utilization pattern for ammonia, nitrate, nitrite and phosphorous of the isolates RJ1, RJ2, RJ3 and RJ4 in synthetic pond water depicted in A, B, C and D respectively.

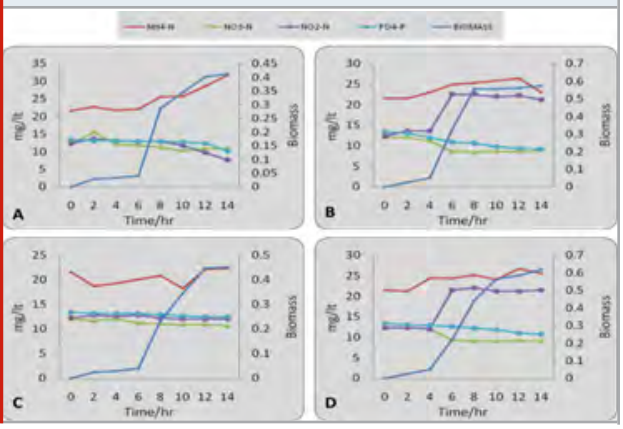
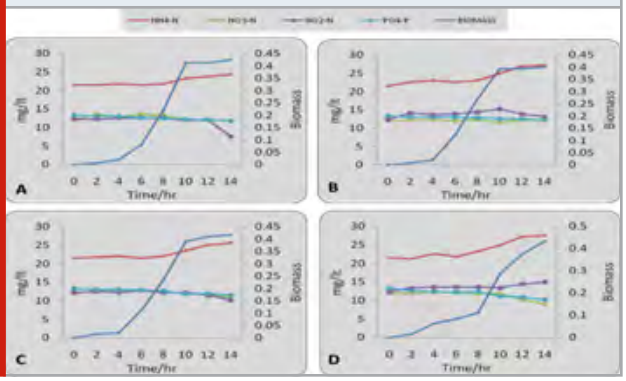


Figure 3: Nutrient utilization pattern for ammonia, nitrate, nitrite and phosphorous of the isolates RJ5, RJ6, RJ7 and RJ8 in synthetic pond water depicted in A, B, C and D respectively.



Although we have incorporated all the nutrients so for nitrification and denitrification the directly the NO3-N, NO2-N were consumed not the NH4-N. Thurlow et al. (2019), observed reduced nitrate-nitrogen (75%) and total nitrogen (43%) in catfish pond water treated with *Bacillus* velezensis AP193. A long-term incubation may result to

further demineralization as observed in our study. But mostly *Bacillus* spp. are recommended as ammonifiers by its taxonomic classification and also there may be a strain variation as the reported experiments are not for the same bacterial species used as in our study.

Figure 4: Nutrient utilization pattern for ammonia, nitrate, nitrite and phosphorous of the isolates RJ9, RJ10, RJ11 and RJ12 in synthetic pond water depicted in A, B, C and D respectively.

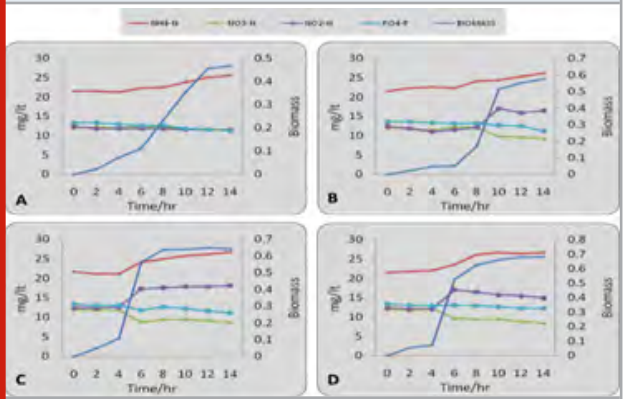
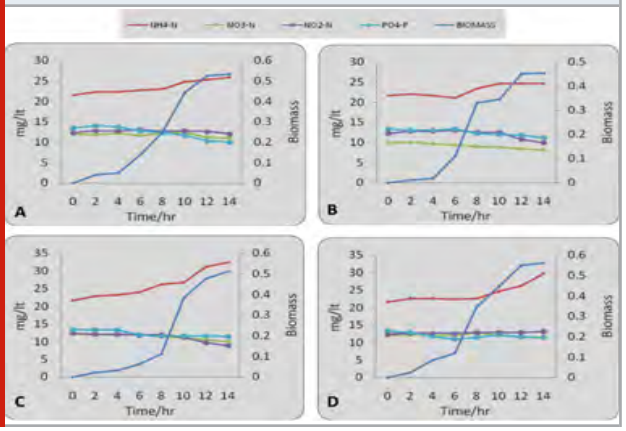


Figure 5: Nutrient utilization pattern for ammonia, nitrate, nitrite and phosphorous of the isolates RJ13, RJ14, RJ15 and RJ16 in synthetic pond water depicted in A, B, C and D respectively.



In phosphorus metabolism RJ2 was found to be utilize highest phosphorus in synthetic pond water with 32% decrease in phosphorus concentration whereas most of the strains showed no change in the activity. Like nitrate accumulation phosphate also leads to algal bloom in fish culture systems (Lalloo et al., 2007) as required for biological processes. The increase in phosphate concentration leads to eutrophic water (Luo et al., 2016; Reddy et al., 2018) and Bacillus spp. have been observed as potential phosphate solubilizers. In many studies it has been observed that *Bacillus* spp. can reduce phosphate ions and 81% reduction was reported (Reddy et al., 2018) in a study having equal proportions of *B. subtilis, B. mojavensis* and, *B. cereus*.

Also, it was evidenced in the presence of pathogens also (Lalloo et al., 2007) In a pond treated with *Bacillus* spp. decreased phosphorus levels was found as compared to control of shrimp culture ponds (Wang et al., 2005). Total phosphorus reduction was also documented in catfish ponds treated with B. velezensis (Thurlow et al., 2019). The finding of this study proved that *Bacillus* spp. are ammonifiers, denitrifies and have the ability to mineralize phosphorous, however *in vivo* study in aquaculture ponds are necessary to draw final conclusion.

Conflict of Interests: We declare that no competing interests exist among the authors of this article.

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The Role of Allium sativum Extract in Treating *Aeromonas* Infection of *Labeo rohita*

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ABSTRACT

The *Aeromonas* species of gram negative bacteria is a motile, pathogenic species which is generally seen in the various bacterial diseased fishes that are known to host various fresh and brackish water fish species that are generally seen in abundance globally and are generally found in cold to temperate zone regions and seen to be cultured in the warm water fish. The content of interest in this study is to analyze the inhibitory properties of the phytochemical extract of the *Allium sativum* against the *Aeromonas* species of diseased host fish *Labeo rohita*. Extracts of above that are sensitive to *Aeromonas* are taken and minimum inhibitory concentrations (MIC) of the extracts were added and analyzed by various qualitative methods. The results and data can then be analyzed statistically using ANOVA and the phytochemical screening shall be done using the standard procedures of estimation. Extracts of aforementioned phytochemicals are seen to possess the potential as therapy against *Aeromonas* which causes Aeromoniasis of Rohu that are having an opportunistic and global status and whose cure and inhibitory effect by the plants natural defense system over Aeromonas shall show a great advantage for various aqua culturists in better progress and production in its part as Rohu being a freshwater fish is a food for many. In this assessment, the vital organic functions of *Allium sativum* L. are briefly highlighted with supportive working mechanisms. Further, the mechanism is then discussed how it is seen to be applicable on the treatment of Aeromonas causal diseases of *Labeo rohita*.

KEY WORDS: AEROMONAS, ROHU, PHYTOCHEMICALS, AQUA CULTURISTS, OMEGA-3-FATTY ACIDS.

INTRODUCTION

The development of the population has led to rapid growth that has led to competition in every sphere of life i.e.; more land, more resources of everything, more food etc. So, the fittest is always considered to thrive in this competitive world and thus, for the present scenario there is an increasing demand for the basic materials like the food and the food products and where in one sphere is the aquatic ecosystem is coming into great demand thus, the seafood is acquiring its popularity too. Freshwater is seen to add up a larger amount of its production day by day in the diet conscious and developing scenario of the world food production both in variety as well as biomass. The freshwater or the anadromous fish is seen to be a better option as compared to the meat as it has a lesser lipid content and more unsaturated fat and rich source of omega -3-fatty acids and aids better digestion due to the presence of various digestible protein in it. The major producers of aquaculture species are identified as Asia, Europe and Africa (Mazumdar et al., 2015).

ARTICLE INFORMATION

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Rohu (*Labeo rohita*) a major Indian carp is one of the most commercially significant species in this group of freshwater species and also a very popular cuisine ingredient in the Indian communities (Mazumdar et al., 2015). But the various studies have shown that the Rohu commercialization in the aquaculture sector is having a persistent threat because of various pathogens like the *Aeromonas* species. The bacterial *Aeromonas* species which are the rod shaped bacterial pathogen that have been generally identified as a potential pathogen in causing infections not only in the fishes but also in various amphibians , reptiles but also affect the humans as well as the former organisms act as a carrier of that infection. The infectious pathogen.

Aeromonas is generally isolated from the surface waters, estuarine water, gills , eggs, freshwater, ventral muscles and stomach of fresh water prawns(Rahim, & Aziz, 1994), various consumable products ,in the diseased or healthy fish,excretory products of hominids and animals, thus they have concluded by various studies to be omnipresent in the aquatic ecosystems(Dar et al., 2016).Further they are also cultured in an environment where warm water fish thrive so , they are generally seen to be everywhere as mentioned before too(Chopra et al., 1993) (Nordmann & Poirel , 2002). Further the *Aeromonas* is of no commercial advantage as various studies indicate it as a "Developing foodborne pathogen" as at ambient temperature and ubiquitous nature gives it a great boost in it developing as an active spoiler of fish and meat. The conditions if are favorable too also as they enhance the chances of better disease development by these species in the fish due to the stress situations like overpopulation(because of the method like polyculture of fish) , rough handling , cross contamination , meagre water quality thus leading it to cause major epidemic outbreaks and causes various biological hazards.

Over several years it has been seen through global studies that the seafood is an important foundation of various foodborne outbreaks so, if this foundation is tackled smartly then it won't be able to spoil the environment or cause any detrimental changes in the aquatic ecosystem. According to a study it has been reported that the European Union (EU), in 2015 had many foodborne outbreaks of which 10% has been seen to be linked to the seafood. Moreover the disease is not coming into light due to the various unreported cases (Auth, 2016). Today upto this present time also the awareness on the growth and pathogenic prospective properties of the *Aeromonas* species in the seafood and seafood products that are a major food to be ingested and projected for raw consumption is very limited. Insufficient studies are concentrated w.r.t to the presence of this pathogen in lightly processed Ready-To-Eat (RTE) seafood products such as sushi (Atanassova et al., 2008) (Pinto et al., 2012) (Hoel et al., 2015), cold smoked salmon (Hudson & Mott, 1993) and molluscan shellfish (Silva et al., 2018).

Thus, the dangerous pathogen can be controlled using various plant resources one of which to be studied here is the *Allium sativum* ordinarily named as the garlic i.e. is seen to have a greater application on the fish culture and helps in stimulating the defence system activity as well in a progressive way and it is one such traditional power packed herb that has the antimicrobial activity (Liza et.al, 2018). The various phytochemicals seen in the extraction are the flavonoids, phenolic, saponin, alkaloids and tannins that are seen to show better results in antimicrobial activity through various studies and will help to inhibit the growth of *Aeromonas* bacterial growth in the Rohu fish (Lekshmi et al., 2015). As Rohu is a majorly available fish in Northern as well as Central India and as well its meat is mostly consumed as it is plentifully filled with the vitamins like A(a group of fat-soluble retinoids),B and C(ascorbic acid) and omega -3-fatty acids whose better management against this disease shall give better health benefits to consumer too.

This study here aims to summarize the status of *Aeromonas* as a probable foodborne pathogen, and to deliberate the importance of *Allium sativum* in the enhancement of defence system of Rohu and thus, inhibits the bacterial growth and thus help in the better commercial benefits in the field of aquaculture by use of completely natural, traditional herb with least side effects and provide benefit as a whole thus providing welfare of human, and protect the environment and aqua species as well thus showing largely, the garlic as a tremendous regularly ingestion food source as it's a power pack ingredient possessing multidiverse bioactive sulfuric compounds and that has encouraging claims in the advancement of this as a great functional foods or nutraceuticals for the better management of the fish diseases like Aeromoniaisis, Epizootic ulcerative syndrome, fatal septicaemia, ascitis , ulcerations etc., so that this natural ingredient being a traditional herb can be given inputs as a nutritional ingredient to save the aquatic beings and the organisms depending on that too.

2. Rohu (*Labeo rohita*)

2.1 Scientific name: *Labeo rohita* (Hamilton, 1822)

2.2 Taxonomic Classification

Phylum	Vertebrata
Subphylum	Craniata
Superclass	Gnathostoma
Series	Pisces
Class	Teleostomi
Subclass	Actinopterygii
Order	Cypriniformes
Division	Cyprini
Suborder	Cyprinoidei
Family	Cyprinidae
Subfamily	Cyprinini
Genus	*Labeo*
Species	*rohita*

2.3 Morphological characteristics

2.3.1 Scales

- Moderate in size.

- Found along the lateral line with the presence of around 40 to 42 scales.
- Scales are seen to be arranged in a transverse manner from dorsal fin origin to the base of ventral.
- Around the predorsal scales 14-17 scales are found and along the caudal fin there are 20 scales

2.3.2 Barbels

- Concealed in the lateral grooves are a pair of short, thin maxillary barbles.

2.3.3 Measurements

- The fish has a head of about 4.5 to 5, depth of 4 to 4.7 in length.
- Anterior half of head has the eye located in it
- Obtuse snout devoid of labial folds with an inferior mouth and distant inner fold to each lip.

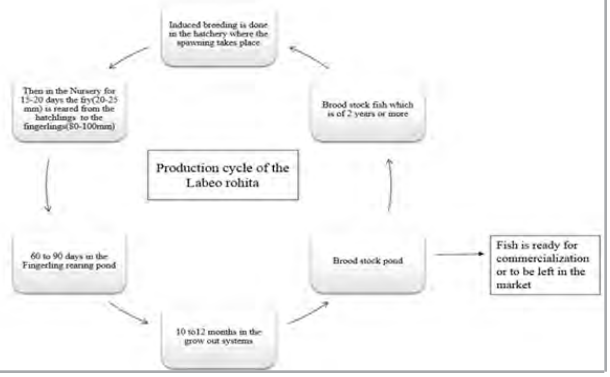
2.3.4 Colouration

- Back is observed to be bluish with sides and beneath having a silvery colour .
- Breeding season brings in a rod shaped marking on each scale with black and greyish fins.
- Body colour is changeable according to its habitat i.e. greenish black back observed in the fishes living among the weeds
- Reddish eyes are studied.

2.3.5 The production and harvesting of Rohu fish

- When quite small they are generally cultured in the aqueous environment in pond and tanks.
- A type of technique called repeated netting is seen at the end of the fish culture period.
- Almost all the seed required for the culturing of fish is done through induced breeding of Rohu.
- Hypophysation is a common practice done here since 1957, several synthetic commercial formulations.
- For seed production a common hatchery that's used here is a circular one constituting of the spawning or the breeding tank, hatching or the incubation reservoir and further stored water and supply system is done before hand.
- The weight ratio of the female is to male ratio is normally kept at 1:1 and 1:2 by number.
- For next 15 to 20 days a nursery stage is done for raising fry, followed by a two to three months phase for fingerling development.
- Then the fingerlings undergo rearing phase where the 6mm three day old hatchlings, , are reared to become a fry of 20-25 mm in small earthen nursery ponds of 0.02-0.1 ha.
- The nursery-raised fry of 20-25 mm are further reared for two-three months to 80-100 mm (6-10 g) fingerlings in earthen ponds of 0.05-0.2 ha.
- Around 60 to 70 percent fingerlings are seen to survive and then the Rohu is grown in such a way and then followed by harvesting.
- Several stocking in addition to harvesting procedures are practised, the size is seen and the harvesting of larger sizes (300 - 500 g) is commonly begun after six-seven months of culture, and the smaller ones are sent back to the pond for better progressive size attatinment.

Figure 1: Production Cycle of Rohu



2.3.6 Handling and processing: Among the major Indian cultivated carps Rohu is seen to be a significant species. Even these species are found to be large commercialised and fresh water species which are further cleaned by water thoroughly, and packed along with crumpled ice pieces in 1:1 ratio inside rectangular plastic crates (frequently of 60 cm x 40 cm x 23 cm size). Packed fish with ice are sent to longer distances in proper shielded vans throughout India around 3000 km by land transport of these species. Some non-existent and value-added fortification method are still non-existent in such high marketing countries of this species of Rohu.

2.3.7 Production costs: Rohu being a low valued species is always tried to give minimum input costs and a minimum of the supplementary feed, seed , fertilizers are tried to be utilised with maximum overturn.

3. The biology of Aeromonas species: The *Aeromonas* is generic name which comes under one of the five genera of the family of Aeromonadaceae., order Aeromondales ,class Gammaproteobacteria, Phylum Proteobacteria and thus belongs to the Kingdom Bacteria which was once considered as part of the family Vibrionaceae, which has two genera, namely, Vibrio and *Aeromonas* (Inglis et al., 1993). It is found to be gram-negative autochthonous aquatic bacilli, which are oxidase-catalase-positive and facultative anaerobe. Generally the members of this genus are categorised into 2 major groups ,one of which is immotile, fish-infecting, psychrophilic immotile strains that comprises of *Aeromonas* salmonicida.

The extra larger group comprises of larger, motile, mesophilic *Aeromonas* species, and generally infect the humans (Martin et al., 2005 , Martino et al., 2014 ,Pang et al., 2015).Reclassification of this species is going on as described above. The 2005 type of the Bergey's Manual of Systematic Bacteriology, described that there were around 14 species under this genus and formerly known as the Enteric group 501, then later on categorized as *Aeromonas* diversa (Martin et al., 2005 , Minana et al., 2010) Old classification was differentiated based on the 16S rRNA of the and DNA-hybridization groups (Coloston et al., 2018). So far, *Aeromonas* are having 36 species described since 1943 - out of which 19 are

detrimental and human pathogens (Hoel et al., 2019). The various species are listed below in the underlying table1.

Incidence of Aeromonas diseases in Rohu: In these recent years of detailed fish study the aquaculture zone where the various fishes, shrimps, humans, etc. are being affected due to the various pathogens of which a dangerous non-commercial pathogen of the *Aeromonas* spp. is causing a persistent hazard due to this fish pathogen. According to a study done by Gowhar et al., 2016, various biochemical studies are a crucial part in the management of various fish pathogens like of the *Aeromonas* spp. As we know the pathogens which are disease causal agents are generally saprophytic in nature and they evolve into pathogenic species only if there is certain negligent practices, higher pollution levels, unbalanced physiological conditions, nutritional deficiency or other stress enhancers like the non-optimum water quality, congestion and various anthropogenic activities which are great opportunities to trigger the *Aeromonas* infections in fish in the field of aquaculture (Mishra et al., 2017).

Figure 2: Phytochemicals in Garlic (Bautista et al., 2005)

Name of the species	References
Aeromonas allosaccharophila *Aeromonas aquatica* *Aeromonas finlandiensis*	Martinez et.al,1992 Beaz et.al,2015
Aeromonas aquatilis, *Aeromonas crassostreae,* *Aeromonas enterica, Aeromonas*	Figueras et.al,2017
Aeromonas australiensis *Aeromonas bestiarum*	Roman et.al,2013 Ali et.al,1996
Aeromonas bivalvium	Galbis et.al,2007
Aeromonas cavernicola	Martinez-Murcia et.al,2013
A. caviae , Aeromonas eucrenophila	Schubert et.al,1988
A. dhakensis	Beaz-Hidalgo et.al, Huys et.al, 2002 Martinez-Murcia et.al, 2008,2013
Aeromonas diversa	Miñana-Galbis et.al,2010
Aeromonas encheleia	Esteve et.al,1995
Aeromonas fluvialis	Alperi et.al,2010
A. hydrophila	Stanier et.al,1943
Aeromonas jandaei, Aeromonas trota	Carnahan et.al,1991
Aeromonas media	Allen et.al,1983
Aeromonas molluscorum	Minana Galbis et.al,2004
Aeromonas lacus, Aeromonas lusitana	Martínez-Murcia et.al,2016
Aeromonas piscicola	Beaz-Hidalgo et.al,2009
Aeromonas popoffii	Huys et.al,1997
Aeromonas rivipollensis	Marti et.al,2015
Aeromonas rivuli	Griffin et.al,1953
A. salmonicida	Griffin et.al,1953
Aeromonas sanarellii	Alperi et.al,2008
Aeromonas schubertii	Hickman-Brenner et.al,1988
Aeromonas simiae	Harf-Monteil et.al,2004
Aeromonas sobria	Popoff et.al,1981
Aeromonas taiwanensis, *Aeromonas tecta*	Demarta et.al,2008
A.veronii	Hickman-Brenner et.al,1987

The various parameter based studies done by Gowhar et al., (2016) in the pond environment further explored during the present study was thus showed that the field of aquaculture is prone to a variety of diseases due to inapt farm management systems, and further the various fish species mostly the study they did was *Labeo rohita* showed greater susceptibility of fish to get attacked by various pathogenic infections is enhanced (Sayed, 2006) and variety of movable *Aeromonas* Spp. has been thus detected in the aquatic fish and its habitat (Kaper et al.,

1981; Carlos et al., 1986; Hatha et al.,2005). Various human activities leads to the spread of thus pathogen from one organism to other similarly such kind of scattering when seen in the pathogenic organism like A. sobria by excreta can completely disturb the entire pond ecosystem and be carried through food chain to various organisms and thus reach the humans thus leading to a mechanism of plasmid interchange between the bacterial species that freely then keeps on spreading and facilitating its strains elsewhere and can thus result in a higher occurrence of numerous antibiotic resilient strains and the development of this fish disease in the fish (Chang & Bolton , 1987).

Medicated supplements to treat this cycle further helps the pathogen of *Aeromonas* to spread and further lead to an outbreak in the waterbody by the further production of various virulent and resistant strain types(Gowhar et. al.,2016) (Redmayne,1989). *Aeromonas* species leads to septicaemia with extensive skin lesions, dermal oedema , musculature disintegration is seen and worsening of the internal organs like liver, spleen and muscles. The visible symptoms of the diseased fish include lethargic movement accompanied with pale red gills, blackening at the scraped areas. Tissue sections further revealed focal haemorrhage and various necrotic changes in the haematopoietic tissue of the liver, glomeruli and renal tubules in kidney, hyperplasia of gill lamellae, swelled intestinal mucosal epithelium and mild deteriorating changes of myocardium which are generally observed by the fish affected by the *A. hydrophila* type (Manoj et al., 2010). According to the studies done by Gowhar et al., 2016 the results they obtained from the Vitek database directed the probabilities of various species of *Aeromonas* as of identification of then they have concluded to have found about 95 to 99 percent of A. veronii and a percentage of 69 to 83 percent of the species of *A. hydrophila*, *A. caviae* and *A. sorbia* (Cai et al.,2012) and about 93 percent of the A. sobria (Dar et al., 2016).

4.1 Isolation and Identification of Aeromonas: From a study done by Fricker and Tompset out of 563 fish and meat samples were obtained from retail outlets and investigated and concluded the isolation of mesophilic *Aeromonas* species from around 287 samples .The symptoms of *Aeromonas* disease affected fish species were observed by them and were further isolated from gills, eggs, stomach and ventral muscles of fresh water prawns available in Bangladesh (Zeaur & Aziz, 1994). A study on food mock-ups showed a 10.47% percent positive results for aeromonads. Out of 99 fish samples 22 (22.22%) were found positive, in which *A. hydrophila* (66.6%), A. sobria (27.27%), A. caviae (9.09%) were found positive (Leitao et al., 1991).

Further experimental studies done by Gowhar et al., showed that the characterisation of being *Aeromonas* species is primarily done by seeing the various symptoms that this *Aeromonas* bacterium shows on the *Labeo rohita* then they isolated the fish pathogenic bacteria by using a culture dependent approach by using a spread plating technique .Later on they swabbed the fish surface for bacteria segregation, and then the inoculums were spread over the agar rich nutrient medium (Dar et al.,2016, Jingram 2007 ,Tilak 1987, Noor et al.,2014 ,Austin & Austin,2012) and kept it at 25°C-30°C incubation for a duration of 2-3 days (Spanggard et al.,2000; Harbi & Uddin 2004 ,2005). Further they isolated and purified the stocks and further the morphological and biochemical characteristics analysis done by them showed the *Aeromonas* species on the nutrient agar plate. In this way they are detected over the fish species as they did in the case of *Labeo rohita*.

4.2 Treatment of Aeromonas diseases in Labeo rohita: Aeromoniasis, a disease cause by *Aeromonas* species is a gram negative, facultative anaerobe or food borne pathogen among the aquatic species that has caused great menace and havoc among the aquatic ecosystem. No epidemiological evidences are actually in availability so its very difficult to treat or completely eliminate the once culture facilities and/or aquatic species that are infected (Chauhan, 2014; Angahar, 2016; El-deen et al., 2018). Aeromoniasis or motile aeromonas septicaemia disease, tail rot and fin rot, eye and ulcer diseases in fish hatchery can be effectively controlled by removal of infested eggs from the troughs or incubation gutter. Prevention is the better method of controlling this disease.

Therefore, the diseases caused by the *Aeromonas* can be best prevented by good aquaculture management practices such as:

- Ulcers could be prevented by removal or complete terminalisation of the diseased fish, also the ponds should be sterilised with a solution of potassium permanganate at 0.5 parts per million ,and supplemented feed of sulphadiazine at 100mg/kg or terramycin 75-80 mg/kg could be given for a period of 10-12 days.
- Dropsy could be prevented by sterilizing the pond with the potassium permanganate solution of quantity 1 parts per million and potassium permanganate bath treatment of 5 ppm for a time interval if 2 minutes.
- Epizootic ulcerative syndrome could be prevented by the lime usage of quantity of 200kg/ha and application of 0.1 ppm of CIFA ,India product called CIFAX .
- Proper pond sanitation is the key to prevent such pathogens growth.
- Motile *Aeromonas* Septicaemia can be treated by using medicated ration containing 2 to 4 g of oxytetracycline/Kg of feed per day for 10 days.
- Experimental vaccination for prophylaxis against infection of *A. hydrophila* has against infection of *A. hydrophila* has been examined.
- Fish immunized either intramuscularly or intraperitoneally with vaccine showed protection against challenge. The agglutinating antibody titter increased in the serum of immunized fish but no commercial vaccine has been developed.

4.2.1 The application of antibiotic and chemotherapy in the management of Aeromonas: As the world is

progressing there is a greater demand for modernised methods where the application of antibiotics and chemotherapy is in demand for its effective property to deal with various types of fish disease's treatment and its prophylaxis .The valuable inputs of antibiotics and chemotherapy is due to its faster results that stands as a greater advantage of this type. But the major obstruction is its environmental threats and the development of antibiotic resistance where the specific *Aeromonas* species has learnt the skills to defeat the drugs designed against them. As the controlling of the *Aeromonas* species in aquaculture observed that *A. hydrophila* isolates from contaminated aquatic species were not sensitive to chloramphenicol. Further changes in the outer cell membrane and extrinsic proteins is observed in the *Aeromonas* salmonicida due to the opposite reaction of quinolones and tetracycline on it and with numerous attempts of resistance of it towards the pathogen (Barnes et al., 1990; Wood et al., 1986). The extensive use of antibiotics against bacterial pathogen thus, helps in the development of resistance by helping them knowing the nature of drugs.

Earlier when multi-resilient *A. hydrophila* were seen to be resistant to antibiotics like penicillin and ampicillin, but sensitive towards chemical agents like aminoglycosides, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, quinolones, and second- and third-generation cephalosporin but the frequent usage of these agents leads the pathogen to develop better resistance globally (Igbinosa et al., 2012; Vivekanandhan et al., 2002). In a study done by Parker and Shaw (Parker and Shaw, 2011) many virulent factors like hemolysins, aerolysins, proteases, adhesins, enterotoxins, phospholipase and lipase helped in developing the resistance. As a study done by Stratev and Odeyemi (2016), a table below has described how the *Aeromonas* species in *Labeo rohita* is seen to have developed the resistance against the following antibiotics as described in Table 2.

Table 2. Antibacterial agents against Aeromonas

Anti-bacterial agent against Aeromonas spp.	Resilient strains in % o btained from Labeo rohita
Amoxicillin	52.7
Ampicillin	100
Chloramphenicol	5.5
Nalidixic acid	1.8
Novobiocin	94.5
Oxytetracycline	40
Polymyxin-B	41.8

So, whatever ways as explained the use of antibiotic or chemotherapy or chemical agents like potassium permanganate ,etc uses in spite of its safety may be limited in its applicability due to the high cost of acquiring effective concentration of which is as high as 30,000 mg/L (Hashemi et al., 2012).Many rules and regulations are to be followed with the usage of such agents mostly to be taken care by the aqua culturists and in most cases users often misuse or overuse the required dosage which may cause the target pathogen to develop resistance to the chemical antibiotic applied. Chemicals being in addition, these chemicals are theoretically seen to be hazardous to the users, the fish, the consumers and ultimately the environment and leading to a complete imbalance of the complete ecological balance .Moreover these dangers calls for an urgent steps for the safety of consumers of such fish and our dear environment as the world is on its path of destruction due to various anthropological behaviours.

5. Alternative Treatment of Aeromonas Bacterial Diseases of Labeo rohita by phytochemicals of Garlic (Allium sativum): The plants and the plant products are considered to be the best alternative therapy in the treatment of the various diseases in the aquaculture industry due to the increasing drug filtrate deposition and development of new resistant pathogens in the treated fish due to application of various antibiotics and chemical therapies in the treatment of bacterial diseases and also pose a greater health hazard to the humans and other animals who come in contact with the diseased fish. It is imperious that an alternate treatment for *Aeromonas* is to be industrialized, such that they are an ecologically safe, effective and economically feasible technique of disease management. Many peasant local fish sellers and poor farmers are not able to afford such costly and high paid antibiotics or drugs so, the use of medicinal plants or herbs is moreover, a better option as it will increase the period of production which will ultimately increase the production cost. So, plants being a relatively, non-toxic option, and the fish can be sold and treated at any time and thus, are found to be very safe to be consumed at any time (Ilondu et al., 2009). Other advantages of medicinal plants are plant materials are inexhaustible, harmless, readily available, decomposable and can be treated as food for fishes.

One such anti-phiogistical, seasoning herb is the Garlic (*Allium sativum*) which is the pleasantly available highly pungent smelling natural antibiotic against the bacterial *Aeromonas* spp. in *Labeo rohita*. Garlic (*Allium sativum* L.) is a extensively consumed spice all over the world which is a rich source of various diverse bioactive phytochemicals compounds, such as allicin, alliin, diallyl sulfide, diallyl disulfide, diallyl trisulfide, ajoene, and S-allyl-cysteine. Significant research done on the garlic shows that they are a power pack of bioactive ingredients that are seen to reveal various cure against the most dangerous comorbidities as it acts a great antioxidant, anti-inflammatory agent , anti-bactericidal, anti-fungal, cardiovascular, immune-stimulating , anticancer, Hepatoprotective, gastro protective, anti-diabetic, anti-obese, neuroprotective, and renal protective possessions.

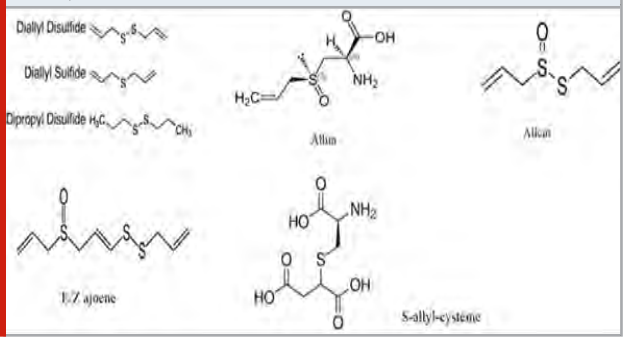
It is powerful ingredient with various organic sulphides, saponin, phenolic complexes, and polysaccharides

(Bose et al., 2014, Diretto et al., 2017, Sahi & Bansal, 2020).It is even a known traditional medicine in China (Jacob & Narendhirakannan, 2019). In comparison to the fresh garlic ,the black garlic type is being greatly focused as they have, higher level of polyphenol and flavonoid content, in addition to restored antioxidant properties(Kimura et al.,2017).

Table 5.1. Systematic classification of *Allium sativum* L. (Garlic)

Kingdom	Plantae
Sub-kingdom	Tracheobionta
Super-division	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Lilidae
Order	Liliales
Family	Liliaceae
Genus	*Allium*
Species	*sativum*

Figure 2: Phytochemicals in Garlic (Bautista et al., 2005)



5.2. Important bioactive compounds or phytochemicals of Garlic (Allium sativum): Diallyl thiosulfonate (allicin), , diallyl disulfide (DADS),dipropyl sulphide , E/Z-ajoene, S-allyl-cysteine (SAC), diallyl sulphide (DAS) , diallyl trisulfide (DATS)and S-allyl-cysteine sulfoxide (alliin) are the major organosulfur compounds found in garlic (Yoo et al.,2014 , Kodera et al.,2017 , Yoo et al.,2014 , Mansingh et al.,2018).

5.3. Biochemistry of Garlic (Allium sativum) showing the presence of various chemicals: Furthermore, analysis 85 percent fructose, 14 percent glucose, and 1percent galactose were found in garlic. It was known through many research studies that normal raw garlic when given thermal treatment changed to black garlic (Liang et al., 2015). Further thermal processing of black garlic further reduced the sugar levels in it. Also, higher temperature and lower humidity levels enhanced the contents of polyphenols and the total flavonoids in black garlic (Kim et al., 2013). Various studies also suggest that the garlic chemical analysis also suggest that around contains thirty three sulfur compounds, numerous enzymes,seventeen amino acids, and minerals like selenium. The pungent smell and the medicinal effects of garlic is specially due to S-compounds present in it.

One of the most important compounds,is found to be allicin (diallylthiosulfinate or diallyl disulfide) which is obtained only when garlic is crushed ; as an injury to the garlic bulb activates the enzyme alliinase, which metabolizes alliin to allicin. Allicin is later broken down to vinyl thinner which occurs within hours at room temperature and within minutes during cooking. Allicin, which was first chemically isolated in the 1940's, has antimicrobial effects against many viruses, bacteria, fungi and parasites. Garlic oil, aged garlic and steam-distilled garlic doesn't have substantial amounts of aliin or allicin, but instead contain various products of Allicin alteration; not a bit appears to have as much physiologic action as fresh garlic or garlic powder (Londhe et al., 2011).

5. 5. Role of Allium sativum in treating Aeromonas infection of Labeo rohita: Garlic has a wide-ranging variety of anti-bacterial benefits as the garlic is generally seen to have natural antibiotic property and is one of the effective natural immunostimulants ,has antioxidant properties (Rahman,2003) It generally, stimulates the phagocytic cells and proliferates the bactericidal activities and stimulates the natural killer cells, complement, lysozyme, and the antibody responses of fish. The stimulation of these immunity of fish is related with increased protection against infectious disease in fish. As studies made by Lau, 1991 the macrophages accelerate phagocytosis under the influence of garlic. The studies made by Martins et al. when fish diets were supplemented with garlic augmented the RBCs number, hemoglobin level, hematocrit, WBCs number, and thrombocyte number. Studies made Nya suggest that garlic supplementation induced significant changes in serum total protein and globulin in rainbow trout (Nya et al., 2009).

Innate immunity , the serum total protein, albumin, and globulin contents also is seen to increase (Jha et al.,2007). Thus , when increased protection against an immediate challenge with *A. hydrophila*, showing the protective properties of garlic and thus, this property of rainbow trout done can be also used to prevent the *Aeromonas* causal infection in *Labeo rohita*. Studies done by Cavallito and Bailey (1944) showed that the antibacterial properties of crushed garlic could be accredited mainly to allicin component of garlic. Studies done by Diab, Nya and Austin (2009) showed the garlic as a best anti-bacterial agent against *A. hydrophila* in freshwater where they had testified that the usage of garlic-complemented diets for 14 days directed to a marked reduction in death with *A. hydrophila* in fishes. 4 percent mortalities were only seen in groups fed 0.5 and 1percent garlic-mixed feed compared to 88 percent mortality in the control group (Nya & Austin,2009).

Table 5.4. Important biological activities of *Allium sativum* (Shang et. al, 2019)	
Antioxidant Activity	Phenols and saponin; raw garlic>> cooked garlic;fermented garlic like black garlic>> crude garlic; anti-oxidant enzymes like heme oxygenase-1 (HO-1), glutamate-cysteine ligase modifier (GCLM) subunit through the nuclear factor erythrobia-2 related factor 2 (Nrf2)-antioxidant response element (ARE) pathway, which sheltered human endothelial cells against oxidative stress.
Anti-Inflammatory Activity	Inflammatory mediators are inhibited by garlic usasge where the mediators could be nitric oxide, Tumour necrosis factor and (Interleukin) and also has a great potential to treat diseases interrelated to inflammation like the auto-immune disease arthritis as the toxic levels are low in this natural ingredient of garlic.
Antimicrobial Activity	Oil obtained from garlic was seen to be the main antimoneran ingredient that terminates the moneran cell wall structure by inhibiting its metabolic pathway.
Modulating Immune System	Polysaccharides of garlic are seen to promote the immunity as it normalizes the expressions of Interleukins(6,10), tumor necrosis factor-α, and interferon- γ in RAW garlic and also macrophages are secreted a lot.
Cardiovascular Protection	A great agent in the keeping our pumping organ fit is by helping in reduction of blood pressure levels, cholesterol levels, and other heart related problems stay at bay due to garlic consumption in the powdered form.
Anticancer Activity	Various cancers like the colorectal , urinary bladder and intestinal related are avoidable due to the Allium intake as it 1. Metabolic regulator of Cancer-causing Substances 2. Cell Growth and Proliferation are blocked by it 3. Inducing Apoptosis 4. Suppressing Angiogenesis 5. Inhibiting Invasion and Migration
Anti-Diabetic Activity	Repressed pancreatic cell wound, oxidative stress, and pathological changes in streptomycin-induced type 1 diabetic rats, were seen that helped in the management of Diabetes-type2.Thus, Allium components manages our sugar levels too thus, preventing the conditions like insulin resistance.
Anti-Obesity Activity	Anti-obese ingredient garlic is seen to act as cutter of a high-fat diet on the weight of body and breaksdown the fat depository adipose tissue in hyperlipidaemia rats. In addition, the oral administration of (Fermented lactic acid bacteria garlic buds) LAFGE reduced the weight of high-fat diet male C57BL/6J mice and reduced their epididymal, retroperitoneal, and mesenteric adipose tissue mass.

Various studies done by other researchers also suggest same results for this like suggested by (Sahu et al.,2007) that infectious *A. hydrophila* which affected the *Labeo rohita* fingerlings, showed that when the 0.1 and 0.5 percent garlic added groups showed the maximum level of survival (85%) as compared to the controller group (57%) .Study done by Aly and Mohamed (2010) also suggested that *O. niloticus* when fed a 3% garlic-supplemented feed showed a considerably better survival rate (85%) after a challenge with *A. hydrophila* . Zhang (2003) studies measured the inhibitory effects of garlic on two isolates of *A. hydrophila*, AH_1 and AH_1, in vitro and found that the minimum inhibitory concentrations (MICs) were 15.6 and 1.95 mg/ml respectively.

When Chen(1977) studied the 50 mg/diet of garlic on the infected Silurus soldatovi meridionalis ,AH_1 strain was seen to be under control but neither isolate was eliminated completely. Experiments done by Rahman et al. (2009) , evaluated the efficacies of antibiotics and medicinal plants on three common bacterial fish pathogens: *A. hydrophila*, P. fluorescens, and E. tarda found that 8 milligram per mL of garlic when given to the young Thai silver barb (*Barbonymus gonionotus*) showed the finest recovery rate of around (90 percent) during the 10-day trial period. A study done by Dash et al.,2014 suggested that the garlic when compiled with mineral oil, showed a modified adjuvant preparation is well proficient of augmenting defence in the *L. rohita* against *A. hydrophila* infection where the garlic was found to intensify the serum agglutinating antibody titer combined with a greater hemolysin movement of the serum and also increased the survival rate post-challenge thus, suggesting the garlic to be an excellent immunoadjuvant as well as its effect on stimulation of specific and nonspecific immune response stimulation , and seen to be a significant response in lesser dose of garlic might be the contribution of one compound or multiple compounds present in garlic.

CONCLUSION

Garlic a great traditional spice with a great power pack plant with various bioactive compounds and thus, has many beneficial activities being a great renal protector , cardiovascular protector, anti-obese , anti-inflammatory , etc. Therefore, garlic and its phytochemicals are encouraging as functional foods or in the field of nutraceuticals for the therapy of diverse diseases. In the studies to be suggested for future, a greater demand for better evaluation, extraction and separation of biological components of garlic for better performance in biological functions are suggested and to check its intake levels at a safety parameter and much more clinical experiments are to be paid attention to the ill effects/protection of garlic.

Thus, the above experimental set ups done by various renowned researchers showed great quality of work and how the *Aeromonas* can be inhibited but can't be totally eliminated from the infected *Labeo rohita* fish and thus, giving us a better aspect to work on so that the field of aquaculture which is facing such a great pressure due to these pathogens by the simplest , traditional ,healthiest , eco-friendly methods, that they are given more preference and opportunities to work on this garlic isolated phytochemicals to combat this infectious up to some extent at least naturally. However, to prove its complete mechanism and its adjuvant potential, advanced study with continuing trials and on detection of the most active compound of garlic is very essential for saving our aquatic nature and for out practical approach towards the protection of the environment of the harsh anthropogenic activities

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A Review on Recent Advances in Rearing of the Larval Parasitoid Bracon hebator (Say)

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ABSTRACT

Larval parasitoid, *Bracon hebator* (Say) is being utilized in various bio-control research, developmental and extension units for management of lepidopteran peast in their larval stages. *Larval parasitoid B. hebator* ranks first in the management of lepidopteran insects. Production of *B hebator* nutritional requirements have to be taken into account because nutritional deficiencies have been linked with such vague symptoms like poor growth rate, lowered fecundity or reduced body weight. As it is one of the most effective parval parasitoid for different lepidopeteran pests various dietary formulations were developed in the present study with differet combinations and the effects of these formulations on the growth, development, reproduction of *B hebator* were reviewed.

INTRODUCTION

With the increasing demand for environmental safety and global demand for pesticide free food An eco-friendly methods of pest management is highly essential. As a result of which, biological control method has achieved the new international trends, which helps in conservation and sustainable use of biological resources as compatible a manner as possible. *Bracon hebetor* (Say), the larval parasitoid is considered as one of the potential biological control agents against larval stage of lepidopteran pests. It is a gregarious ecto-parasitoid completing its larval stage on different species of Lepidoptera (especially Pyralidae) in their larval stage. It has already been successfully utilized in integrated pest management. Most of the species of Pyralidae are agricultural pests on some field crops and storage crops. The most important species of those insect pests are *Ephestia kuehniella* (Z.), *E. cautella* (Walk), *Galleria mellonella* (L.), *Achroia grisella* (F.), *Helocoverpa armigera* and *Corcyra cephalonica.*

The efficiency of biological control depends upon the ability of the production of relatively inexpensive biological control agents of insect pests. The production of beneficial insects, especially parasitoids, has improved substantially in recent years. The life table is one of the tools, used in quantitative analysis and in estimation of populations. So the present study was mainly focused on the effect of different hosts on the developmental time, longevity, fecundity and life table parameters of B. hebetor. The aim was to find the most suitable hosts for rearing *B. hebetor* to use as effective biological control agent.

RESULTS AND DISCUSSION

Bracon hebator is reared in "Sandwich method". About 100 pairs (both males and females) freshly emerged adults from "Bracon pupal card" are caged in rearing jars measuring about 15*10 cm. A cotton swab soaked in 50% honey is provided to the side of the jar wall as a adult food. The mouth of the jar is then screwed with piece of muslin cloth by rubber band. About twenty numbers of 5th instars caterpillars of rice moth or any other lepidopteran larvae of similar size (particularly black headed caterpillar) are placed over the muslin cloth

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and another piece of cloth and another piece of cloth is tightly tied over the first by rubber band. The female parasitoids lay eggs on the host larvae throughout the muslin cloth. Before laying eggs they inject venom to the larval body. The injected venom paralyze the body of the larva and also prevent them from rotting. The parasitized larvae are removed by forceps after 24 hours of exposure and are arranged over an art paper strip. The paper strip is inserted into a specimen tube and kept in a horizontal rack. New batch of caterpillars are placed over the first layer of muslin cloth and the process continues. The *Bracon* eggs hatch in 1.5 to 2 days. The larva feed in the *Corcyra larva* for 3-4 days. During pupation, the Bracon larvae leave the host (*Corcyra*) larva and pupate on the paper strip.

The host (*Corcyra*) turns black and are removed by forceps. The cocoons (pupa) present in the paper strip can be stored in refrigerator for 3-4 weeks. Some pupal cards are used for further rearing of Bracon. Rest are released in the field. For effective release of the parasitoids in the field, pupal cards are stapled or tied with the coconut leaflets @ one card per ten plants (100 cocoons per 10 plants) for controlling black headed caterpillars. The major species of the parasitoids are *Bracon hebator* and *B. brevicornis*. The parasitoids may also be released in the adult stage @5-10 adults / palm or 500-1000 adults / ha. Two or three releases may be required depending on the intensity of pest infestation. The first release is initiated in the first week of February or depending upon the pest intensity.

The parasitoids may also be deployed against leaf eating caterpillars of different crops (such as leaf folder in paddy, leaf worm, semi looper and leaf roller in cotton etc) at the dose and intervals mentioned above. We can sell a card @ Rs. 15/- per card each containing approximately 100 cocoons. Pre treatment and post treatment pest population and/or damage may be compared. Larva may be collected before and after release of parasitoid. They may be reared separately in the laboratory for adult emergence and per cent parasitization may be calculated. It shouldn't be treated with insecticides. Mahadavi et al. in 2011 in their studies showed that carbaryl had more adverse effects on population parameters of the parasitoid compared to abamectin. Field studies are needed to determine the total effects of the pesticides on *Habrobracon hebetor*.

Sadat and Bandani in 2014 studied the effect of different lepidopteran hosts, *Ectomyelois ceratoniae, Plodia interpunctella, Ephestia kuehniella, Helicoverpa armigera* and *Malacosoma disstria* on the biological parameters of this *Bracon*. Comparatively, the parasitoid performed better on stored product pests, such as *Ephestia kuehniella* and *Plodia interpunctella*, than field crop pests, such as *Helicoverpa armigera* and *Manduca disstria* in terms of percentage egg hatch, rate of development, off-spring sex ratio and adult dry mass. The greatest activity of the quality and quantity of the proteases and α-amylase was recorded in the gut of those parasitoids that were reared on stored product insects (*P. interpunctella* and *E. kuehniella*). It is concluded that stored product insects, which feed on a diet rich in sugar and glycogen, provide physiological conditions that are more suitable for the parasitoid than field crop insects, which feed on diet rich in terpenes and tannins.

Magro and Parra in 2003 has studied the biology of the ecto-parasitoid Bracon hebetor Say on seven different types artificial diets under controlled environment and compared it with its biology on its natural host *Anagasta kuehniella* (Zeller). Though the life cycle duration (egg–adult) and female longevity was not significantly different but failure of the pupa to produce a protective cocoon during the pupal phase was observed in 60% of the larvae developed on the artificial diet indicating that natural host are superior as compared to artificial diet are good option for rearing of these larval parasitoids. Farag et al. in 2015 studied on the life table of Bracon hebetor adult reared on three different hosts Greater wax moth (*Galleria mellonella*), Mediterranean Flour Moth (*Ephestia kuehniella*) and Rice moth (*Corcyra cephalonica*) Stainton. The developmental time was significantly shortened when parasitoid reared on G. mellonella. The total number of eggs deposited by female of B. hebetor reached its maximum of (395.11 eggs) on *G. mellonella* comparing to (93.5 and 56 eggs) on *E. kuehniella* and *C. cephalonica* respectively. Ashfaq et al. in 2011 given a refined rearing technique for Bracon hebetor on large scale on a host, waxmoth, *Galleria mellonella*, at different temperature and humidity percentages. In the light of their experiments, they have concluded that pupal stage is the best stage of storage of the parasitoid for its timely releases (as long as 4 weeks at 50C). They concluded the temperature range of 25-30°C is found to be best suitable temperature for the rearing of the parasitoid.

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A Brief Outline on Nanobiopesticides

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ABSTRACT

Biopesticides which are derived from biological substances such as plants, microbes, etc. aims to control, kill and destroy the harmful pests. They serve their purpose in a target specific way, with no harm to the environment. Nanotechnology which deals with particles less than 100 nm is an emerging field nowadays which is applied in various fields including agriculture. This technology is used to modify the biopestides into nano sized particles to increase its specifity towards insects pests. Plants compounds having pesticidal activities can be used to synthesize nanobiopesticides in various methods like biological,physical or chemical. In this review the importance of different nanobiopesticides and their action have been discussed.

KEY WORDS: BIOPESTICIDES, NANOTECHNOLOGY, NANOBIOPESTICIDES.

INTRODUCTION

Huge crop losses occur due to insect pest attack which leads to the development of synthetic chemical pesticides. But they did not proved to be promising as large chemical accumulation occur in soil which deteriorates the health of soil, plants as well as animals.

In this situation, nanotechnology emerged as a new field whose materials size is less than 10-9. Scientists have been using nanoparticles for plant growth stimulation, insect-pest control, disease diagnosis in plants, post-harvest management (1, 2). Due to hazardous effects of chemical pesicides the market for biopesticides have developed a little. Biopesticides are substances made of biological substances which control, kill, and destroy the insect –pests. Pesticides obtained from plants are known as Botanicals which act as natural defense against harmful plant pests since many decades.

Nanotechnology and its Application: Nanotechnology consists of materials which have a dimension less than or equivalent to 100 nm. Nanoparticles can be prepared from organic or inorganic sources by physical, chemical or biological methods. The field of nanotechnology is hugely developing nowadays creating tremendous impact on agricultural and medical field. Owolade (2008) showed that nanobiopesticides, nanomicrobicides, are being used efficiently in agriculture (3). Nanotechnology has various applications in the field of chemical, agricultural, medical, cosmetic industries among many others. Metallic and polymeric nanoparticles have been used in controlling various insect pests destroying foods.

Biopesticides and their advantages: The type of pesticides which are obtained from natural sources such as animals, plants, microbes such as fungi, bacteria etc. are known as biopesticides. Biopesticides include neem oil, canola oil from plants as well as fungi like *Beauveria* sp. or bacteria like *Pseudomonas* sp. and Bacillus sp. Biopesticides are advantageous than chemical pesticides in various ways such as specific and slow mode of action, safer to the humans and environment, do not form residues in soil,

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breakdown rapidly in the environment, low risk to non-target organisms, broad spectrum of action (4). Due to these advantages biopesticides should be used in place of chemical pesticides.

Biopesticides based on Nanotechnology: Plant kingdom is a rich source of organic compounds which are used as medicines, hallucinogens, pesticides etc. Thus the plants with medicinal and pesticidal values should be exploited through nanotechnology to suppress harmful plant pests. Plants can be utilized as an important source of nanoparticle based biopesticides. Compounds from different plants have been used to synthesize nanoparticles which have various beneficial applications on plants have been listed in Table 1.

Table 1. Nanoparticle based biopesticides derived from plant compounds

Plant name	Nanoparticles synthesized	Mode of action
Anacardium occidentale (Kaaju)	Au, Ag, Cu	Insecticidal (5)
Azadirachta indica (Neem)	Ag, Cu	Insecticidal (6)
Brassica campestris (mustard)	Zn, Ag	Insecticidal mainly beetles (7)
Capsicum annum (chilli)	Cu, Ag, Au	Beetles (8)
Curcuma longa (turmeric)	Ag, Zn	Pesticidal (9)
Euphorbia sp.	Ag, Pt	Insecticidal (10)
Ocimum tenuiflorum (tulsi)	Ag	Insect repellent (11)
Ricinus communis (castor)	Au, Ag	Pesticidal (12)
Fenugreek	Ag	Insect repellent (13)
Pyrethrum	Au, Ag	Bees (14)

CONCLUSION

To protect our environment from deleterious effects of synthetic pesticides, use of botanical based nanopesticides should be done by farmers. Nanobiopesticides application should also be started in field experiments, research works for biotic stress control. It provides a good alternative to the animal and environmental safety.

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On Farm Trials of Low Gi Rice Variety RNR 15048 in Gajapati District of South Odisha

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ABSTRACT

Large population in India consume rice as staple food and are suffering from type-2 diabetes. In general, most of the rice varieties have the GI of around 73 (white rice - polished) and 68 (brown rice - unpolished) which is responsible for a person to become diabetic. The rice variety RNR 15048 developed by Professr Jayashankar Telangana State Agricultural University is having Glycemic index of 51%. On farm trials were conducted during Kharif 2009 with low GI rice variety RNR 15048 in 290 farmers' fields of 10 villages of Gajapati district, Odisha. The farmers adopted both dry seeding and transplanting method of crop establishment, used different aged seedings for transplanting between July 16 to Aug 31 and applied varying levels of fertilizer. There was an increase of 5 % of paddy in dry seeded crop over that of transplanted crop. The grain yield of the crop sown between July 1 to 15 has recorded 26 and 11% higher yield (4228 kg ha^{-1}) than that of June 10-20 and June 20 to 30 respectively. The mean yield improvement observed was 3 % at higher N and P2O fertilizer application (113 kg N and 58 kg P_2O_5 ha^{-1}) (4379 kg ha^{-1}) as compared to low N and P2O fertilizer applied (43 kg N and 23 kg P2O5 ha^{-1}). The grain yield of rice was higher with transplanting between 16 to 31 July (5092 kg ha^{-1}) which was 18, 21 and 49 % higher over that transplanted between Aug 1 to 10, 11to 20 and 21to 31. There was decrease in grain yield with increase in average age of seedlings from 30 to 36 and 43 days. The grain yield of transplanted rice was higher in Lingipur village where soil N and P were lower as compared to village Chandanakhala where soil fertility was higher and grain yield was lower than that in former village.

KEY WORDS: AGE OF SEEDLINGS, DRY SEEDING, FERTILIZER LEVELS, KHARIF SEASON, TIME OF SOWING, TIME OF TRANSPLANTING.

INTRODUCTION

In India, as many as 50 million people are suffering from type-2 diabetes. Majority of them consume rice as staple food. In general, most of the rice varieties have the GI of around 73 (white rice) and 68 (brown rice). Several studies have reported that higher intake of rice is strongly associated with type 2 diabetes (Blaak et al., 2012 and Hu et al., 2012). The low GI rice varieties are packed and sold in India at a high price of rupees 90 to 120 per kg under the name of different brands. With technical support from M.S. Swaminathan School of Agriculture, the Last Mile Distribution India Pvt Ltd (LMDC) taken up the production of low GI rice variety in the farmers' fields of Gajapati district, Odisha. The Gajapati district is having 60% tribal population and 90% people depending on agriculture and the district ranks one among the 50 most backward districts of the country. The farmers were provided with the seed and technical advice on daily basis by the production team through app-based monitoring (Kalgudi) and regular SMS.

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The paddy crop is grown in 90% of the cropped area (4.46 million hectares) in Odisha (agriodisha.nic.in). The productivity of paddy ranges from 3750 to 4500 ha-1. The poor yield of rice crop is due to dependence on rainfall and irrigation through diversion of stream water for rice cultivation. The crop is cultivated as dry seeded and transplanted (Reddy and Panda, 1988). The crop suffers from weeds in dry seeded rice and delayed transplanting with aged seedlings in transplanted rice. The crop is fertilized at sub optimal level and rarely Zinc is applied. The varieties grown are coarse varieties like MTU 1001 and MTU 1010. The RNR 15048 (Telangana Sona) is a fine variety with 120-125 days duration, having tolerance to BLB and suitable for late sowing (July) (Vyavasaya Panchangam, PJTSAU, 2019). If sown early in June, it takes more time to mature and grows taller thereby there is scope for lodging.

The cultivation of low glycemic index variety was taken up to improve the productivity and income of farmers in the backward area of Gajapati district. The farmer's meetings were organized in 10 villages of 8 Panchayats of Gosani and Gumma blocks of Gajapati district, Odisha and convinced them to take up the production of low Glycemic Index paddy variety RNR 15048 (Prasanthi Prabhakaran Sobhana, et al., 2019). To facilitate the farmers in getting higher paddy production and realization of more income through technological guidance and marketing, the school has developed a network through which each individual farmer was contacted and his field was monitored through App Kalgudi.

MATERIAL AND METHODS

During 2019, an area of 480 acres of 290 farmers in 10 villages of Gajapati district, Odisha was cultivated with low GI paddy RNR 15048. The farmers were supplied foundation seed of RNR 15048. Before start of the season, the farmers were trained on production technology of this variety. The technical support was provided through the faculty of M.S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Odisha and marketing team of Gram Tarang Foods Pvt. Ltd. The Last Mile Distribution India Pvt. Limited has developed network through which each individual farmer was contacted and his field was monitored through App Kalgudi.

The soil test was done in all the fields of the farmers for knowing the nutrient status of field. All the farmer's production activities were monitored by a specially designed social-networking app, named 'Kalgudi'. All the farmers were connected through this app and further it was connected to the technologists as well as monitoring team of Last Mile Distribution India Pvt. Limited. The Kalgudi app shows the GPS location and the time of posting of any photo or video, which made the monitoring team to respond promptly. Whenever, farmers needed any support, messages were communicated immediately. Regular field visit by monitoring team and update of each field on daily basis was done by the team and SMS alert was given to all farmers.

The package of practices was explained to the farmers in June before the start of the season. The recommended package of practices of RNR 15048 include sowing of nursery after July 10, transplanting in August first fortnight using 25-30-day old seedlings, application of 100 kg N, 60 kg P2O5 and 50 kg K2 O ha^{-1}. The farmers taken up the dry seeding at different times (Table 2) and provided water as and when required with stream water. In transplanted crop, the nurseries were raised from June last week to fourth week of July. The crop was transplanted with different aged seedlings on different dates (Table 5 and 6). The data of 58 farmers were collected and analyzed for different agronomical parameters as per the production package to assess the yield performance of RNR 15048.

RESULTS AND DISCUSSION

Dry seeded rice: Of the 58 farmers whose data was used for study, twelve farmers adopted dry seeding and 46 farmers adopted transplanting method of rice cultivation. There was an increase of 5 % of paddy in dry seeded crop over that of transplanted crop (Table 1).

Table 1. Effect crop establishment methods on grain yield of RNR 15048 in farmers' fields of Gajapati district, Odisha during Kharif 2019

Crop establishment method	Yield, kg ha^{-1}
Dry seeding	4357 (12)
Transplanting	4153 (46)

Figures in parenthesis are the number of farmers

Time of sowing: The time of sowing has considerable influence on grain yield of rice. The crop sown between June 10 to 20 has recorded 26 and 11 % lower grain yield than that sown between June 20 to 30 (3828 kg ha-1) and June 20 and 30. On the other hand, the grain yield of the crop sown between June 20 to June30 has recorded 14% higher yield (4228 kg ha-1) than that sown on June 10 to 20.

Table 2. Effect of dates of sowing on grain yield of of Dry seeded rice variety RNR 15048 in farmers fields of Gajapati district, during kharif season 2019

Date of Sowing	Yield, kg ha^{-1}
10 to 20 June	3359 (1)
20 to 30 June	3823 (8)
1 to 15 July	4228 (3)

Figures in parenthesis are the number of farmers

Fertilizer levels: The grain yield obtained in Bagusala village where soil fertility especially N and fertilizer applied was low as comparable to that in Lingipur village where soil N and P contents (Table 3) and fertilizer N applied were higher. The yield and nutrient levels observed in three villages followed in the order of Machamara > Kharsanda > Katalkhaita (Table 4). The mean yield improvement observed was 3% at higher soil and fertilizer N and P2O5 level (4379 kg ha-1) as compared to low N and P2O5 fertilizer applied plot having low N content (Table 4). This could be due to environmental conditions and rainfall received during kharif season. However, no relation could be found between soil fertility and fertilizer response which need further research.

Table 3. Grain yield of Dry seeded rice variety RNR 15048 at varied soil fertility and fertilizer application in farmers' fields of different villages of Gajapati district, during kharif season 2019

Village	Soil fertility			Fertilizer applied, kg ha-1			Grain yield, kg ha-1
	N	P	K				
Bagusala	20	10	198	82	30	60	4447(2)
Katalkaitha	80	22	298	115	58	52	3359(1)
Kharasandha	93	4.1	642	115	58	52	3789(1)
Machamara	160	18.2	890	115	58	52	3868(1)
Lingipur	101	9	472	102	58	52	4408(7)

Figures in parenthesis are the number of farmers

Table 4. Effect of fertilizers applied and soil fertility on grain yield of Dry seeded rice variety RNR 15048 in farmers' fields of Gajapati district, during kharif season 2019

Parameter	Fertilizer applied, kg ha^{-1}			Soil fertility, kg ha^{-1}			Grain Yield, kg ha^{-1}
Fertilizer	N	P_2O_5	K_2O	N	P	K	
high	113	58	54	102	9.3	498.2	4379 (10)
Low	43	29	53	80	40.8	769.5	4246 (2)

Figures in parenthesis are the number of farmers

Table 5. Grain yield of transplanted rice variety RNR 15048 in farmers' fields of different dates of planting in Gajapati district, during kharif season 2019

Date of Transplanting	Yield, kg ha^{-1}
16 to 31 July (6)	5092
1 to 10 Aug (21)	4298
11 to 20 Aug (18)	4225
21 to 31 Aug (1)	3415

Figures in parenthesis are the number of farmers

Transplanted crop

Date of Transplanting: The grain yield of rice was higher with transplanting between 16 to 31 July which was 18% higher over that transplanted between Aug 1 to 10 (Table 5). There was no difference in grain yield between the crop transplanted between Aug 1 to 10 and Aug 11 to 20. However, the grain yield decreased considerably (49%) when the crop was transplanted between Aug 21 to 31 as compared to that transplanted between August 21 and 31.

Age of seedlings: There was decrease in grain yield with increase in age of seedlings from 30 to 36 and 43 days. The decrease in yield was 20 and 23% when seedlings of 36 and 43 day old seedlings were used as compared to 30-day old seedlings (Table 6). Higher grain yield was reported by using 25 day old seedling due to more number of tillers and thereby panicles (Vijayalaxmi et al., 2016).

Fertilizer application: In transplanted rice, the N fertilizer application ranged from 110 to 115, P2O5 ranged between 52 to 57 and K2O varied between 47 to 55 kg ha-1 in all the farmers' fields except one field. The grain yield of transplanted rice in Lingipur village was higher as compared to that in Chandanakhala village. The soil N

and P were lower in the former village as compared to latter village.

Table 6. Grain yield of transplanted rice variety RNR 15048 in farmers' fields of different age of seedlings in Gajapati district, during kharif season 2019

Age of Days Seedling,	Average age of Seedlings, Days	Yield, Kg ha -1
21-30	30	5263 (13)
31-40	36	4226 (18)
41-50	43	4072 (13)

Figures in parenthesis are the number of farmers

The dry seeded crop resulted in 5% higher yield than transplanted rice. In general, the early establishment of the crop with dry seeding under favorable rainfall gives enough anchorage to the seedlings and establishment, higher ear bearing tillers and thereby grain yield (Reddy et al., 1993, Reamulu et al., 2020). However, the dry seeded crop needs proper weed management (Lateef Pasha et al., 2011, Kadiyala et al., 2012). The higher grain yield is due to higher panicles per unit area and thereby higher yield as that of transplanted conditions. For RNR 15048, sowing in July first fort night has been found suitable as the crop sown during this period has given 26 and 11 % higher yield over that sown between June 10 to 20 and June 20 to 30. Under dry seeded conditions, higher fertilization resulted in 3 % increase in yield over that of low level of fertilization. In South Odisha and adjoining areas of North Coastal A.P., the rice crop is grown under dry seeded as well as transplanted conditions (Reddy et al., 2006). The present study suggests that the low GI rice variety RNR 15048 is suitable for both dry seeded and transplanted conditions.

The Professr Jayashankar Telangana State Agricultural University recommended variety RNR 15048 (Telangana Sona) for late planted conditions in Telangana state (Vyayasaya Panchangam, PJTSAU 2019). Contrary to this, the grain yield of rice was higher with early transplanting between 16 to 31 July as compared to that in August. Further, transplanting with younger seedlings (30 Days) has resulted higher yield than older seedlings (36 and 43 days). The Recommended level of fertilizer has given 3.4 to 4.4 t grain yield. The yield obtained with RNR 15048 was 400 kg ha-1 higher than that of farmers yield in nearby fields. The on-farm trials suggest that growing of low glycemic index (GI) variety RNR15048 is suitable for kharif under both dry seeded and transplanted conditions. Sowing of RNR 15048 from July first fortnight in case of dry seeded rice gives higher yield than that sown at later date. Further, transplanting in July with 30-day old seedlings with recommended fertilizer level of 100 kg N, 60 kg P205 and 50 kg K20 ha-1 gives better yield.

Table 7. Grain yield of transplanted rice variety RNR 15048 at varied soil fertility and fertilizer application in in farmers' fields of different villages of Gajapati district, during kharif season 2019

Village	Soil fertility, kg ha^{-1}			Fertilizer applied, kg ha^{-1}			yield
	N	P	K	N	P_2O_5	K_2O	
Bagusala	76	64	430	115	57	112	3838(1)
Bomika	167	17	371	115	57	55	4330(18)
Chandanakhala	186	32	715	115	57	52	4006(3)
Kharasandha	88	36	528	110	52	47	4389(11)
Lingipur	71	7	521	115	57	52	5131(7)
Machamara	160	18	880	115	57	52	3827(4)
Patikota	130	12	350	115	57	52	3561(1)
R.Sitapur	93	9	475	115	57	52	3465(1)

Figures in parenthesis are the number of farmers

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Molecular Surveillance and Cellular Homeostasis

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ABSTRACT

A web of events which are inter-related and highly regulated determines the fate of gene expression. Essentially, these vital events are broadly categorized into two types, namely, post-transcriptional and post-translational events. Primarily, processing mechanisms of pre-mRNA including polyadenylation, capping, splicing and modifications of RNA through changes in chromatin [Small interfering RNAs (siRNA), long non-coding RNAs, micro RNAs (miRNA)] are included under post- transcriptional events. Protein modifications including sumoylation, ubiquitination and phosphorylation are some of the events which occur at post-translational level. Both post-transcriptional and post-translational events are considered to be constitutive and also are aggravated by endogenous and exogenous factors. Particularly, in plants, regulation of gene expression is yet to be fully understood. These molecular events ensure proper occurrence of the factor(s) required which would ultimately modulate several downstream cellular processes. This review primarily focuses on some of the key post-transcriptional and post-translational events which are significant in deciding the fate of eukaryotic gene expression.

KEY WORDS: REPLICATION, MESSENGER RNA, POST-TRANSCRIPTIONAL MODIFICATIONS, PROTEIN MODIFICATIONS.

INTRODUCTION

Exterior framework of an organism typically referred to as the phenotype, is generally determined by the functional proteins, though their sequence is encoded in DNA. The genetic expression is regarded as one of the elemental process which plays a vital function in the changeover of the complex genome to a substantial life. Since genetic expression process is a strongly regulated event, so any sort of mis-regulation may escort to distorted physical life which includes a variety of genetic diseases. Till date, it is pretty well recognized that the genetic expression is synchronized at a variety of levels and these miscellaneous mechanisms are well included as a food web. The regulatory mechanisms controlling gene expression is primarily divided into two main types (1) post-transcriptional mechanism and (2) post-translational mechanism. Additionally, upstream of these two events, DNA is mostly synchronized at the transcriptional level prior to entering into the transcription event.

Quality control of RNA at the post-transcriptional level is a very important concern for all organisms to ensure precise gene expression, both qualitatively and quantitatively (Ohtani and Wachter 2019).Transcriptional process has been expansively premeditated as compared to that of post-transcriptional and post-translational events for the reason that of scientific aspects. It is apparent that transcription is one of the essential and instinctively imperative steps within the multistep processes involved in regulation of gene expression and also the scientific methods to decode transcriptional regulation are very well recognized.Post-transcriptional regulation mechanisms engage diverse events such as

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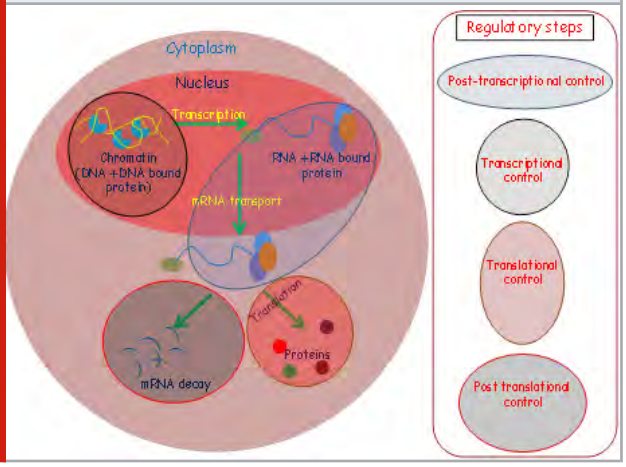
messenger RNA processing which includes 5 end capping, polyadenylation and intron splicing. Export as well as localization of messenger RNA, messenger RNA decay, and translation of messenger RNA are also included (Fig. 1).

Figure 1: Multiple layers of regulatory events meant for gene expression



Regardless of this array of regulatory mechanisms, lone thing in general is that they eventually control where and when a messenger RNA is translated to protein. As a result, translation and its regulation are extremely fundamental to post-transcriptional regulation of gene expression (Panigrahi et al., 2021). The regulatory mechanisms controlling post-translational events are known as Post-Translational Modification (PTM), essentially which refers to several types of covalent and enzymatic modifications of proteins occurring following to their synthesis. Post-synthesis of proteins through ribosomes, they endure PTM in order to shape the mature and functional protein product. PTMs may arise on the side chains of amino acid or else at the N- or C- terminal of proteins (Pratt et al., 2006). Phosphorylation is quite a regular mechanism for modulating the enzymatic activity and is also most common PTM (Khoury et al., 2011).

Countless eukaryotic proteins harbourcarbohydrates attached through a process called as glycosylation, which mostly induces stabilization and protein folding, thus allowing the newly synthesized proteins to perform regulatory functions. Alliance of lipids commonly recognized as lipidation, chiefly aim a specific protein or a part of it which is adhered into the cellular membrane. Additional forms of PTMs comprises of cleavage, as in the case of synthesizing a mature form of protein by processing a given pro-peptide. Occurrence of disulfide bonds, formed due to cysteine residues, also is referred to as a type of PTM (Lodish 2000). PTMs also occur as a result of oxidative stress (Dalle et al., 2006, Panda et al., 2016, Panigrahi et al., 2016, Panigrahi and Sahoo 2016). Protein aggregates formed post protein degradation may be referred to as carbonylation which primarily targets the newly synthesized protein. Modifications in unambiguous amino acids can thus be applied as biomarkers signifying oxidative dent (Grimsrud et al., 2008).

RNA processing and export: Prior to the transfer of mRNA from nucleus to the cytoplasm for getting accessible to the translational apparatus, it needs to experience a sequence of dispensation steps: firstly, at the 5' end, the messenger RNA bears a cap like structure, then, splicing event initiates leading to the splicing out of introns harbouring in the pre-messenger RNA, finally a specific 3' end of the mRNA is synthesized, usually referred to as polyadenylation. Every event advance co-transcriptional and influences each other (Proudfoot et al., 2002). Initially, m7G cap is added at the 5' end of the budding mRNA and occurs following synthesis of 20-30 nucleotides. This event primarily is a complex process and occurs in a three-step process. At first, the guanosine mono phosphate (GMP) domain from the GTP is supplemented into the foremost nucleotide present in the pre-mRNA and then, GMP is methylated specifically at location N7.

For the stability of mRNA and translation process, the m7G cap is imperative. Inside the nucleus, the m7G cap gets associated along with the cap binding complex (CBC). CBC contains two subunits and after being moved into the cytoplasm, it forms a complex with the translation initiation factor 4E (eIF4E), which is essentially an indispensable tread in the initiation process of translation. Since the coding sequences (exons) of a large amount messenger RNAs incase of the higher eukaryotes are broken up by the presence of introns, thus these group of introns needs to be chopped off of the pre-messenger RNA to create functional messenger RNA. Consensus and conserved sequences are required for splicing of the mRNA, which primarily marks the exon-intron limits, and the spliceosome, commonly referred to as the catalytic complex carries out the enzymatic reactions to eliminate the introns and ultimately ligate the flanking exons. Five small ribonucleoprotein particles (snRNP U1, snRNP U2, snRNP U4, snRNP U5 and snRNP U6) make up the spliceosome unit. Usually, snRNPs are made up of a small nuclear RNA, commonly referred to as snRNA, and associated proteins, many of which are accessory proteins.

Preferably, almost hundred of proteins are believed to be engaged as factors, primarily involved in splicing event (Jurica and Moore 2003). Splicing reaction undergoes catalysis process and this event is reliant on RNA-RNA, protein-protein and RNA-protein interactions. Moreover, the unconventional use of exons, referred to as alternative splicing can also add to the formation of protein diversity by allowing a single gene to fabricate manifold isoforms (Matlin et al., 2005). The majority of messenger RNAs also bear a definite structure, a poly (A) tail at the 3' end. In higher eukaryotes, mRNAs coding for histone proteins lack poly (A) tail, but this is absent in yeast (Fahrner et al 1980). Polyadenylation at the 3′ end occurs in two steps: firstly, the newly synthesized messenger RNA is cleaved at the site mostly where the polyadenylation is destined to initiate, and then processed for poly (A) creation. In

resemblance to the splicing, the polyadenylation protein complex is required for poly (A) tail configuration and also explicit sequence-elements above the pre-messenger RNA. In case of mammalian cells, the position of cleavage mostly lies flanked by ahexamer motif (AAUAAA) along with a GU-rich downstream element, DSE.

This hexamer is essentially associated by the cleavage and polyadenylation specificity factor (CPSF). The downstream elements associate with the cleavage stimulatory factor:cleavage factor I and cleavage factor II are also obligatory. While both the poly(A) polymerase (PAP) and the cleavage and polyadenylation specificity factor are mandatory for cleavage of the pre-mRNA and poly(A) addition respectively, the cleavage stimulatory factor(CstF) is also indispensable for the endonucleolytic cleavage to occur, and CstF together with the CPSF are indulged for recruiting CF I and also the CF II. The synthesis of poly (A) tail occurs in the same way both in the case of yeast and mammalian cells. The protein factors concerned largely bear orthologous components, but also explicit accessory machinery that are specifically found in any one of the species. Additionally, in case of yeast cells, the AAUAAA hexamer pattern is replaced by an erratic A-rich element and instead 3 polyadenylation complexes are present. Cleavage Polyadenylation Factor (CPF), which bears numerous factors which are homologous to CPSF and also the cleavage factor IA (CF IA), poly(A) polymerase and cleavage factor IB (CF IB).

The rising poly (A) tail is associated by the poly (A)-binding protein (PAPB). The PABP is mainly thought to persuade the ultimate length of the poly (A) tail, positively by invigorating the processivity of poly (A) polymerase, and negatively by associating with the poly (A) nuclease (PAN) (Magnus et al., 2003). Moreover, PABPs are concerned with nuclear export and are also imperative for the launch of translation. The poly (A) tail is critical for quite a lot of superfluous mechanisms regulating post-transcriptional events, occurring in the cellular cytoplasm. The translational state can also be standardized via cytoplasmic polyadenylases and steadiness of a range of target messenger RNAs by manipulating the length of the poly (A) tails. The preeminent illustration is most likely that of translational regulation of the maternal messenger RNAs in case of oocytesof Xenopus, stockpile in a translationally subdued state with extremely petite poly (A) tails, which become polyadenylated when activated and as a outcome of which, translated messenger RNA undergoes decay by several exonucleolytic events which is usually preceded by a reduction of the 3 end poly (A) tail (Parker and Song 2004).

In recent times, poly (A) tails deadenylation has also been exposed to ensue in micro RNA-mediated regulation (Giraldez et al., 2006, Wu et al., 2006). The very last component in the expedition from the nucleus (space of transcription process) into the cytoplasm is the nuclear export of the mature messenger RNA. Export occurs through the nuclear pore complex and happens in the perspective of messenger ribonuleoprotein complexes (mRNPs). Messenger ribonuleoprotein complexes embrace messenger RNA and several associated RNA-binding proteins, which associate to the messenger RNA during the progressing steps (Aguilera et al., 2005). Separately from the aforesaid, Cap binding complex or poly (A) binding proteins, like RNA-binding proteins consist of SR (serine/arginine rich) and hnRNP(heterogeneous nuclear RNP) proteins, the exon junction complex (EJC), which are a group of proteins encumbered onto the messenger RNA, mainly at the upstream region of exon-exon junctions, as a end result of the pre-messenger RNA splicing.

These protein components are imperative for the organization of the mRNP complex with the nuclear pore complex and the shuttling from nucleus into the cytoplasm, and a few of them settle, allied with the messenger RNA as it is moved out, whereas others are constrained within the nucleus. Moreover, nuclear export is an central step in quality control, as damaged or un-processed messenger RNAs are not only ineffective, but also potentially detrimental, if incase gets translated within the cytoplasm. Only physiological messenger RNAs are transferred into the cytoplasm from their site of synthesis and particularly this surveillance step is very closely united to RNA processing and the composition of mRNP. Yet again, it needs to be highlighted that, regardless of the introduction of messenger RNA transcription and other downstream chronological events occurring in the cell are well integrated among each other and are not independent in temporal and spatial perspective (Proudfoot et al., 2002, Aguilera et al., 2005).

Significance of translational regulation: A varied number of reasonable benefits do occur since the translational regulation is perfectly fitted. Most importantly, the translational regulation happens as an immediate retort without the requirement of undergoing the several processes involved in regulating gene expression such as transcription, processing of messenger RNA or even export of messenger RNA. Moreover, the regulatory mechanism of translation is a reversible in nature since it involves quite a lot of reversible protein structure alterations like, the phosphorylation of several initiation factors. Control of translational machinery is very much inevitable, particularly in the systems where transcriptional regulation is not promising like in the case of reticulocytes; they lack nucleus, RNA viruses or oocytes.

Most importantly, the translational regulation is primarily, spatial control of gene expression inside the cell (Johnston 2005, Sahoo et al., 2020a,b,c,d,e,f). The significance of dedicated translational regulation is realized especially for localized protein assembly within neurons or else throughout the development process, since transcriptional regulation is limited to the cellular nucleus. For regulating gene expression, translational regulation is a superior alternative owing to its flexible nature. There are numerous molecular targets for regulation of translational process, which

ultimately affects the efficiency of translational event for numerous or a few messenger RNAs. Most remarkably, for fine tuning of gene expression cells regulate the translational machinery, as there are several number of genes such as GADD45α or TNF-α which are regulated at the transcriptional and translational level.

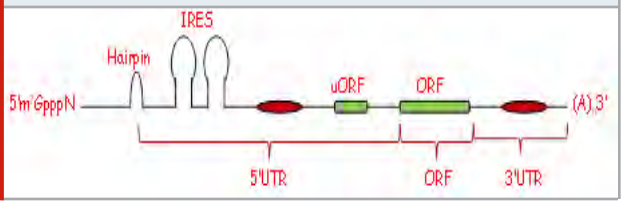
Effectors for regulation of translation: Initiation factors, messenger RNA (mRNA) and the ribosome: Translational control is well regulated at a comprehensive level as well as in a messenger RNA specific manner (Gebauer and Hentze 2004). Primarily, large-scale regulation affects the effectiveness of translation machinery of many messenger RNAs through a common switch-on and switch-off of translation process. The translation of a subset of mRNA is affected by mRNA-specific regulation. Mainly, translational regulation allows or forbids the union of the messenger RNA with that of the translational apparatus. A fundamental target in several regulatory mechanisms is the cap binding protein, eIF4E which binds to many inhibitory proteins, resulting in the unavailability of the messenger RNA.

Comprehensive regulation of translation is universally mediated through such modifications, especially of the translation initiation factors. An additional target for translational regulation is the messenger RNA itself. The cis-regulatory elements associate with trans-acting factors (Fig. 2). The cis-regulatory elements present on the messenger RNA could be present anywhere along the messenger RNA, but typically for the translational regulatory factors, these vital elements are present in the 5' UTR or 3'UTR. Translational regulatory events mediated by messenger RNA, occurs mostly by numerous regulatory proteins, which primarily bind to the cis-regulatory elements of a given messenger RNA.

The ribosome itself may also become one of the targets of translational regulation. Quite a lot of its protein constituents undergo post-translational modifications. An exemplar is the phosphorylation process of ribosomal protein S6 mediated by ribosomal S6. It has been reported that the phosphorylation of ribosomal protein S6 fallout in an augment in translation initiation. Ribosomal proteins also undergo a post-translational modification, ubiquitinationand methylation. Many studies points towards the heterogeneity of ribosomes; the cell is able to construct a range of different kinds of ribosomes, which essentially differs in terms of paralogue composition and post-translational modifications, and many a times dedicated ribosomes could also play a role in the translational regulation of specific subsets of messenger RNAs (Yu et al., 2004).

Novel components in translational control: Processing bodies and micro RNAs: Recently, two novel ways to direct messenger RNA turnover at the post-transcriptional level have gripped an immense deal of consideration. The discovery of processing bodies localized in the cytoplasm of a cell, which were originally considered as foci inside the cell with a high concentration of enzymes meant for messenger RNA decay, has been a significant outreach in the scientific field (Bashkirov et al., 1997). The added detection is that of small RNAs, which may amend the permanence and translation of targeted messenger RNAs (Bartel 2004). Processing bodies are most likely a site of messenger RNA decay (Fig. 3). Processing bodies were first characterized by several groups using several scientific techniques such as microscopy, as factors involved for the perishing of messenger RNA decay and other factors like LSM, XRN1, DCP1 and DCP2 accumulate in the foci (Bashkirov et al., 1997).

Figure 2: Cis-acting sequences influence translation initiation of specific messenger RNAs.

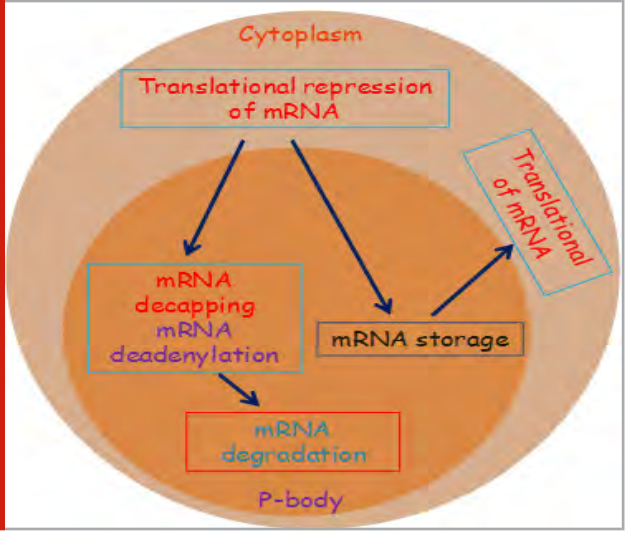


The messenger RNA decay in case of eukaryotes is mainly controlled in diverse ways mostly by exonucleolytic or endonuleolytic pathways.Degradation occurring through exonucleolytic pathway is typically initiated by deadenylation of the poly (A) tail of the messenger RNA. Then the transcripts will be tarnished from their 5′ ends mostly by the exonuclease such as XRN1, subsequent elimination of the 5' cap, known as decapping. On the other hand, the exosome complex can debase transcripts from their 3′ ends prior to decapping. Factors involved in the nonsense-mediated decay process, which are responsible for the hasty dilapidation of messenger RNAs with a untimely stop codon are also found in mammalian processing bodies (Conti and Izaurralde 2005). Nonsense-mediated messenger RNAdecay (NMD), which is possibly the best-known translation-dependent regulatory mechanism specifically in eukaryotes, selectively destroys messenger RNAs as a means of post-transcriptional gene control (Kim and Maquat 2019). Control can be for the purpose of ensuring the quality of gene expression.The relation between the processing bodies and messenger RNA turnover rate is still captivating.

The precise mechanism how messenger RNAs shuttle into the processing bodies and become repressed from translation is not yet clearly deciphered(Panigrahi and Satapathy 2020a).Small RNAs are mostly riboregulators that have significant roles in most of the eukaryotes. They inhibit the gene expression by acting either on DNA to direct sequence abolition and chromatin remodeling, or on the RNA to direct cleavage and eventually regulate the translation expression (Vaucheret 2006). Micro RNAs (miRNAs) and short interfering RNAs (siRNAs) are the two categories of small RNA molecules that appeared as regulating factors of messenger RNA stability and translation. Both the miRNAs and siRNAs are short RNAs ranging around 20-27 nucleotides and are differentiated based on their biogenesis.

miRNAs are principally derived from longer precursors that mostly comprise of a ~75 nucleotide improperly based hairpin segment. siRNAs are of comparable length but are derivative of absolutely complementary RNA precursors. During RNA interference (RNAi), siRNAs which are exogenously introduced target messenger RNAs for cleavage in an endonucleolytic manner (Tomari and Zamore 2005, Panigrahi and Satapathy 2020b). In case of animal cells, a large amount miRNAs are only partly complementary to their target messenger RNAs and the down-regulation of translational product of the target is typically greater than the down-regulation of its messenger RNA profusion, which suggests regulation at the stage of translation (Panigrahi and Satapathy 2020c).

Figure 3: Processing bodies (P-bodies): The site for mRNA decay.



Post-translational modifications of proteins: Post-translational modifications (PTMs) of proteins largely involve covalent alterations that occur subsequent to the translation process. The newly synthesized nascent proteins are eventually exposed to a string of specific enzyme-catalyzed alterations located on their backbones or side chains. Two extensive types of protein post-translational modifications occur. The first type includes every enzyme-catalyzed addition of a few kinds of chemical groups, typically an electrophilic part of a substrate, towards the side chain residue of a protein. This modified side chain is generally electron rich and act as a nucleophile in the transfer process. The second type of PTMs is covalent cleavage of the peptide backbones in proteins. It occurs either by protease action or less commonly mediated by autocatalytic cleavage. A lot of diversifications can be seen in the side chains of amino acids (Walsh et al., 2005).

Covalent modification of proteins: Fundamentally, there are five most frequent types of covalent additions occurring to proteins. They are acylation, phosphorylation, alkylation, oxidation and glycosylation, which are generally catalyzed by dedicated post-translational modification enzymes. Thus, the protein products obtained in this mode result into making up subsets of the complete proteome of an organism commonly referred to as the acyl proteome, the phosphoproteome, the alkyl proteome, the oxidized proteome and the glycoproteome. Most remarkably, every sub proteomes add to extensive diversity (Walsh et al., 2005, Ray et al., 2020, Behera et al., 2020, Jena et al., 2020, Das et al., 2020).

Post-translational modification: reversible and irreversible: Because of the cellular requirement of a meticulous covalent modification occurring in a protein, reversibility and irreversibility of the specific protein modification is critical. The epitome of reversible modification is mainly the protein phosphorylation, reliable with its advancement to the foremost role in protein-based signaling in eukaryotes. All PTMs apart from alkylation have committed enzymes. Mostly, large enzyme families mediate the amputation of several covalent modifications. The enzymes which are involved in acylation, reverse phosphorylation and glycosylation are mostly specific hydrolases, while cleavage of disulfide bonds is mediated by reductases(Walsh et al., 2005).

CONCLUSION

The self-fidelity and unswerving post-transcriptional and post-translational mechanisms make sure of a safe and sound pathway for the genetic makeup, primarily the DNA of an organism and carries out the critical changeover of the DNA into a functional protein which eventually results into an healthy physiological environment within the cell. This inter-relationship and interdependence prevailing among different molecular events similar to a spider's web provides the foundation for a fault free "Central Dogma" of molecular life. A number of events occurring within a cell irrespective of the nature of product to be formed, surveillance mechanisms ensure the fidelity. These molecular events are essentially very critical for maintaining the homeostasis within the cell. For instance, maximum number of immune related genes, both in plants and animals, are tightly regulated by the quality control mechanism, NMD.

NMD ensures that during normal and healthy conditions (pathogen unchallenged condition); these immune genes do not synthesize their protein counterparts. This regulation critically saves a lot of energy for the organism by not synthesizing unwanted protein factors. Whereas, the same NMD process shuts off when the organism is challenged with any sort of pathogen and allows the expression of protein factors responsible for defense mechanism. In a nutshell, understanding and deciphering the role of different post-transcriptional and post-translational events would be critical for future benefits.

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CRediT authorship contribution statement:
Annapurna Sahoo: conceived the idea, wrote the manuscript, have read and approved the final manuscript before submission. Kunja Bihari Satapathy: conceived the idea, have read and approved the final manuscript before submission.

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Potent Phytomolecules Against the RNA Dependent RNA Polymerase of the SARS-COV-2

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ABSTRACT

The 2019 Novel coronavirus (2019-nCoV) threatens public health. The 2019-nCoV is also referred to as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Specific drug against the virus is yet to be discovered. Development of pharmacophores for proficient treatment against severe acute SARS-CoV-2 is challenging. The solved cryo-EM structure of SARS-CoV-2 RNA dependent RNA polymerase (RdRp) can be used as one of the primary target molecule and possible inhibitory ligands may be screened using in silico molecular docking. Primarily phytochemicals can be screened to detect any potential bio active molecules. In silico molecular docking revealed that the phytochemical, Quercitin may effectively bind to the active site of the SARS-CoV-2 main protease.

KEY WORDS: 2019-NCOV, SARS-COV-2, SARS-COV-2 MAIN PROTEASE, DOCKING, PHYTOCHEMICALS.

INTRODUCTION

Coronavirus (COVID19) has become a critical public issue across the global since December 2019 which was suspected to be originated from a wet market in Wuhan, Hubei province, China (Chen et al., 2020, Huang et al., 2020). More than 6 million cases have been reported in 213 countries and territories.SARS-CoV-2 belongs to the beta corona-virus genus, closely related to the previously identified severe acute respiratory syndrome corona-virus (SARS-CoV) (Lu et al., 2020, Wu et al., 2020). It was named as a coronavirus because corona represents crown-like spikes on the outer surface of the virus. Coronaviruses are minute in size (65-125 nm in diameter), enveloped viruses with a single-standard RNA genome. COVID19 ranges from 26 to 32 kilobases which makes it the largest RNA virus. There are four subgroups of coronaviruses family alpha (α), beta (β), gamma (γ) and delta (δ) coronavirus. Among these types, only alpha and beta CoV can infect humans.

The coronavirus disease 19 (COVID-19) is a highly transmittable and pathogenic viral infection caused by sever acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Genomic analysis revealed that SARS-CoV-2 is phylogenetically related to severe acute respiratory syndrome-like (SARS-like) batviruses; therefore bats could be the possible primary reservoir. Public Health Emergency of International Concern (PHEIC) was declared by the World Health Organization (WHO) owing to its fast rate of transmission within the humans (Chen et al., 2020, Chan et al., 2020, Li et al., 2020). Spike protein (S) of SARS-CoV-2 interacts with human Angiotensin-converting enzyme 2 (ACE2). All coronaviruses contain specific genes in ORF1 downstream regions that encode proteins for viral replication, nucleocapsids and spikes formation. The glycoprotein spikes on the outer surface of coronaviruses are responsible for the attachment and entry of the virus to host cells.

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The receptor binding domains (RBD) is loosely attached among virus; therefore, the virus may infect multiple hosts. Other coronaviruses mostly recognize aminopeptidases or carbohydrates as a key receptor for entry to human cells while SARS-CoV and MERS-CoV recognize exopeptidases. The entry mechanism of a coronavirus depends upon cellular proteases which include, human airway trypsin-like protease (HAT), cathepsins and transmembrane proteases serine 2 (TMPRSS2) that split the spike protein and establish further penetration changes. MERS-coronavirus employs dipeptidyl peptidase 4 (DPP4), while HCoV-NL63 and SARS-coronavirus require angiotensin-converting enzyme 2 (ACE2) as a key receptor. SARS-CoV-2 possesses the typical coronavirus structure with spike protein and also expressed other polyproteins, nucleoproteins, and membrane proteins, such as RNA polymerases, 3-chymotrypsin-like proteases, papain-like protease, helical, glycoproteins, and accessory proteins.

The spike protein of SARS-CoV-2 contains a 3-D structure in the RBD region to maintain the van der waals forces. The 394 glutamine residues in the RBD region of SARS-CoV-2 are recognized by the critical lysine 31 residue on the human ACE2 receptor. COVID19 causes respiratory diseases in human, from the common cold to more rare and serious diseases such as the severe Acute Respiratory Syndrome (SARS) and the Middle East respiratory Syndrome (MERS), both of which have high mortality rates and were detected for the first time in 2003 and 2012, respectively. According to the WHO, this contamination is spreading with human to human contact, droplets, and fomites. Crystal structure of the SARS-CoV-2 main protease (Mpro) proves to be an exceptional ground for screening specific ligands (Liu et al., 2020). SARS-CoV-2 main protease can be beleaguered for developing antibodies, diagnostics and vaccines. Reportedly, Mpro and other known viral proteins including RNA dependent RNA Polymerase (RdRp) are defining features paving the path of virus from entry to infection in the host cell (Wrapp et al., 2020, Lung et al., 2020, Sahoo et al., 2020a,b,c,d,e,f, Ton et al., 2020).

Moreover, Mpro can also be an effectual target to diminish the viral replications within the host cells since it facilitates the synthesis of functional viral proteins. The effectiveness of traditional medications on the restriction of COVID-19 growth does not have any scientific back up as of now, since the underlying molecular mechanisms are unclear. The phytochemicals are fundamentally bioactive compounds and has the potential to amend cellular physiology. Plants are enriched with tremendous defense response capabilities (Panda et al., 2016, Panigrahi et al., 2016, Panigrahi and Sahoo 2016, Panigrahi and Satapathy 2020, Panigrahi et al., 2021). Elaborated defense mechanism(s) in plants need to be explored (Panigrahi and Satapathy 2020a, 2020b, 2020c). Here, we report few phytochemicals which has the ability to bind to the active site of the SARS-CoV-2 RdRp as revealed by the *in silico* molecular docking and thus further studies may reveal the effectiveness of phytochemicals to be used as COVID-19 therapeutics.

METHODS

Viral Protein Structure and Phytochemical dataset collection: The 3D structure of RdRp was accessed from Protein Data Bank accession 6VYO (Fig. 1). The structure data files of the phytochemicals under study were retrieved from the EMBL-EBI database (Table 1). For instance, the accession CHEBI:16243 corresponds to the Quercitin (Fig. 2) was obtained. Consequently both the protein and the ligands were used for *in silico* analysis.

Molecular docking: For the *in silico* molecular docking, BIOVIA's Discovery Studio docking method was used for molecular docking. The catalytic pocket of the RdRp protein was specified and targeted for binding of the ligand. CDOCKER Energy and CDOCKER Interaction Energy signify the affinity of the ligands with the protein receptors. Basically, high positive values of the CDOCKER Energy, CDOCKER Interaction Energy and a diminutive difference between the CDOCKER Energy and CDOCKER Interaction Energy are considered to be the most favourable (Behera et al., 2020, Das et al., 2020, Jena et al., 2020, Ray et al., 2020).

RESULTS AND DISCUSSION

Structure-based virtual screening refers to *in silico* identification of potential chemical molecules out of large number of compound libraries, which have high affinity to proteins of known structure, based on the binding of the small molecule with the protein binding pocket. It was found that quercitin binds to the active pocket of the SARS-CoV-2 RdRp (Fig. 3), as apparent from higher CDOCKER energy and CDOCKER interaction energy. Since, simple active bio molecule like quercitin effectively binds into the active pocket of the RdRp under *in silico* conditions it is quite possible to design pharmacophore molecules based on the structural and functional identity of quercitin and eventually can be used in the pharmaceutical sector. Chemical synthesis of quercitin can be cost effective as compared to the isolation process from specific plants.

Figure 1: 3-D Structure of the SARS-CoV-2RdRp showing the active site of the protein.

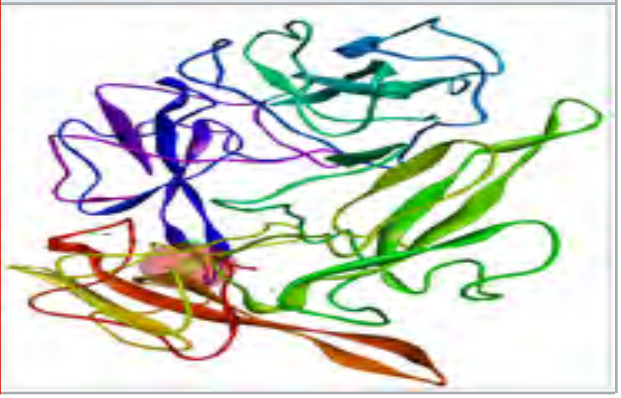
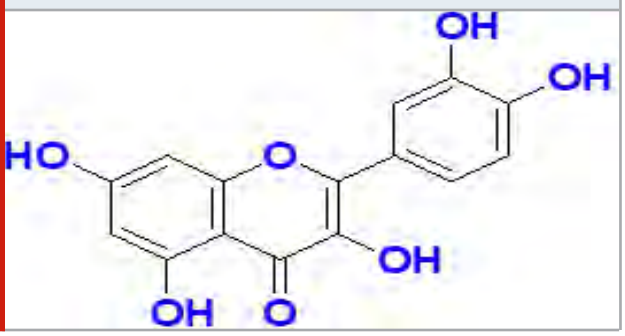


Table 1. Cdocker Energy And Cdocker Interaction Energy values generated for the interaction of several phytochemicals with the active site ofSARS-CoV-2 RNA dependent RNA polymerase (RdRp).

Sl. No.	Phytochemicals	C DOCKER ENERGY	C DOCKER INTERACTION ENERGY
1	Ferulic acid	9.16158	11.6305
2	Genistein	10.4129	19.1312
3	Quercetin	10.57	14.6
4	Glutathione	16.4	7.76
5	Luteolin	7.23	10.5
6	Caffeine	5.45	14.1

Figure 2: Chemical structure of Quercitin



CONCLUSION AND FUTURE PERSPECTIVES

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. Some phytochemicals have been used as poisons and other as traditional medicine. This work is mainly focused on identification of the particular phytochemical responsible for inhibiting and controlling of COVID-19. The current *in silico* molecular docking based study reveals that quercitin can target the reported SARS-CoV-2RdRp. It would be extremely noteworthy being confirmed in vivo. It is crucial to develop diagnostic tools, potential therapeutics and antibodies selectively for the COVID-19 proteins. Phytochemicals like quercitin is commercially available and thus may be effectively prescribed to circumvent the current global scenario. Essentially, this study makes an attempt to reveal simple phytochemicals like quercitin which can be employed for designing novel therapeutics (Fig. 4).

CRediT authorship contribution statement: Gagan Kumar Panigrahi: conceived the idea, performed the experiments, analyzed the results, wrote the manuscript, have read and approved the final manuscript before submission. Kunja Bihari Satapathy: conceived the idea, analyzed the results, have read and approved the final manuscript before submission.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Figure 3: The active site of the SARS-CoV-2 RdRp with Quercitin. 3a: Phytochemical, Quercitin. 3b: Free form of RdRp. 3c:RdRp associated with the ligand, Quercitin. 3d: Magnified image showing the association of the Quercitin with the RdRp. (The white colored arrow and the red colored arrow indicate the active site of the RdRp and binding of Quercitin respectively).

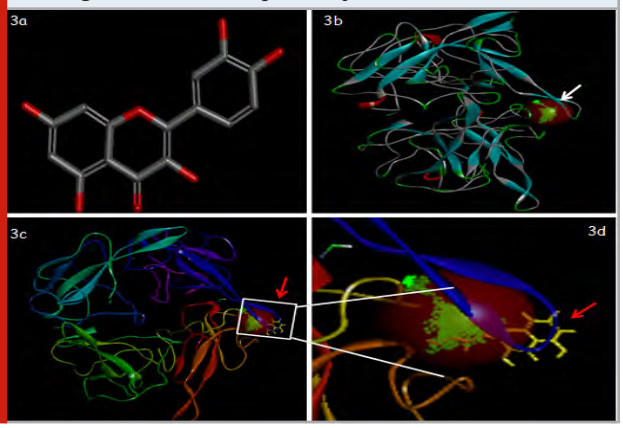
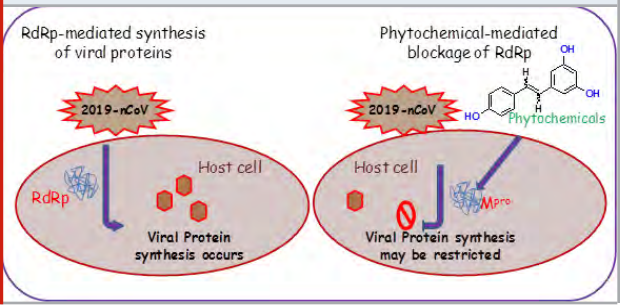


Figure 4: Phytochemicals inhibit the activity of COVID-19 RdRp.



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In Silico Molecular Docking-Based Screening Reveals Phytomolecules Against SARS-COV-2 Main Protease

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ABSTRACT

In past two decades, the globe has faced many infectious disease outbreaks. 2019 Novel Corona-virus (2019-nCoV) or the severe acute respiratory syndrome Corona-virus 2 (SARS-CoV-2) emerged as a global risk and put the entire globe into unrest. Unavailability of specific drug against the virus is more imperative. This demanding situation requires development of bio molecules for competent treatment against the SARS-CoV-2. The crystal structure of SARS-CoV-2 main protease (M^{pro}) may be used for fast *in silico* docking and novel pharmacophores can be discovered. This may result into identification of active bio-molecules largely phytochemicals. In silico Molecular Docking revealed that the phytochemical, Gallic acid effectively binds to the active pocket of the SARS-CoV-2 main protease.

KEY WORDS: 2019-NCOV, SARS-COV-2, SARS-COV-2 MAIN PROTEASE, DOCKING, PHYTOCHEMICALS.

INTRODUCTION

The pandemic situation caused due to the 2019-nCoV represents a severe public health calamity across the globe. The city of Wuhan was the epicentre where the outbreak of this human pathogen emerged, and resulted to human ailment, termed as COVID-19 (Chen et al., 2020, Huang et al., 2020). SARS-CoV-2 belongs to the Beta corona-virus genus, closely related to the previously identified severe acute respiratory syndrome corona-virus (SARS-CoV) (Lu et al., 2020, Wu et al., 2020). Public Health Emergency of International Concern (PHEIC) was declared by the World Health Organization (WHO) owing to its fast rate of transmission within the humans (Chen et al., 2020, Chan et al., 2020, Li et al., 2020).

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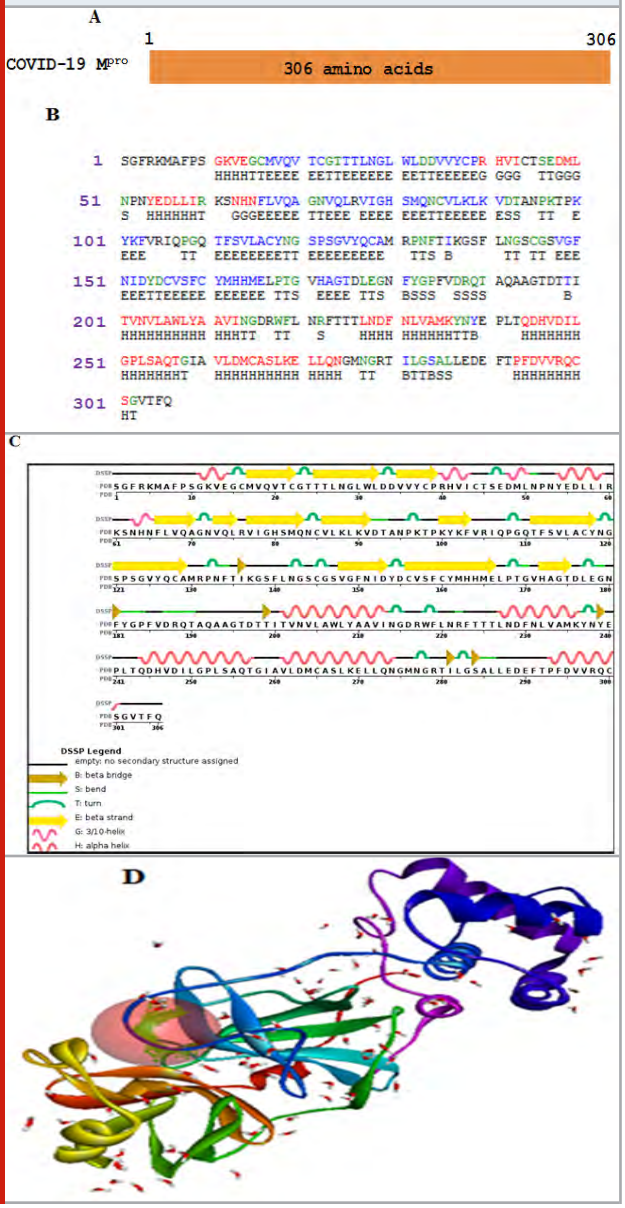
The virus is very contagious and infectious occurs through droplets from coughing and sneezing and touching infected surface. Its genome comprises of nearly 30,000 nucleotides and encoded by 4 structural proteins. Those are Nucleocapsid protein, Membrane protein, Envelope protein, and Spike protein. The virus possesses a positive single stranded RNA. It attacks human cells and converted them into factories of viruses. The capsid protein helps in its replication and transcription. Crystal structure of the SARS-CoV-2 main protease (M^{pro}) proves to be an exceptional ground for screening specific ligands (Liu et al., 2020). SARS-CoV-2 main protease can be beleaguered for developing antibodies, diagnostics and vaccines. Reportedly, M^{pro} and other known viral proteins are defining features paving the path of virus from entry to infection in the host cell (Wrapp et al., 2020, Lung et al., 2020, Sahoo et al., 2020a,b,c,d,e,f, Ton et al., 2020).

Moreover, M^{pro} can also be an effectual target to diminish the viral replications within the host cells since it facilitates the synthesis of functional viral proteins. The effectiveness of traditional medications on the restriction of COVID-19 growth does not have any scientific back up as of now, since the underlying molecular mechanisms are unclear. Plants are enriched with tremendous defense

response capabilities (Panda et al., 2016, Panigrahi et al., 2016, Panigrahi and Sahoo 2016, Panigrahi and Satapathy 2020a, Panigrahi et al., 2021). Elaborated defense mechanism(s) in plants need to be explored (Panigrahi and Satapathy 2020a,b,c). The phytochemicals are fundamentally bioactive compounds and has the potential to amend cellular physiology. Here, we report few phytochemicals which has the ability to bind to the active site of the SARS-CoV-2 main protease as revealed by the *in silico* molecular docking and thus further studies may reveal the effectiveness of phytochemicals to be used as COVID-19 therapeutics.

Figure 1: Amino acid sequence of COVID-19 M^{pro}. (A) Mpro contains 306 amino acids. (B) Sequence and Define Secondary Structure of Proteins (DSSP) image of COVID-19 M^{pro}. (C) Sequence chain image of COVID-19 Mpro. (D) 3-D Structure of the SARS-CoV-2 M^{pro} showing the active site of the protein. Images 1(B) and 1(C) were generated from the Protein Data Bank.



METHODS

Viral Protein Structure and Phytochemical dataset collection: The 3D structure of M^{pro} was accessed from Protein Data Bank accession 6M03 (Fig. 1). The structure data files of the phytochemicals under study were retrieved from the EMBL-EBI database (Table 1). For instance, the accession CHEBI:30778 corresponds to the Gallic acid (Fig. 2). Consequently both the protein and the ligands were used for the *in silico* analysis.

Molecular docking: For the *in silico* molecular docking, BIOVIA's Discovery Studio docking method was used for molecular docking. The catalytic pocket of the M^{pro} protein was specified and targeted for binding of the ligand. -CDOCKER Energy and -CDOCKER Interaction Energy signify the affinity of the ligands with the protein receptors. Basically, high positive values of the CDOCKER Energy, CDOCKER Interaction Energy and a diminutive difference between the -CDOCKER Energy and -CDOCKER Interaction Energy are considered to be the most favourable (Behera et al., 2020, Das et al., 2020, Jena et al., 2020, Ray et al., 2020).

Table 1. Cdocker Energy and Cdocker Interaction Energy values generated for the interaction of several phytochemicals with the active site of SARS-CoV-2 main protease (M^{pro}).

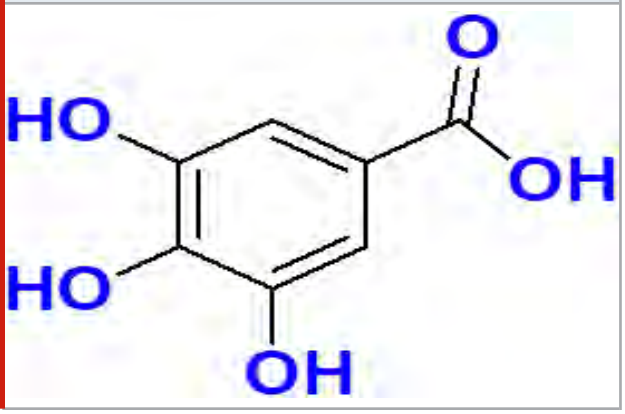
Sl. No.	Phytochemicals	C DOCKER ENERGY	C DOCKER INTERACTION ENERGY
1	Resveratrol	13.04	25.59
2	Phenyl isothiocyanate	13.76	16.31
3	Myricetin	23.39	24.93
4	Caffeine	10.2	18.61
5	Benzyl isothiocyanate	11.54	13.34
6	Kaempferol	20.69	26.33
7	Genistein	15.46	23.32
8	Daidzein	12.16	20.03
9	Theobromine	9.58	14.85
10	Quercetin	24.08	27
11	Pelletierine	10.51	18.36
12	Alliin	11.97	17.96
13	Gallic acid	23.43	20.24
14	Ellagic acid	14.56	24.23
15	Pelargonidin	9.62	29.89
16	Isorhamnetin	16.62	21.62
17	Epicatechin	19.37	27.87
18	Coumarin	11.6	14.69
19	Ferulic acid	17.07	20.79
20	Glutathione	38.56	28.77
21	Sulforaphane	18.51	16.9
22	Salicylic acid	14.28	16.4
23	Eugenol	8.19	18.38
24	Apigenin	19.26	24.2
25	Luteolin	22.63	25.96

RESULTS AND DISCUSSION

Through the process of molecular docking i.e.*in silico* molecular docking, some phytochemicals have shown their effectiveness against the particular disease. As the crystal structure of SARS-CoV-2 has been solved i.e. main protease (M^{pro}), it can be considered as the root way for the screening of inhibitory ligands to detect bioactive molecules. Through *in silico* molecular docking, Gallic acid has remarkably shown the effectiveness against

COVID-19 by binding to the active sites of SARS-CoV-2 main protease (M^{pro}). It was found that Gallic acid,a common phytochemical, specifically binds to the active pocket of the SARS-CoV-2 M^{pro} (Fig. 3), as apparent from higher -CDOCKER energy and -CDOCKER interaction energy. Since, simple active bio molecule like Gallic acid effectively binds into the active pocket of the Mpro under *in silico* conditions it is quite possible to design pharmacophore molecules based on the structural and functional identity of Gallic acid and eventually can be used in the pharmaceutical sectors. Chemical synthesis of Gallic acid can be cost effective as compared to the isolation process from specific plants.

Figure 2: Chemical structure of Gallic acid



CONCLUSION AND FUTURE PERSPECTIVES

As the coronavirus outbreak became the nightmare for the whole human society and the devastation caused by it is unpredictable and beyond imagination, the world has not left with enough time to discover a new drug or vaccine for it due to the requirement of sufficient time. Due to its highly contagious nature, it is considered as global pandemic within no time by taking many lives of people. But future studies on Gallic acid may become the building block for the medication and treatment against the SARS-CoV-2. The current *in silico* molecular docking based study reveals that Gallic acid can target the reported SARS-CoV-2 M^{pro}. It would be extremely noteworthy being confirmed in vivo. It is crucial to develop diagnostic tools, potential therapeutics and antibodies selectively for the COVID-19 proteins. Phytochemicals like Gallic acidis commercially available and thus may be effectively prescribed to circumvent the current global scenario. Essentially, this study makes an attempt to reveal simple phytochemicals like Gallic acid which can be employed for designing novel therapeutics (Fig. 4).

CRediT authorship contribution statement: Annapurna Sahoo: conceived the idea, performed the experiments, analyzed the results, wrote the manuscript, have read and approved the final manuscript before submission. Kunja Bihari Satapathy: conceived the idea, analyzed the results, have read and approved the final manuscript before submission.

Figure 3: The active site of the SARS-CoV-2 main protease (M^{pro}) interacts with Gallic acid. 3a: Phytochemical, Gallic acid. 3b: Free form of the M^{pro}. 3c: M^{pro} associated with the ligand, Gallic acid. 3d: Magnified image showing the association of the Gallic acid with the M^{pro}. (The white coloured arrow and the red coloured arrow indicate the active site of the M^{pro} and binding of Gallic acid respectively).

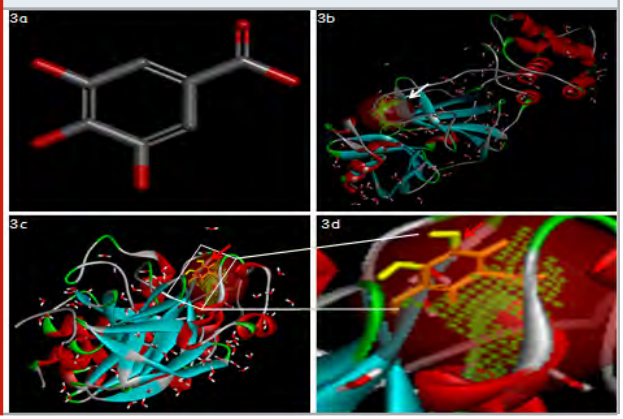
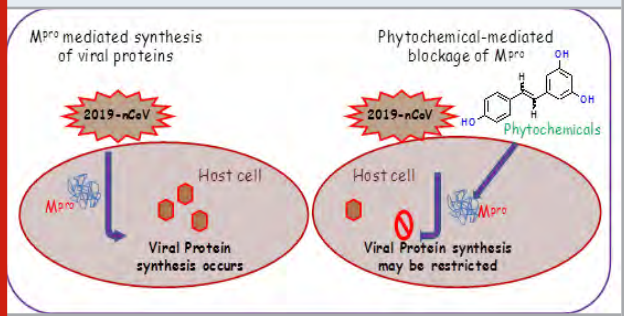


Figure 4: Phytochemicals inhibit the activity of COVID-19 M^{pro}.



Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Screening of Phytochemicals Against Cancer Biomarker: An In Silico Approach

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ABSTRACT

Heparan sulphate proteoglycans (HSPGs) including Glypicans are primarily involved in critical cellular pathways. Essentially, these groups of proteins are located on the cell membrane and modulate signalling pathways resulting in the cell growth process. Strikingly, the protein level of Glypican-1 (GPC-1) rises primarily during pancreatic cancer thus can be considered as a potential clinical biomarker. GPC-1 may also activate further downstream events, supporting the cancerous phase. To restrict the activity of GPC-1, several bio molecules can be deployed, of which the phytochemicals can be the best alternative. Molecular docking-based screening of a few phytochemicals revealed that the organosulfide class of phytochemicals effectively associate with the active site of the GPC-1 and hence harbours diagnostic and therapeutic potentials against pancreatic cancer.

KEY WORDS: GLYPICAN-1, HEPARAN SULPHATE PROTEOGLYCAN, ORGANOSULFIDES, PANCREATIC CANCER, PHYTOCHEMICALS.

INTRODUCTION

Cancer still being considered as a global health problem owing to failure in restricting the casualty it causes (Ferlay et al., 2019). Though the key oncogenic signaling mechanisms are revealed as of now, being intracellular in nature, they are inaccessible to the antibodies (Bailey et al., 2018; Sanchez-Vega et al., 2018). HSPGs reside at the cell surface and in the extracellular matrix (ECM) are essentially the proteins of interest (Knelson et al., 2014; Nagarajan et al., 2018; Christianson et al., 2013). Glypicans belong to the HSPG family. Mostly, glypicans are involved in enhancing the extracellular growth, enabling overgrowth of human cells ultimately leading to cancer (Sarrazin et al., 2011). Even, glypicans are highly expressed in few cancers, regulate angiogenesis thus facilitate the tumourigenesis as apparent from several genetic evidences (Filmus et al., 2008; Fico et al., 2011; Li et al., 2018). GPC-1 is highly expressed in cancerous cells and found in the peripheral blood, thus can be a key biomarker (Lu et al., 2017; Matsuda et al., 2001; Davies et al., 2004; Suhovskih et al., 2013; Hara et al., 2016; Lewis et al., 2018).

GPC-1 knockdown inhibits the response of mitosis to fibroblast growth factor-2, thus pointing out the molecular mechanism of GPC-1 in promoting cancer (Matsuda et al., 2001). GPC-1 also plays a significant role in modulating the VEGF-A and TGF-β signalling pathways (Olsson et al., 2006; Aikawa et al., 2018; Liu et al., 2018, Sahoo et al., 2020a,b,c,d,e,f). Owing to the cleavable nature of the GPC-1 and a secreted soluble component, it is noticeable in the peripheral blood, thus acting as a potential marker (Wang et al., 2019). In order to develop a prospective diagnostic tool or therapeutic agent against GPC-1, several approaches can be considered. Using phytochemicals can also be one of the best alternatives.

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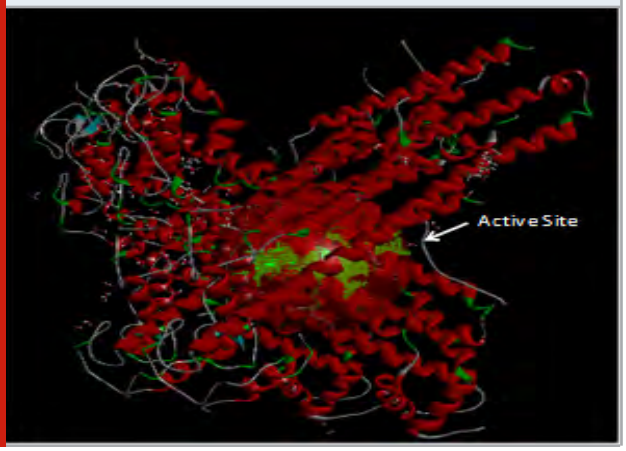
Several categories of phytochemicals are found in the plant parts like fruit, leaf, stem, root, flower and bark which contain immense pharmaceutical functionalities (Jakubowski et al., 1997). Plants possesses sophisticated defense response mechanism(s) which still needs to be elucidated clearly (Panda et al., 2016, Panigrahi et al., 2016, Panigrahi and Sahoo 2016, Panigrahi et al., 2021, Panigrahi and Satapathy 2020a,b,c). For treating diseases like cancer, phytochemical compounds like tocopherols, carotenoids, anthocyanins, phenolics etc. are effective (Altemini et al., 2017; Naczk and Shahidi2006). Several phytochemicals act as natural antioxidants, which supplements the need of the human body (Boots et al., 2008). Across the globe, it is recommended for consumption of fruits and vegetables, primarily to improve the state of health (Vivekananthan et al., 2003). We primarily screened a few phytochemicals, which are not yet globally recognized for being used against GPC-1, using a molecular docking method (BIOVIA).

Figure 1: The 3-D structure along with the active site of the GPC-1



METHODS

Viral Protein Structure and Phytochemicals dataset collection. From the Protein Data Bank (accession: 4AD7), the 3D structure of Glypican-1 protein was accessed (Fig. 1). For docking with the target protein, Glypican-1, ten numbers of phytochemicals were considered and structure data files were used for the purpose.

Molecular docking: *In silico* molecular docking was done by using the BIOVIA's Discovery Studio docking method (Behera et al., 2020, Das et al., 2020, Jena et al., 2020, Ray et al., 2020). The catalytic pocket of the GPC-1 protein was generated and subsequently targeted for ligand interaction.

RESULTS AND DISCUSSION

The positive values of the CDOCKER Energy and CDOCKER INTERACTION ENERGY represent the affinity of the ligands with the receptor proteins. Ten numbers of phytochemicals (Table 1) against the GPC-1 protein revealed that phenyl isothiocyanate, benzyl isothiocyanate and resveratrolare potential binding ligands as evident from their higher CDOCKER ENERGY and CDOCKER INTERACTION ENERGY (Table 1).

Figure 2: Chemical structure of the Phenyl isothiocyanate, Benzyl isothiocyanate and Resveratrol.

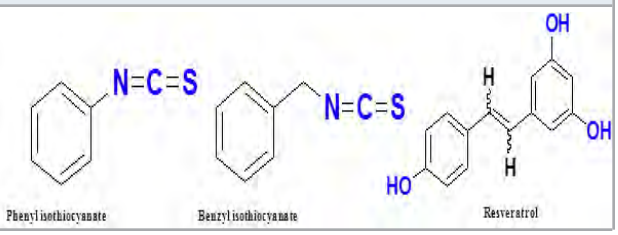
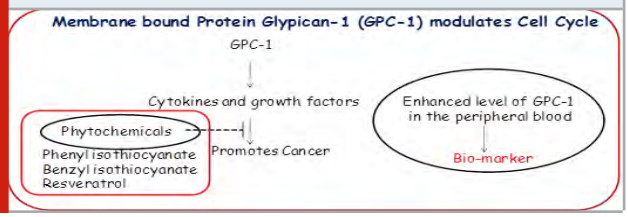


Table 1. List of phytochemicals tested for their binding affinity with the GPC-1.

Ligand			Receptor		Interaction Status		
Class	SDF accession	Phytochemical	Protein	PDB accession	Docking Result	CDOCKER Energy	CDOCKER Interaction Energy
Flavonoids	CHEBI:28775	Hesperidin	Glypican-1	4AD7	Negative	Not applicable	Not applicable
	CHEBI:23053	Epicatechin			Negative	Not applicable	Not applicable
	CHEBI:9400	Tangeretin			Negative	Not applicable	Not applicable
Organosulfides	CHEBI:85103	Phenyl isothiocyanate			Positive	11.78	14.17
	CHEBI:17484	Benzylisothiocyanate			Positive	15.02	17.41
	CHEBI:28411	Allicin			Negative	Not applicable	Not applicable
	CHEBI:47807	Sulforaphane			Negative	Not applicable	Not applicable
Anthocyanin	CHEBI:71682	Cyanidin			Negative	Not applicable	Not applicable
	CHEBI:6674	Malvidin			Negative	Not applicable	Not applicable
Stylbenes	CHEBI:45713	Resveratrol			Positive	21.23	33.55

Figure 3: Phytochemicals can be effectively used for blocking the activity of the GPC-1.



These are very common and easily available. Phytochemicals including Hesperidin, Epicatechin, Tangeretin, Allicin, Sulforaphane, Cyanidin and Malvidin did not show affinity for the active site of the GPC-1 as the docking results were failed. The chemical structure of ligand molecules (Fig. 2) showing positive affinity for the GPC-1 can be studied extensively and related synthetic molecules can be developed for wide range applications in the cancer therapeutics.

CONCLUSION AND FUTURE PERSPECTIVES

In silico molecular docking based study reveals several novel candidate molecules which can target the Glypican-1 protein. It would be highly significant being confirmed in vivo. Specific phytochemical targeting GPC-1 can be employed in two ways. Firstly, these phytomolecules may act as drug by blocking the specific sites of GPC-1, ultimately inhibiting the downstream pathways. Secondly, cost effective medical device can be developed to diagnose early stages of cancer by targeting marker proteins like GPC-1. Phytochemicals including phenyl isothiocyanate, benzyl isothiocyanate, resveratrolmay be effective. Since, glypicans are highly significant in modulating the growth factor signaling and promote cancerous activity; they should be restricted being over activated by blocking their active site (Fig. 3). Early diagnosis being a critical issue in several cancers, appropriate ligands can be developed to be used as a diagnostic tool.

CRediT authorship contribution statement
Annapurna Sahoo: conceived the idea, performed the experiments, analyzed the results, wrote the manuscript, have read and approved the final manuscript before submission. Kunja Bihari Satapathy: conceived the idea, analyzed the results, have read and approved the final manuscript before submission.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Antibacterial Properties of Simple Chemically Fabricated ZnS/Graphene Composite

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ABSTRACT

In the present work a simple wet chemical method has been adopted for synthesis ZnS and ZnS-Graphene composite in low temperature range of 50-90 0C. XRD analysis shows cubic phase of thus prepared sample and morphology analysis by SEM indicates mono dispersed particle morphology. The graphene composite has been prepared and investigated, which was found to show enhanced antibacterial activity towards E.Coli in comparison to neat ZnS.

KEY WORDS: SEMICONDUCTING MATERIAL, GRAPHENE COMPOSITE, ZINC SULPHIDE, ANTIBACTERIAL ACTIVITY.

INTRODUCTION

To combat bacterial infections, antibacterial agents are widely used because of their powerful outcomes. Reagarding this, many researches has been focussed on the search for new antibacterial agents to combat the gradual developing resistance against potent antibiotics. However studies have indicated that widespread use of antibiotics has led to emergence of bacterial resistance to multiple drugs. Such abuse of antibacterial drugs are known to be the cause of development of superbacteria which are reportedly resistant to nearly all antibiotics owing to presence of gene NDM-1. In addition, reckless use of antibiotics has led to various health hazards to public health such as superbugs that do not respond to any existing drugs . Therefore, this necessitates a search for effective antibacterial materials. Regarding this, different nanoparticles have been used as alternative to antibiotics, which is because of the fact that nanoparticles are capable of preventing microbial drug resistance in certain cases .

Now-a-days intensive research is going on composite materials in which the matrix is usually a conducting substrate such as conducting polymer, graphene, metal powder etc. Further, it is known that, combination of such semiconducting nanomaterials with some typical conducting polymer matrix is expected to improve some of their useful properties such as thermal, mechanical properties, electro chemical properties and physicochemical qualities because of synergistic effects.

In this regard, graphene stands out as the most promising candidate to be a major filling agent for composite applications. At very low loading of fillers, such matrix have found to improved in multifunctional aspects in comparison to bare filler material. These materials are additional advantages like their lightness and simple and easy method of processing. In addition, these also make the material stronger for various multifunctional applications with simultaneous improvement in the physicochemical qualities of the host matrix upon distribution. This has additional benefits of increased interfacial bonds between the layers of graphene and the guest filler matrix. Such a bonding leads to reinforcement

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of improvement in synergistic properties in composites such as catalytic as well as antibacterial activities.

Synthesis method: All the Chemical reagents were of analytical grade without further purification and they are listed billow. Graphene powder, hydrogen peroxide, zinc nitrate, sodium sulfide, methylorange powder, sodium nitrate, potassium permanganate, these reagents werepurchased from Nice chemical private limited, Sulphuric acid, disodium salt of ethylene diaminetetraacetic acid (EDTA) and sodium dodecyl sulphate (SDS) wereprocured from CDH chemicals private limited, while Sodium borohydrade and Ethylene diamine were taken from Spectrochemprivate limited. The bacterial strain Escherichia coli (HO25), Nutrient agar, Nutrient broth,Mueller-Hinton agarwas provided from the Department of paramedics, CUTM.Odisha.

Preparation of ZnS: In a typical procedure 0.73 g of Zinc nitrate and 5g of EDTA/ 5mL of Ethylene diamine was added into 50 mL of water at 50 °C under stirring for half an hour. After that 5.9 g of SDS (5 times of CMC) was added followed by 0.6 g of sodium sulfide and temperature was increased up to 50 °C at reflux condition for 2hours. Then it was filtered and washed for several times to remove excesses amount of soluble impurities. Then the resulting mixture was allowed to dry in the vacuum oven at 60°C. The same procedure was repeated at 70 and 90 °C

Preparation of Graphene Oxide (GO): Aqueous suspension of GO was synthesized by modified Hummers method. In a typical procedure, 1.5g of graphite powder and 1g of Sodium nitrate was slowly added into 34 mL of sulphuric acid under stirring in an ice bath at below 10oC. After 30min 6g of potassium permanganate was added slowly, after 2 h of continuous stirring the resulting dark green suspension was removed from the ice bath to maintain normal temperature then 100 mL of water was slowly added to it under stirring which results in an increase in temperature up to 98°C. after that 10 mL Hydrogen peroxide was added, after addition of hydrogen peroxide a yellowish brown solution was obtained and it allowed to settle for hours, then the final mixture was separated and washed with water for several times to remove excess of sulphuric acid along with soluble impurities. After that the resulting black pasty mass was allowed to dry vacuum oven at 60 °C

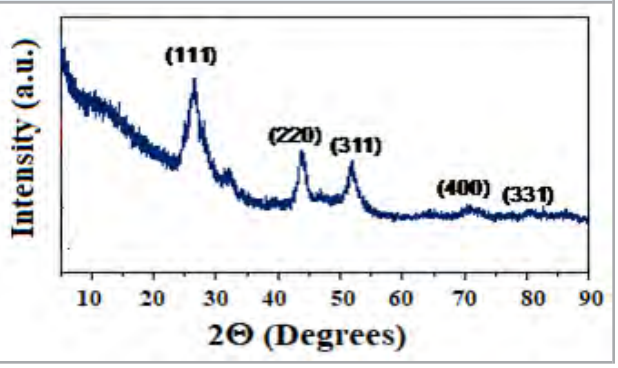
Preparation of reduced graphene oxide: To prepare reduced graphene 0.5 g of graphene oxide was suspended into 50 mL of water followed by the addition of 0.07 g of sodium borohydrade at 90 °C under reflux condition for 2 hrs with continuous stirring.

Preparation of ZnS-Graphene: ZnS-Graphene nanocomposite was synthesised by wet chemical method. Here 0.5 g of graphene oxide, 0.73 g of Zinc nitrate and 5 g of EDTA (or 5mL) of Ethylene diamine was added into 50 mL of water at 50°C under stirring for half an hour. After that 5.9g of SDS (5times of CMC) was added followed by 0.6 g of sodium sulfide and temperature was increased up to 90°C at reflux condition for 2hours. Subsequently 0.07g of sodium borohydrade was added into it, after 30min it was filtered and washed for several times to remove excesses amount of soluble impurities. Then resulting mixture was allowed to dry in vacuum oven at 60°C. Similarly ZnS-Graphene nano composite was prepared.

Antibacterial Study: The bacterial activity was studied by chromogenic medium (chromagar) and the antibiotic sensitivity profile was determined by using Mueller-Hinton agar (MHA*); the pH of the medium was adjusted to 7.0 and sterilized all media and glass wares at 25 lbs pressure and 120 °C temperature in an autoclave. Subsequently 20ml of agar medium was poured into either 100mm or 150mm petri-dishes and let's dry in a laminar air flow to solidify, which takes up to 20min.

Characterization: The structural characterization of the particles were done by X-ray diffraction (XRD) employing Philips X-ray DiffractometerXpert with CuKα radiation in the 2θ range of 5°-90°. Figure 1 and 2 shows XRD patterns of ZnS prepared with different templating agents viz EDTA and Ethylenediamine individually at 50, 70 and 90 oC. The XRD showing peaks at 2θ= 19.085, 28.151, 29.34, 34.55, 43.94, 51.83, indicate cubic phase of the sample according to JCPDS card 77-2100. The prominent peaks were seen to be broadened indicating the nanocrystallinity of all the samples. The analysis of XRD pattern stated that ZnS nanoparticles possess The average crystallite size determined using Debye Scherrer's equation,

Figure 1: XRD pattern of synthesized ZnS particle prepared with EDTA at (a) 50 °C, (b) 70 °C and (c) 90 °C.



$$D=K\lambda/(\beta \cos \theta)$$

Where K, λ, β, and θ are Scherrer constant, wavelength of X-ray radiation target used, maximum peak width in half height and angle of diffraction respectively. The presented data shows the composite synthesized at 90 oC with EDTAand EN, have crystallite size 92 nm and 98 nm respectively. In case of the graphene composite, there is a notable degree of decrease in intensity of the peak at 2θ centered around 25, which indicates appreciable degree of exfoliation of the graphene sheets [29-31].

Scanning Electron Microscope (SEM): The surface structure of the synthesized ZnS nanoparticles prepared with different temperatures were studied with the help of SEM. Figure 4 clearly reveal that the ZnS nanoparticles are irregular in shape and uniformly distributed. Figure 5 shows the SEM images of ZnS-Graphene nanocomposite from which it is evident that ZnS nanoparticles tend to be regularly distributed on the graphene platelets.

Figure 2: SEM images of ZnS in presence of EDTA at 90 °C

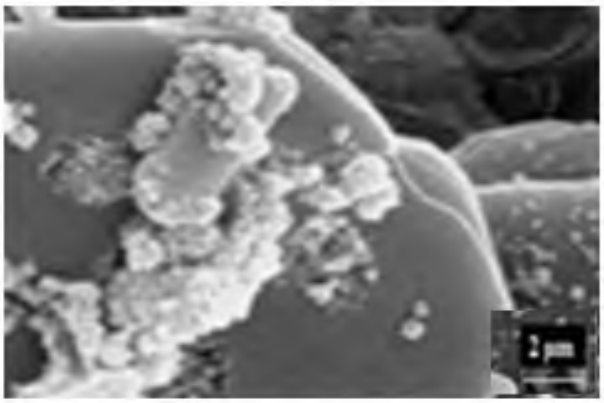


Table 1. Zone of inhabitations against *E.Coli* with respect to relevant specimens

Sl. No.	Types of compound	Zone of inhabitation (Diameter in mm)
1	ZnS/EDTA/90 °C	14
2	ZnSEN/90 °C	13
7	ZnS/Gr/EDTA	21
8	ZnS/Gr/EN	24

Antibacterial activity: The antimicrobial activity was studied using *Escherichia coli* bacteria. The inhibition zone was measured in millimetres around the 'well' having ZnS nanopowder synthesised at different experimental conditions along with graphene composite and has been represented in Figures 9 and 10 and corresponding inhibition zones observed are represented in Table 1. The inhibition zone. was found with ZnS which was prepared with EN at a temperature of 90 oC, which was 17 mm against *E. coli* and that in case of corresponding graphene composites was found to be 20 mm. It should be noted here that in case composite, the amount of ZnS was very small in percentage (~30% or nearly one third) and inspite of this it is showing appreciable antibacterial activity towards *E-coli* bacteria.

Moreover it is important to mention here that, the antibacterial activity performed using neat graphene only, does not indicate any inhibition zone. Thus, from above observations it can easily be concluded that the graphene composites inevitably shows greater antibacterial activity in the present case, which is ascribed due to synergistic effect [32-34]. The ZnS prepared with EDTA at temperature 50 °C had a smallest inhibition zone of 13 mm. While the composite which prepared with EDTA doesn't shows any inhabitation zone. This fact possibly due to formation of some type of organic coating around the sample due to which it inhibits the antibacterial activity and needs further research.

CONCLUSION

ZnS nanoparticles was successfully synthesised by wet chemical processes. XRD data and SEM images confirms that particle size varies at different temperatures as increase in temperature particle size increases. The uniformity of distribution of particles on graphene sheets has been confirmed by morphology analysis. Further, It also has been observed that the antibacterial activity also increases with higher particle size, which in turn, is higher in case of the graphene composite in comparison to neat compound.

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Effect of Nickel on Germination, Seedling Growth And Biochemical Alterations of *Sesamum orientale* L.

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ABSTRACT

Environmental pollution by toxic metal has accelerated dramatically since the beginning of the industrial revolution. The primary source of this pollution include the burning of fossils, mining and smelting of metaliferous ores, municipal waste, fertilizers, pesticide and sewage. Toxic metal contamination of ground water and soil, which poses major environmental and human health problems, is currently in need of an effective and affordable technological solution. In this study, germination was conducted in Til (*Sesamum orientale* L.) in order to find out the effect of Ni toxicity on its germination, growth and biochemical parameters. The seeds were germinated in six different concentrations of nickel chlorode solution having 0-50 mg/l of nickel. The pot culture experiment was done with different concentrations (10, 20, 30, 40 and 50 ppm) of nickel. It was noted that the Seedling vigour index, Metal tolerance index were found to be reduced and the percentage of phytotoxicity was increased and biochemical parameters showed a declining trend with increasing Ni concentrations. The seedlings treated with Ni showed decreased chlorophyll and soluble protein content as compared to control while increased proline content was observed as compared to control.

KEY WORDS: NICKEL, GERMINATION, BIOCHEMICAL CHANGES, PHYTOTOXICITY, SEEDLING VIGOUR INDEX.

INTRODUCTION

Heavy metals are significant environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. The term ''heavy metals'' refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration. There are 35 metals that concern us because of occupational and residential exposure, out of which 23 are the heavy elements or "heavy metals". Heavy metal can include elements lighter than carbon and can include some of the heaviest metals [1]. Metals such as aluminium, arsenic, cadmium, cobalt, chromium, copper, lead, manganese, mercury, nickel, selenium and zinc have been considered as the major environmental pollutants and their phytotoxicity has been established [2, 3, 4, 5].

It has been reported that metals such as cobalt, copper, chromium, iron, magnesium, manganese, molybdenum, nickel, selenium and zinc are essential nutrients that are required for various biochemical and physiological functions. Inadequate supply of these micronutrients results in a variety of deficiency diseases or syndromes. Elevated concentrations of both essential and nonessential heavy metals in soil and water can lead to toxicity symptoms and growth inhibition in most plants [6, 7, 8]. Absorption, translocation and accumulation of heavy metal ions of Hg, Pb, Cr and Cd by plants, reduce qualitative and quantitative productivity of the species and cause serious health hazards through the food chain to other life forms [9, 10, 11, 12, 13, 14, 15]. Different heavy metals at supra-optimal concentrations have been shown to inhibit various metabolic process in plants

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resulting in their reduced growth and development [16, 17, 18, 19, 20, 21]. As nickel is an important heavy metal pollutant, this work was undertaken with an objective to determine its effect on the growth and development of *Sesamum orientale* L.

MATERIAL AND METHODS

Selection of plant material: *Sesamum orientale* L. belongs to an angiospermic family Pedaliceae and commonly called Til/Rassi is one of the most ancient oil seeds crop known to mankind. Sesame plays an important role in human nutrition. Most of the sesame seeds are used for oil extraction and the rest are used for edible purposes. Seeds of *Sesamum orientale* L. were obtained from "National Seed Corporation", Bhubaneswar. The seeds were stored in dark and cool place for experimental use.

Experimental Design: The present study was undertaken with nickel chloride solutions at 10, 20, 30, 40 and 50 ppm along with control (untreated). Twenty seeds of *Sesamum orientale* L. each of were surface sterilized with 0.1% mercuric chloride and washed thoroughly with tap water and then with distilled water. Twenty uniform sized seeds were placed in petri-dishes of 10 cm diameter with different concentrations of Nickel chloride solution (10, 20, 30, 40 and 50 mg of Ni) and one with control at a constant temperature of 26 °C. The seeds were submerged in 10 ml of test solutions and Hoagland nutrient solution twice a day. Each treatment was replicated five times. The number of seeds germinated in each treatment was counted on 5 days after sowing and the total germination percentage was calculated. Tolerance index and Vigour index of seedlings were calculated. This experiment was done in triplicates and the data was statistically analyzed and standard errors of mean (SEM) was calculated.

RESULTS AND DISCUSSION

Germination study: Significant changes were found in the germination of *Sesamum orientale* in different concentration of Ni. The germination percentage was decreased with increased concentration of Ni.

Table 1. Effect of Ni on seed germination of *Sesamum orientale* L. Seedling Values of 5 replicate ±SD

Concentration of Ni	Germination (%)	Radicle length (in cm)	Standard Vigour Index	Metal Tolerance Index	Pytotoxicity (%)
0.0 ppm (control)	85±1	8.4±0.3	714	100	0
10 ppm	85±2	8.4±0.2	714	100	0
20 ppm	60±1	7.55±0.25	453	89	10.11
30 ppm	57.5±1.5	7.05±0.05	405.37	83.92	16.07
40 ppm	47.5±1.5	6.55±0.05	311.12	77.97	22.04
50 ppm	37.5±0.5	5.56±0.25	211.87	67.26	32.73

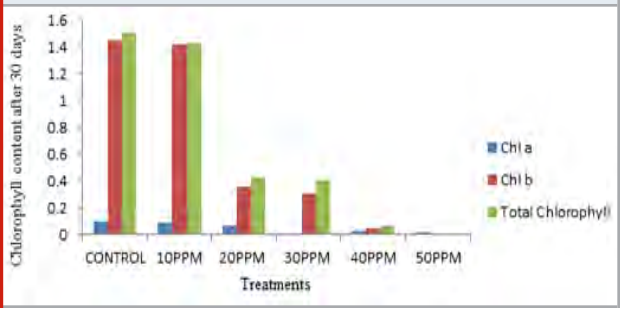
Observations on the germination study of *Sesamum orientale* L. depicted in Table 1. It was clearly indicated that the Seedling vigour index and Metal tolerance index were decreased with increase of concentration of Ni while the phytotoxicity was increased with increase of Ni concentration. The significant decreases in radical length of Sesamum orientale L. seedling suggest that low concentration of Ni was beneficial for seed germination.

Analysis of Biochemical Parameters

Effects on Chlorophyll content of Sesamum orientale L.: The effect of varied concentrations (10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm) of Ni with untreated soil on chlorophyll synthesis in *Sesamum orientale* L. seedlings have been depicted in Fig.1, 2, 3.

Concentration wise, the chlorophyll content after 30 days and 45 days of treatment was found to be increased while after 60 days of treatment the chlorophyll content was decreased.

Figure 1: Effect of different levels of Ni on the Chlorophyll content in *Sesamum orientale* L. after 30 days of treatment.



Effect of Ni on Soluble protein content of Sesamum orientale L.: There was gradual decrease in protein content with rise in different levels of Nickel concentrations.

Protein is produced in less amount when plants are subjected to environmental stress. Here protein content was found to be maximum when plants were subjected

at treatment of 10 ppm. But in both 30 and 45 daysof treatment protein content in 30 ppm concentration are nearly equal whereas the protein concentration was higher in *Sesamum orientale* L. after 60 days of treatment.

Figure 2: Effect of different levels of Ni in Chlorophyll content in *Sesamum orientale* L. after 45 Days of treatment

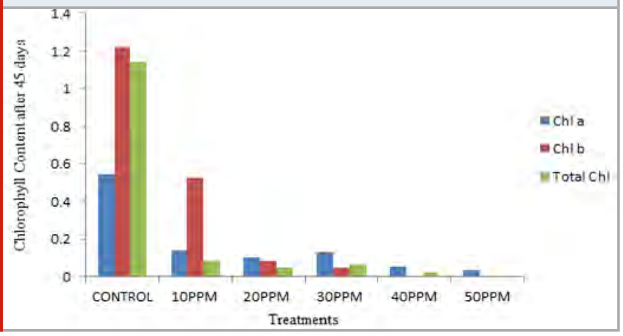
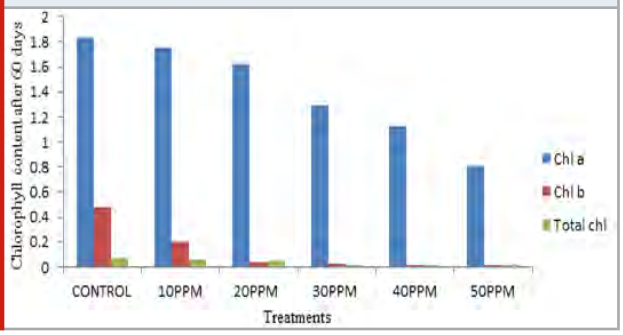


Figure 3: Effect of different levels of Ni on the changes in Chlorophyll content in *Sesamum orientale* after 60 days of treatment.



Effect of Nickel on Proline content of Sesamum orientale L.: Proline is produced in high amounts when plants are subjected to environmental stress. There was a gradual increase in proline content with rise in different levels of Ni which might be due to heavy metal stress.

The possible cause might be the stimulation of some of the enzymes of the proline biosynthetic pathways by Ni which caused the pronounced synthesis of proline at different level of Ni concentrations. There was a gradual increase in proline content with rise in levels of concentration of Ni. The maximum proline content was observed in seedling treated with 50 ppm of Ni and minimum in control irrespective of the days of treatments. Interestingly the proline content was observed to be highest after 45 days of treatment in all the concentrations of Ni.

CONCLUSION

Toxicity of heavy metals has received considerable attention partly due to its occurrence in nature and by mining activities. The data on growth parameter study showed that, with the increase in Ni concentration, the growth rate decreased progressively. Nickel at higher levels may inhibit the growth and development directly by inhibition of cell division or cell elongation or combination of both, resulting in the limited uptake and translocation of nutrients as well as water which causes mineral deficiency. At higher concentrations it acts as a toxic metal. From the result of this investigation, it can be concluded that Nickel at lower concentration has a stimulating effect on the germination process and seedling growth and will inhibit the same at higher concentrations.

Figure 4: Changes in Soluble protein content in *Sesamum orientale* L. after 30, 45 and 60 days of treatment.

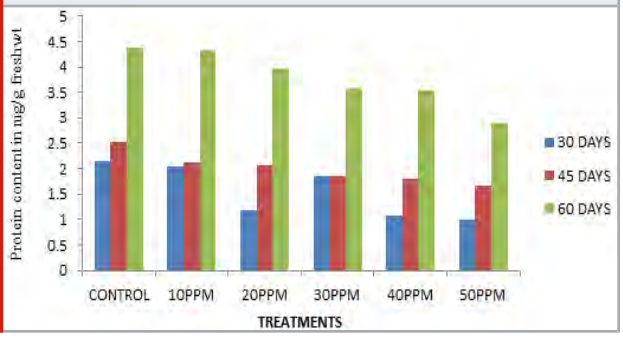
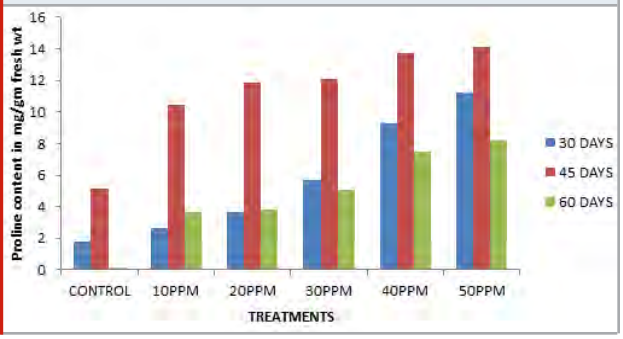


Figure 5: Effect of different levels of Ni on the Proline content after 30, 45 and 60 days of treatment.



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Evaluation of Piprazine Based Nickel Complex as Better BSA Protein Binder

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ABSTRACT

In this work, a Schiff base ligand namely 4-((2-(piprazine-1-yl)ethyl)pent-2-en-2-ol (HL) has been synthesized. Further the reaction between HL and $NiCl_2.6H_2O$ salts has resulted in Schiff base complex with general formula [Ni(L)].Cl. The ligand HL and the corresponding Ni(II) complex was further characterized using various spectroscopic techniques, such as, FT-IR, ESI-MS, and elemental analysis. In the recent scenario, research in the field of protein dynamics is very crucial as these are the major part of the biological systems, and interactions of those macromolecules are responsible for various pathological diseases. Most of the metals inside the biological system shows some kind of affinity to bind with those macromolecules. Herein, it is our unmet need to address the issue by preparing some metal complexes. Therefore, fluorescence spectroscopy was introduced to check the protein binding study of BSA using Stern-Volmer equation.

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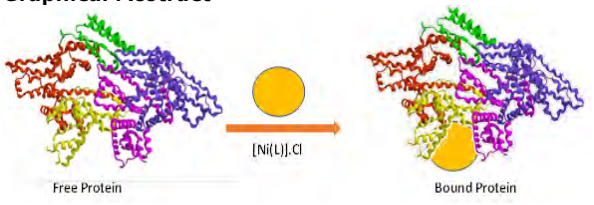

KEY WORDS: SCHIFF BASE, BSA USING STERN-VOLMER.

INTRODUCTION

Biological processes drives to recognize the fundamental principles by the use of transition metals and its functionality due to its diverse nature in the quest of the scientist, which ultimately assistances to advance various structural and more significantly functional model systems.[1-3] Besides studying diverse biological procedures these metal ions are tend to retain the assets like antifungal, antibacterial, antiproliferative, antimicrobial and anticancer activity. These transition metal ions also perform a crucial part in terms of structural organization and overall functionality.[4-6].

Moreover, small metal complexes are quite interactive with essential protein is a current topic of main research as there is adequate potential for the progress of new therapeutic agents predominantly screening antitumor

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activities and the opportunity of conveying these molecules throughout the physiological organization. [6-10] Nickel and copper are two basic examples among the various transition metal ions, which have previously revealed promising capabilities in many of the above stated area.[11-15].

The ability of the serum albumin to bind towards the diverse endogenous and exogenous samples delivers this plasma protein to achieve an effective character for conceivable drug delivery. Thus, the study of the interaction of small molecules with different types of serum albumin is also crucial for the understanding of metallo-pharmaceutical pharmacokinetics and structure–activity relationships. The studies on the interactions between proteins and nickel and copper were not studied much.[16-21]. Thus, we account the synthesis and characterization of a new mononuclear nickel complex [Ni(L)].Cl [where, ligand, HL=1-Phenyl-3-(2-piperazin-1-yl-ethylimino)-but-1-en-1-ol]. The interaction of Ni(II) complex with bovine serum albumin (BSA) also have been considered that displayed favorable consequences with effectual affinity towards the protein.

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MATERIAL AND METHODS

We used the chemicals from Sigma aldrich and applied without additional refinement. A BRUKER TENSOR 27 instrument were recorded Infrared spectra (4000 to 500 cm–1) with using KBr pellets. We used Bruker-Daltonics Mass spectrometric microTOF-Q II mass spectrometer for study. Moreover, the elemental analyses were done with a ThermoFlash 2000 elemental analyzer. Emission spectroscopy were measured in a Horiba JobinYvon (Model: FM-100) made Fluoromax-4p spectrofluorometer from using a 1 cm path length-based quartz cuvette. UV-Visible (200 to 800 nm) spectra were recorded in 0.1 cm path length cell (Hellma, Muellheim/Baden, Germany) using a scan rate of 20 nm min–1 and band width of 1 nm. The ligand, HL and corresponding complexes were characterized using various spectroscopic techniques, such as, FT-IR, ESI-MS, and elemental analysis.

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Synthesis of complex, [Ni(L)].Cl: Ligand, HL (0.062 g, 0.25 mmol) and $NiCl_{2.6}H_2O$ (0.58 g, 0.25 mmol) containing 15 mL of MeOH solution was stirred at refluxing condition for 4 h and the producing light green coloured solution. After that, the solution was concentrated by evaporating the methanol solvent. Green needle shaped crystals were finally obtained after two or three days by keeping the reaction mixture to the slow evaporation at room temperature. Yield: 85%. Anal. Calcd (%): $C_{16}H_{21}ClN_3NiO_5$: H, 4.93; C, 44.74; N, 9.78. Found (%):H, 5.07; C, 44.64; N, 9.26. $[C16H_{21}N_3NiO]+$ (m/z in positive ESI-MS mode) calculated – 330.05 $(m)^+$; obtained – 330.10 $(m)^+$ for $[Ni(L)]^+$. Designated infra-red data on KBr pallets (ν/cm^{-1}): 1603-1604 (C=N), 2951 (N-H) (Figure 2).

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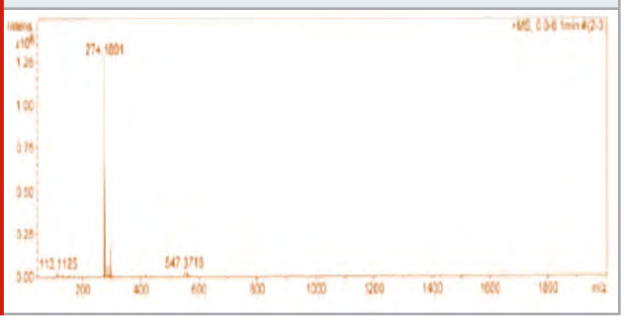

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RESULTS AND DISCUSSION

Synthesis of ligand and complex: The reaction of 1 : 1 molar ratio of amino ethyl piperazine with 1-phenyl-1,3-butanedione in methanol directed to the development of Schiff base ligand, HL (Scheme 1). The ligand can behave as a tetradentate ligand or as a tridentate ligand depending upon its conformation. That is why these types of ligand may be called as flexidentate ligand. Upon reaction of HL with nickel per chlorate in MeOH, a light green colour medium was found. The solution was then dried to the rotary evaporator and the solid compound was collected. A pinch of the sample compound was then dissolved in the acetone for get the nice crystals of [Ni(L)].Cl within 3-4 days at room temperature (Scheme 1).

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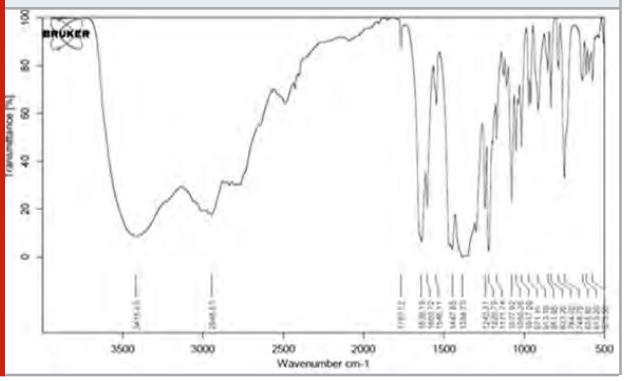

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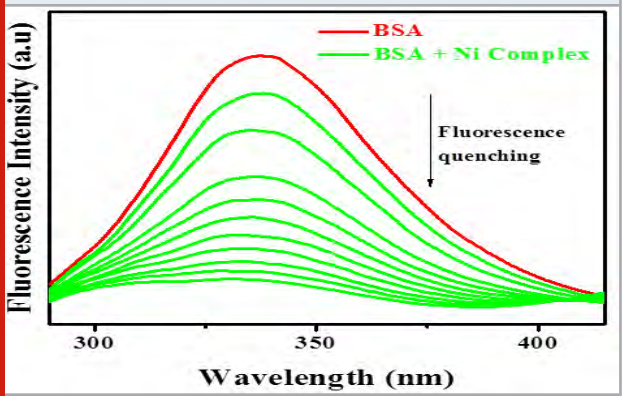
$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV}[Q]$$

where F and F_0 are the emission intensities in the presence and the absence of a quencher, k_q is the rate constant for the bimolecular quenching, τ_0 is the fluorophore's average life time when the quencher is not present and [Q] is quencher (here complex) concentration. K_{SV} is the Stern-Volmer quenching constant in M^{-1}.

Figure 4: Quenching of the fluorescence of bovine serum albumin BSA by Ni(II) complex.



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A Theoretical and Experimental Evaluation of Piprazine Ligand Mediated Zinc Complex Towards BSA Protein Binding

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[2]*School of Vocational Education and Training, Centurion University of Technology and Management, Odisha, India*
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KEY WORDS: SCHIFF BASE, BIOLOGICAL SYSTEMS, METALS INSIDE THE BIOLOGICAL.

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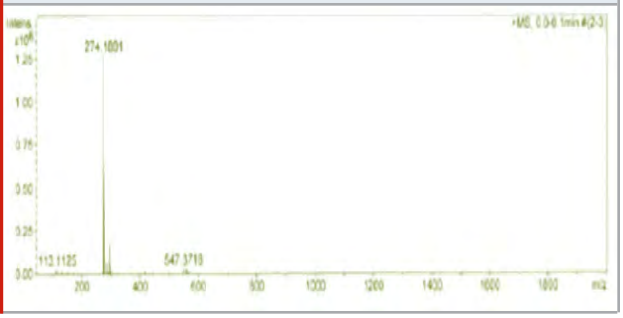

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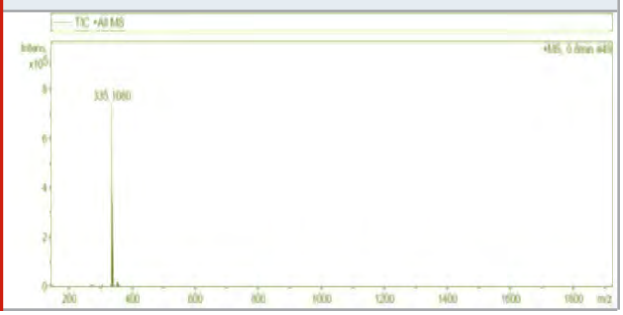

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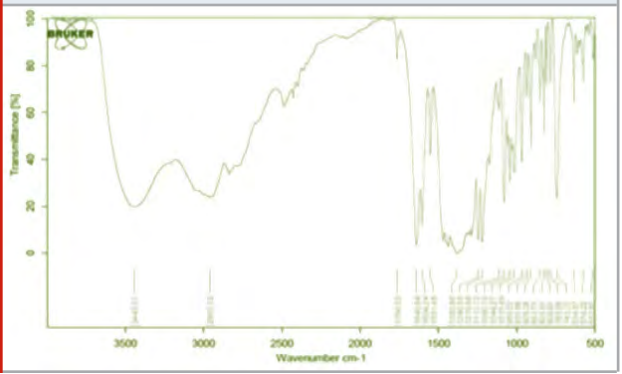

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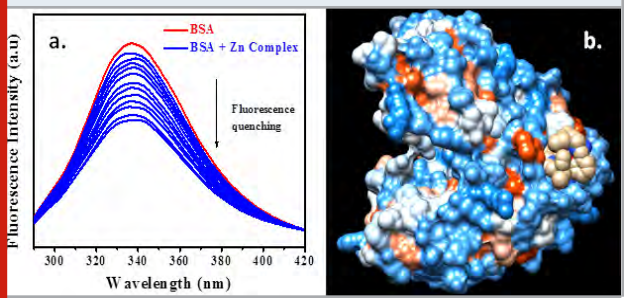
To corroborate the experimental results, the fluorescence quenching affinity further docked with BSA protein (PDB ID: 3V03). The prominent interactions were found with several amino acids of the protein chain, which are basically joined through hydrogen and van der Walls bonding. Thus, we can say that the experimental results are very much similar to the theoretical interpretation.

Figure 4: Quenching of the fluorescence of bovine serum albumin BSA by Zn(II) complex.



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Exploration of Diverse Properties And Gas Sensing Application of Transition Metal Doped Sno_2 Nanocomposites

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ABSTRACT

This manuscript unfolds the structural, surface and optical properties of the transition metal doped stannous oxide in comparison with pure stannous oxide. A simple sol-gel method is used to synthesize all the nanocomposites. It has immense contribution to gas sensing application by knowing its utmost need in current era. The transition metal doped nanocomposites have enhanced gas sensing efficiency than pure stannous oxide.

KEY WORDS: TRANSITION METAL, STANNOUS OXIDE, SOL-GEL, GAS SENSING, OPTICAL PROPERTIES

INTRODUCTION

Nanotechnology is multidisciplinary expanse of investigation and solicitations. It has diverse range of applications from medicine to environmental issues. It is defined as management of matter with at least one dimensional sized ranging from 1 to 100 nanometers. Nano composite is considered as one of significant part of nanotechnology owing to its surface morphology. Nano composites can be defined as multi component materials comprising multiple different (nongaseous) phase domains in which at least one type of phase domain is a continuous phase and in which at least one of the phases has at least one dimension of the order of nanometres. SnO_2 nanoparticles have gained special attention due to its shape and size persuading over properties. SnO2 is a n-type semiconductor having band gap value of 3.6 eV at 300k. It shows transparency in the visible region of the spectrum which enables it to perform in optoelectronic devices, conducive transparent electrodes, supporting system for catalysis, sensor and anti-reflection coatings.

The doping of transition metal in nano composite increases its electrochemical properties by showing better specific capacitance and charge/discharge which make them capable applicants as electrodes in super capacitors combining high energy density with high energy power delivery. The various transition metal doped nanocomposites are fabricated by using simple, cost effective sol-gel method. The transition metal doped SnO2 has extensive usage in gas sensor. Our atmosphere is full of toxic and unwanted gases due to rapid growth of industrialization. Environment can be polluted free by removal of all toxic gas exhausts from automobiles and industries. Gas sensor is the device used to detect varieties of contaminated gas present in air. The gas sensing applications of pure SnO_2 is less effective as compared to transition metal doped stannous oxide. So, doping of transition metal has played intense role in enhancing performance of gas sensor.

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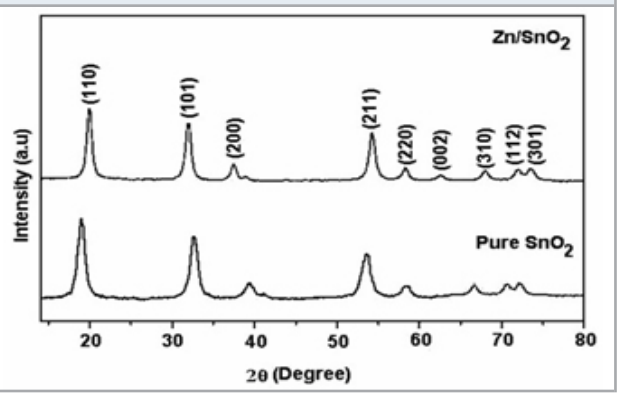
In this study ,the structural, surface morphological, optical properties of the pure SnO_2 and transitional metal doped SnO_2 is explained in systematic manner. The synthesized nanocomposites undergo characterization through different techniques such as XRD, SEM and UV-visible spectroscopy. The analytical study from characteristic spectra helps to outline structural, morphological and optical properties of prepared nanocomposites. The spectra data from X-Ray diffractometry reveals the size of nanocrystal.SEM study helps in figuring out shape of nanostructures by focusing at surface morphology. The interaction between high energy UV rays and nano composite depicts the amount of light absorbed by it and reveals the chemical behavior of prepared sample.

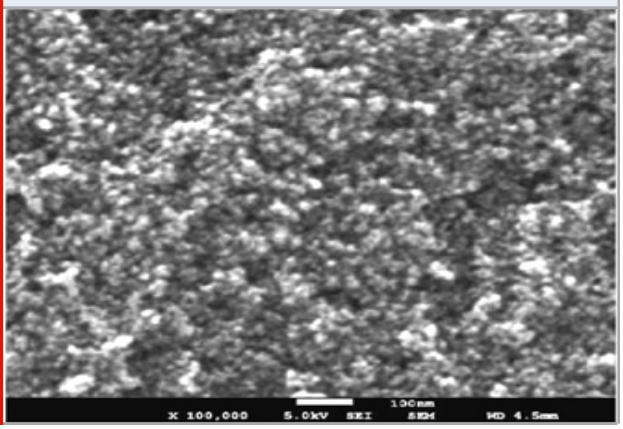
Structural properties: The structural studies of nanomaterial are achieved by considering results from XRD analysis. X-Ray Diffraction (XRD) technology is a non-destructive test procedure employed to investigate structure of nano crystalline materials. It give effort in finding out number of phase present in materials and to reveal information about its chemical composition. The average crystallite size of nanoparticle is obtained by using Scherer formula $D=0.9\lambda/\beta\cos\theta$, where λ depicts X-ray wavelength, θ is the Bragg diffraction angle and β is the FWHM peak performing at the diffraction angle θ. The different phase of SnO_2 is confirmed by all the diffractions peaks. It is noticed that increase in dopant concentration of transition metal in SnO_2 cause shift of peak to a greater angle. SnO_2 nano composite has smaller crystalline size in comparison to the transition metal doped SnO_2. This indicates that in latter case the generation of crystal defect around the dopant imbalance of charge arising from this defect alters the structure of nanomaterial. Some reported data suggested the minimum crystalline size of pure SnO_2 was smaller than transition metal doped SnO_2 which may be due to increase in concentration of dopant increase size of nano crystal and given Figure 1.

Figure 1: XRD characteristics spectra of pure SnO_2 and transition metal doped SnO_2



Surface properties: A scanning electron microscope scans a concentrated electron beam over a surface to generate an image. The electron beam inter mingle with the sample generating ample signals that reveal information about the surface morphology and composition. The transition metal doped SnO_2 nanoparticles is slightly agglomerated particles might be due to lower calcinations temperature. A typical image of SnO_2 nanoparticles given in Figure 2.

Figure 2: SEM image of transitional metal doped stannous oxide [23]



Optical properties: UV spectroscopy is used to determine optical properties of the nanomaterial. It is method employed for the quantitative analysis of the amount of light absorbed by the specified nanosample. It works on the principle of absorption of high energy UV light by molecule followed by electronic excitation which give a spectra. In case of transition metal doped SnO_2 nanocomposites it was noticed from the spectra that absorption edge shifts towards the higher wavelength side as it undergo red shift by increasing dopant concentration. Mostly, particle size decreases undergo decrease in maximum wavelength excitation absorption due to photo-generated electron-hole pairs.

Gas sensing application: The environmental pollution has become matter of concern in the current era. Health issues increases exponentially due to all non-toxic, hazardous gases present in the atmosphere is the main cause of air pollution. By keeping in mind above factor gas sensor has gained attention of research owing to its advantages comprising low cost, compact structure, long life span andsimple circuit. Transition metal doped nano composite has become hotspot in the area of gas sensor [6-9]. A gas sensor is an instrument which converts information of unknown gas into other electrical signals according to specific principles, combining detection principles, material science and processing technology. Though it is widely used in detecting harmful, toxic gases, it has demerits of low response, poor selectivity and high operation temperature. So to overcome above issues doping of transition metal is done by controlling particle size and morphology.

It has been found that doped stannous oxide has higher efficiency than pure stannous oxide. To have an eco-friendly environment, the detection of NO, nitrogen dioxide, ammonia, CO and formaldehyde has become necessary due to their noxious nature and allied threat to the ecology. These pollutants lead to the discrepancy

in atmosphere and global warming. The detection of nitrogen oxide and nitrogen dioxide are the source from ignition and automobiles has become gained huge concern as it is hazardous to flora and fauna species. CO is the tasteless, poisonous generally produced from ignition of fossil fuels and faulty working of apparatus has high toxic effect on the blood stream and nervous system [10]. It is widely used in power transformer in the form of dissolved gas helps in evaluating insulation performance.

So it has extreme significance in the environmental regulation and industrial usage. Ammonia is considered as irritating and corrosive gas whose low concentration cause severe irritation in skin and eyes and high concentration become root cause for caustic damage such as skin burning, eye damage etc. Formaldehyde in huge concentration in air cause throat infection, itching, redness, wheezing and dermatitis. Benzene is highly volatile and easily diffused to the atmosphere leading to create acute and chronic respiratory disease to flora as well as fauna species. Volatile organic compounds are organic substances with enhanced vapour pressure at ordinary conditions. These toxic chemicals have critical and long standing consequences on the health which comprises infection in eye, nose and throat, headaches and nausea. Tremendous researches are done for the development of enhanced sensitive, cost effective, portable sensor with low power intake. The doped nano sample having high surface to volume ratio and hollow structure is considered as model for gas molecule adsorption and storage.

CONCLUSION

This paper outline studies of various properties of transition metal doped stannous oxide in comparison with pure stannous oxide followed by the characteristic spectral interpretations. It has also application in the gas sensor made a revolution in research field. Gas sensing properties include effective nanostructure and morphology which is considered as main ingredient for excellent performances which can be achieved by adding dopant. By comparing it has been behold that transition metal doped stannous oxide has higher efficiency than pure stannous oxide.

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Design and Synthesis of Highly Tunable Inhibitor Against Pathogenic Gram-Negative Bacteria: *Salmonella typhimurium* and *Escherichia coli*

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ABSTRACT

Many of the Gram-negative bacteria are known from decades that cause life-threating diseases in human and many other animals. These bacteria have high tendency to survive in the host body rather quickly due to the presence of protective cell wall which defends from invasion of exogenous toxic agent. Therefore, developing new molecular scaffold with appropriate architectural unit that can circumvent the preventive cell wall of such bacteria is a huge challenge. We report herein, a synthetically simple and elegant, small organic molecule, PA-B-ester which endowed with unique molecular scaffold of prerequisite properties that can easily penetrate through the protective cell wall of the bacteria. We also successfully demonstrated antibacterial activity of PA-B-ester molecule against pathogenic gram-negative bacteria such as: *Salmonella typhimurium* and *Escherichia coli*.

KEY WORDS: GRAM-NEGATIVE BACTERIA, SALMONELLA TYPHIMURIUM, ESCHERICHIA COLI, INFECTED DISEASES, ANTIBACTERIAL ACTIVITY, SMALL ORGANIC MOLECULE, PATHOGENIC BACTERIA.

INTRODUCTION

Gram-negative bacteria such as: Salmonella typhimurium and Escherichia coli are very common pathogens which can infect a broad range of animals. [1, 2] The toxicity effect of gram-negative bacteria is due to the presence of protective cell wall that protect the bacteria from toxic effect of exogenous agent.15 The cell wall of these bacteria is made up of lipopolysaccharide coat (LPS) which strictly prevents the foreign invasion. This unique feature of the cell wall in gram-negative bacteria enables to survive in any adverse environment, such as mammalian intestines. However, most of the gram-positive bacteria devoid of such protective cell wall and therefore, they have poor resistivity in hostile environment than gram-negative bacteria.

Among gram-negative bacteria, *Salmonella* is a major pathogen that infect thousands of lives worldwide even today. [1, 2, 16] It is a rod-shape bacterium having wide range of size varies from 0.4 to 3 µM. The rod-shape is maintained by an actin-like bacterial cyto-skeleton. [3, 4] The pathogenic nature of Salmonella species has reported in many scientific literatures. These *Salmonella* species can cause a broad spectrum of diseases such as: neurological abnormalities, gastroenteritis, and life-threatening Typhoid fever in human being.[5, 7, 11-13] Therefore, *Salmonella* has been a cause to longstanding worldwide health problem and became a reason for significant mortality globally.[6, 11-13] *S. Typhimurium*

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belongs to *Salmonella* and it infects a wide variety of hosts; but however, there are few class of *Salmonella* such as: *S. Typhi, S. Pullorum* and *S. Gallinarum* are exquisitely host-restricted.[6] Furthermore, *Salmonella enterica* serovar *Typhimurium* is also a primarily responsible for food-borne disease as well.[8].

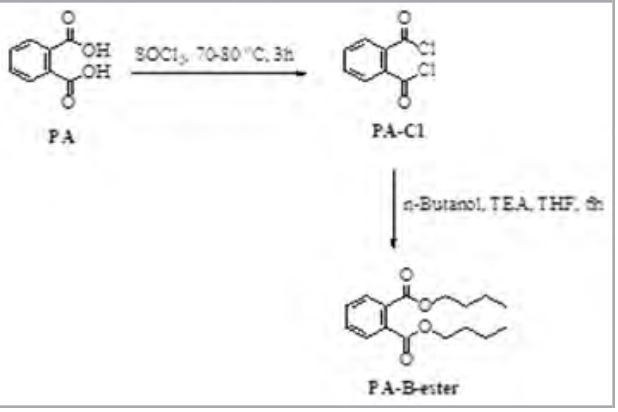
In addition to this, there are another category of gram-negative bacteria, called Escherichia coli which are found in the environment, foods and intestines of people and animal. This is a highly diverse group of bacteria. It has been known in the literature that most of *E. coli* are not harmful, rather helps to keep our digestive track healthy. However, there are few varieties of *E. coli* are highly responsible for causing a broad spectrum of infections such as: diarrhea, food poisoning, pneumonia, urinary tract infections etc. There are different categories of *E. coli* such as: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorragic *E. coli* (EHEC) and enteroaggregative *E. coli* (EAEC) which are responsible for causing diarrhea.[14].

Considering the life-threatening pathogenic effect of gram-negative bacteria, it has been a continuous endeavour from wide spectrum of scientific community to develop novel antibacterial agent. Till today, Most commonly used antibacterial agents are the derivative of quinolones and fluoroquinolones compounds.[9] It is always been a huge challenge for synthetic chemist to develop molecule with well-defined architecture that can strictly inhibit pathogenic gram-negative bacteria. The poor rate of progress is attributed to the protective cell wall of the gram-negative bacteria which demands a precise molecular structure. Therefore, to overcome this difficulty, herein we have developed a new scaffold with stringent architectural component inbuild into the benzene core. We have demonstrated potential antibacterial activity of this molecule against gram-negative bacteria.

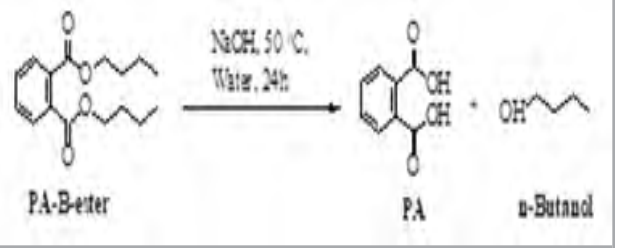
RESULTS AND DISCUSSION

There are numerous coupling reagents (DCC, HATU, HBTU, EDC, SOCl2 etc.) available now a days to convert acid to corresponding amide or ester. But all these amide or ester coupling reagents such as: 1) DCC, 2) HATU, 3) HBTU, 3) EDC are highly expensive and therefore, difficult to afford them for academic research. But on the other hand, thionyl chloride (SOCl2) is another robust alternative for the synthesis of an amide or ester from acid. Furthermore, it is comparatively much cheaper than other coupling reagents. This reagent is pretty simple to handle and moreover, it affords high yield. Therefore, this reagent became central to many of the organic transformation for industrial uses. In light of these potential advantages, we have utilized the thionyl chloride (SOCl2) as a coupling agent to generate a new variety of molecular scaffolds which might inhibit toxic pathogen like Gram (-ve) bacteria such as: *Salmonella* and *E. coli*. (Scheme 1).

Scheme 1: Synthetic scheme for the synthesis of PA-B-ester



Scheme 2: Base hydrolysis of PA-B-ester



In search of an ideal molecular scaffold for the precise installation of n-butanol unit in order to have ester-based molecules, we intended to start with semi-rigid backbone like phthalic acid (PA). We envisioned that benzene ring in PA will provide rigidity along with pi-pi stacking interaction with hydrophobic region of the toxic pathogen which in turn could provide good binding efficacy. In addition to that the ester functionality may help in making hydrogen bonding interaction with hydrophilic part of the pathogen. The remaining butane tail may provide flexibility to the core structure and along with that it may enhance the possibility of cell permeability nature of the molecule. Consequently, synthesizing this kind of molecule may inhibit pathogens. Having these anticipations in mind, we wanted to install the ester moiety into a rigid system, Phthalic acid (PA). Therefore, we persuaded to synthesize compound, PA-B-ester (Scheme 1) with an intention that this compound may show better inhibition property towards pathogen. In the beginning, the precursor compound, phthalic acid (PA) converted to corresponding acid chloride, PA-Cl by treating with thionyl chloride under room temperature for 3 hours (Table 1).

The mixture of water and tetrahydrofuran was chosen as the solvent system to carry out the reaction. Minimum amount of water was taken to solubilize the precursor compound, phthalic acid. The resulted acid chloride, PA-Cl was further treated with n-butanol in the presence of triethyl amine as a base to get the desired product, PA-B-ester. Reaction kinetics was completely monitored by thin layer chromatography (TLC) techniques. But surprisingly,

TLC result analysis showed no new product formation, rater mostly precursor, PA was observed on TLC (Figure 1). We anticipated that the phthalic acid does not convert to corresponding acid chloride due to high transition state energy barrier which may require high temperature rather than room temperature. Therefore, to overcome this challenge we further executed the reaction in the reflux condition keeping all other parameters unaltered. TLC result shows very mild product formation (Figure 1) which is negligible.

This experiment clearly indicating that, not only temperature but also protic solvent like water and methanol play a role in inhibiting the reaction. In presence of methanol we observed almost the similar results like water. Therefore, we turn our attention to start the reaction using only aprotic solvent like THF. To overcome this problem, we designed two experiment in parallel by using THF as solvent. In the first experiment, the reaction was carried out at room temperature in presence of THF as a solvent and kept all other parameter constant. However, in the second experiment the reaction was carried out in reflux condition in presence of THF as a solvent without altering any other parameters. TLC analysis clearly shows new product formation (Figure 1, TLC-4) in both cases. The intensity of product spot observed for refluxed reaction is much brighter than room temperature reaction. These results clearly reveal that the reaction must be carry out in aprotic solvent at refluxed condition to get the optimum product formation.

With this optimized reaction condition, we observed up to 90 % of product yield (Table-2). The products were further purified by column chromatography in 1% methanol and chloroform system. The desired product, PA-B-ester formation was further confirmed by UV-Visible spectroscopy study (Figure 3) by comparing with commercially available starting material. This data clearly showed the formation of product. Elemental analysis data also support the product formation. Again, to cross verify the product formation we carried out the base hydrolysis reaction (Scheme 2) with sodium hydroxide solution in water as a solvent and isolated the hydrolyzed products using column chromatography techniques. After column purification of crude product, we observed two kinds of material. One material is solid and another one is liquid. The solid material expected to be phthalic acid and liquid material may be n-butanol. The solid material obtained after hydrolysis was analyzed by comparing the TLC with commercially available starting material. The TLC result clearly shows, the hydrolyzed product is a starting material (Figure 2).

It was further confirmed by comparing melting point with the starting material. The melting point of the starting material (207 °C) was found to be same as that of isolated product (207 °C). This indicated that the hydrolyzed product is PA (Scheme 1). The liquid material obtained after hydrolysis was analyzed using TLC with reference to commercially available n-butanol compound. TLC result clearly showed both have the same retention time. This result indicated the second hydrolysis product may be n-butanol. It further confirmed by determining the boiling point of the liquid material which was similar to n-butanol. This result confirmed that the resulted hydrolysis product is a n-butanol. Therefore, these results strongly support the formation ester derivative, PA-B-ester.

Figure 1: TLC-1-Reaction carried in water+ THF solvent mixture at room temperature; TLC-2-Reaction carried in water as solvent at reflux condition; TLC-3-Reaction carried in methanol as solvent at reflux condition; TLC-4-Reaction carried out in THF at reflux condition

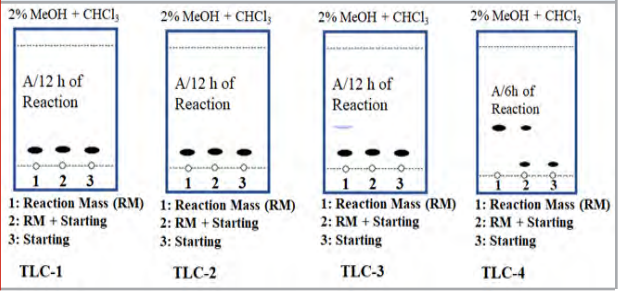


Figure 2: Monitoring the reaction kinetics of PA-B-ester hydrolysis reaction (scheme-2): after 1, 6, and 12 hours of reaction time using TLC.

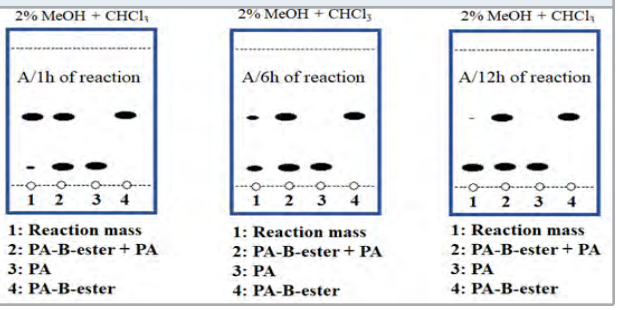
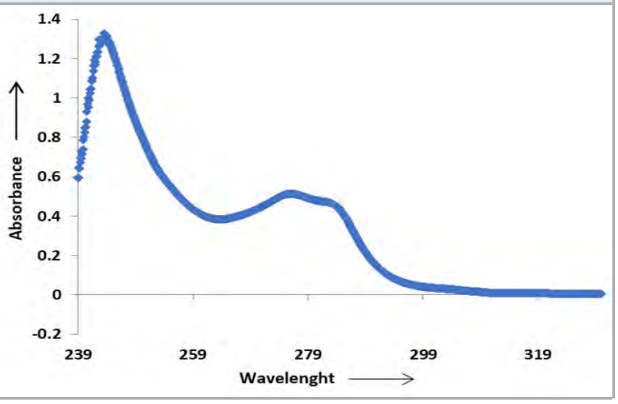


Figure 3: UV-VIS Spectra of the compound, PA-B-ester



The antibacterial activities of PA-B-ester against pathogenic bacteria i.e. *Salmonella typhimurium* and *Escherichia coli* were checked. A standardized concentration of both the inoculums were evenly spread on the surface of two different agar plates. A 500 µM concentration of PA-B-ester was loaded into both the bore

wells and incubated for 24h at 30±2oC temperature. The results clearly showed a very strong inhibition zone of diameter 30.2 mm size against *Salmonella typhimurium* after incubation with PA-B-ester for 24 hours (Figure 4a). This result clearly reveals that, PA-B-ester has potential antibacterial activity against *Salmonella typhimurium*. However, the antibacterial activities of PA-B-ester was found to be relatively lesser for *Escherichia coli* than Salmonella typhimurium which is evident from the inhibition or clearance zones of both the pathogenic strains (Figure 4 a & b).

Table 1. Chemicals used for the reaction

Chemicals	Molecular Weight in g/mol	Quantity	Moles
Phthalic acid $\{C_6H_4(COOH)_2\}$	166.13	5 gm	0.03
Thionyl Chloride ($SOCl_2$)	118.97	6.6 ml.	0.075
THF (C_4H_8O)	72.11	100 ml.	0.09
n-Butanol ($C_4H_{10}O$)	74.121	6.04 ml.	0.81

This antibacterial activity could be due to the presence of well-balanced hydrophobic, hydrophilic and long chain hydrocarbon molecues. Owing to these well-balanced properties of PA-B-ester, might be proficient to cross the bacterial cell membrane resulting in the inhibition of the bacterial strain growth.

Experimental section
MATERIAL AND METHODS

The chemicals and solvents were purchased from Spectro chem Ltd and Sigma Aldrich. All the chemicals were directly used without further purification. Normal phase column chromatography purification was carried out by using MERCK silica gel 60 (particle size: 100-200 mesh). Reactions were monitored wherever possible by thin layer chromatography (TLC). Silica gel G (Merck) was used for TLC and column chromatography was undertaken on silica gel (100–200 mesh) in hexane, hexane–ethyl acetate or chloroform. UV and visible peaks of synthesized organic compound was measured in chloroform as a solvent in the range of 200-400 nm. The walelength (in nm) was taken in X-axis and absorbance in the Y-axis. It shows maximum absorbance at 243.6 nm wavelength. Melting points were recorded in a Fisher-Johns melting point apparatus.

Table 2. Reaction analysis in different solvent system

Solvents	Temperature	TLC Analysis	Yield	Remark
Water + THF	Room Temp. (RT)	No new spot observed	0%	The reaction was not progress in aqueous medium.
	Reflux Condition	No new spot observed	1%	
Methanol +THF	RT	No new spot observed	2-3%	The acid was not converted into acid chloride in methanol solvent.
	Reflux Condition	No new spot observed	10%	
Tetrahydrofuran	RT	New spot observed (not clear)	40%	In RT the rection progress very slowly and is taking long time. Yield is less. But in refluxed condition the rection progress very fast and observed quantitative yield.

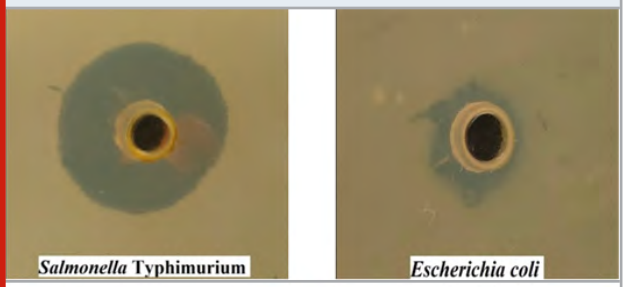
Test organisms: The test bacterial cultures including Escherichia coli (MTCC No.- 614), *Salmonella typhimurium* (MTCC No.-3224) were collected from IMTECH Chandigarh. All the bacterial cultures were maintained in nutrient agar slants. The slants were kept in refrigerator for use during further experiments.

Antimicrobial Assay: A standardized concentration of inoculums with fixed volume was spread evenly or swabbed on the surface of gelled agar plates. A hole which ranges from 6 - 8 mm in diameter was punched with a sterile cork borer aseptically in plates. A fixed volume (50 µl) of the sample solution was then introduced into the bored agar well and incubated at optimum temperature (Bacteria - 30±2°C for 24 hrs) depending upon the test microorganism. [17].

Synthesis of PA-B-ester: Phthalic acid (5g) was taken in a round bottom flask (RBF). To this solid mass, thionyl chloride (6.5 ml) was added dropwise over a period of 1o minutes at 10-15 °C. The temperature of reaction mass was raised to 70-80 °C and stirred it for 3 hours. Slowly cooled the reaction mass temperature to 0-5 °C and added THF (50 ml) into it. To this ice-cold solution, triethylamine was added slowly over a period of 1h. Then n-butanol (6.4 ml) was added into the reaction mass at

0-5 °C. Slowly raised the reaction mass temperature to room temperature and stirred it for 12h. TLC was checked and reaction was found to be completed. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. Finally, the crude compound was purified using column chromatography.

Figure 5: Antibacterial activities of PA-B-ester against pathogenic bacterial stains: (a) Staining of PA-B-ester against *Salmonella Typhimurium*, (b) Staining of PA-B-ester against *Escherichia Coli*.



CONCLUSION

In conclusion, to our knowledge, it is a very unique example where PA-B-ester molecule strongly inhibit the growth of pathogenic gram-negative bacteria like *Salmonella typhimurium* and *Escherichia coli*. We have also thoroughly studied the mechanism of inhibition of this molecule towards these two pathogenic bacteria. This molecule may open up new doorway for the treatment of diseases, causing by these bacteria. To our opinion, this work may provide new insight to design potential molecule of tunable property with respect to protective cell wall of the bacteria which will enhance the cell permeability of the molecule. Thereby, it will inhibit the pathogenic gram-negative bacterial growth.

ACKNOWLEDGEMENTS

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Synthesis of Small Organic Molecule, Pa-P-Ester: A Novel Inhibitor Against Pathogenic Gram-Negative Bacteria; *Salmonella typhimurium* and *Escherichia coli*

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ABSTRACT

Gram-negative bacteria are ubiquitous in nature. These bacteria are responsible for causing many noxious diseases such as: neurological abnormalities, gastroenteritis, and life-threatening Typhoid fever in human being and many other animals. Therefore, Salmonella has been a cause to longstanding worldwide health problem and became a reason for significant mortality globally. The toxicity nature of these bacteria is because of their high tendency to survive in the host body rather quickly due to the presence of protective cell wall which defends from invasion of exogenous toxic agent. Therefore, developing new organic small molecule with ideally disposed functional unit which can easily prevent the growth of bacteria is always demanding. We report herein, a synthetically simple and elegant, small organic molecule, PA-P-ester which having highly tunable prerequisite properties with respect to protective cell wall of the bacteria. The molecule, PA-P-ester shows high antibacterial activity against pathogenic gram-negative bacteria such as: *Salmonella typhimurium* and *Escherichia coli*.

KEY WORDS: GRAM-NEGATIVE BACTERIA, SALMONELLA TYPHIMURIUM, ESCHERICHIA COLI, INFECTED DISEASES, ANTIBACTERIAL ACTIVITY, SMALL ORGANIC MOLECULE, PATHOGENIC BACTERIA.

INTRODUCTION

Gram-negative bacteria such as: *Salmonella typhimurium* and *Escherichia coli* are ubiquitous in nature which are considered as highly toxic pathogen among all other bacteria. These bacteria are having a protective cell wall, made up of lipopolysaccharide coat (LPS). This protective cell wall of these gram-negative bacteria acts as defense wall that prevents the foreign invasion into the bacteria body. As a result, these bacteria can easily sustain in the host body even in highly adverse condition and turn out to be toxic pathogen eventually. However, most of the gram-positive bacteria devoid of such protective cell wall and therefore, they have poor resistivity in hostile environment than gram-negative bacteria.

In day today life these bacteria some way or other impact our lives, even if these are pretty small living organism in the earth.[1] Among many gram-negative bacteria, Salmonella and E. Coli are two major pathogens that infect thousands of lives worldwide even today. [1, 2, 16] These bacteria are existed in different shape such as: spheres, spirals and rods in nature and are having wide range of size varies from 0.4 to 3 µM. The varying shape is due to the presence of actin-like bacterial cyto-skeleton.

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[3, 4] Among many of these gram-negative bacteria, *Salmonella* species are found to be highly toxic pathogen. According to the recent findings, *Salmonella* species are responsible for causing wide spectrum of diseases starting from neurological abnormalities to gastroenteritis to life-threatening Typhoid fever in human being.[5, 7, 11-13] Therefore, *Salmonella* has been a cause to longstanding worldwide health problem and became a reason for significant mortality globally.[6, 11-13].

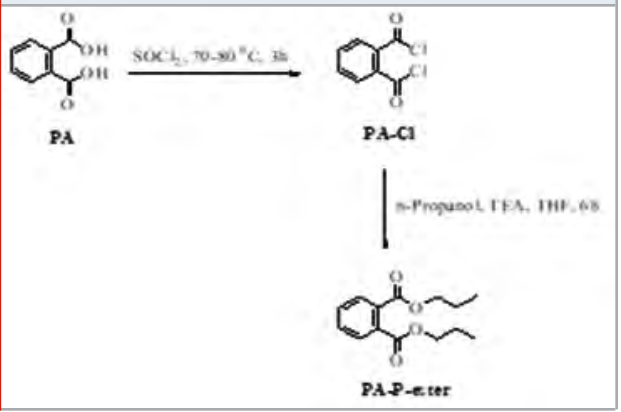
The bacteria S. Typhimurium is a wild in nature which infects a broad range of hosts. However, this specific bacterium belongs to *Salmonella* species. In addition to that, there are few class of Salmonella such as: *S. Typhi*, *S. Pullorum*, and *S. Gallinarum* are exquisitely host-restricted.[6] Furthermore, the bacterium Salmonella enterica serovar *Typhimurium* causes food-borne disease as well.[8] In addition to this, there is a highly diverse group of gram-negative bacteria called *Escherichia coli* which are mostly found in the environment, foods and intestines of people and animal. It has been known in the literature that most of *E. coli* are not harmful, rather helps to keep our digestive track healthy. However, there are few varieties of *E. coli* are highly responsible for causing a broad spectrum of infections such as: diarrhea, food poisoning, pneumonia, urinary tract infections etc. There are different categories of *E. coli* such as: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorragic *E. coli* (EHEC) and enteroaggregative *E. coli* (EAEC) which are responsible for causing diarrhea.[14].

Considering the life-threatening pathogenic effect of gram-negative bacteria, it has been a continuous endeavour from wide spectrum of scientific community to develop novel antibacterial agent. Till today, Most commonly used antibacterial agents are the derivative of quinolones and fluoroquinolones compounds.[9] It is always been a huge challenge for synthetic chemist to develop molecule with well-defined architecture that can strictly inhibit pathogenic gram-negative bacteria. The poor rate of progress is attributed to the protective cell wall of the gram-negative bacteria which demands a precise molecular structure. Therefore, to overcome this difficulty, herein we have developed a new scaffold with stringent architectural component inbuild into the benzene core. We have demonstrated potential antibacterial activity of this molecule against gram-negative bacteria.

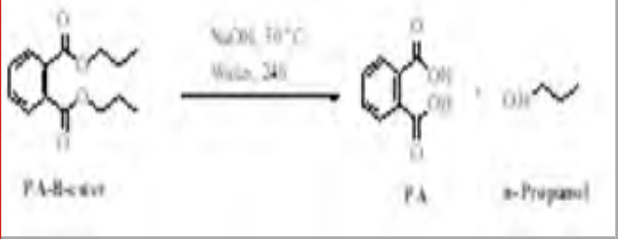
RESULTS AND DISCUSSION

There are several coupling reagents (DCC, HATU, HBTU, EDC, $SOCl_2$ etc.) available now a days to convert acid to corresponding amide or ester. Most of these coupling agents such as: 1) DCC, 2) HATU, 3) HBTU, 3) EDC are highly expensive and therefore, difficult to afford them for academic research. But on the other hand, thionyl chloride ($SOCl_2$) is another robust alternative for the synthesis of an amide or ester from acid. Furthermore, it is comparatively much cheaper than other coupling reagents. This reagent is pretty simple to handle and moreover, it affords high yield. Therefore, this reagent became central to many of the organic transformation for industrial uses. In light of these potential advantages, we have utilized the thionyl chloride (SOCl2) as a coupling agent to generate a new variety of molecular scaffolds which might inhibit toxic pathogen like Gram-negative bacteria such as: *Salmonella* and *E. coli.* (Scheme 1).

Scheme 1: Synthetic scheme for the synthesis of PA-P-ester



Scheme 2: Base hydrolysis of PA-P-ester



In search of an ideal molecular scaffold for the precise installation of n-propanol unit in order to have ester-based molecules, we intended to start with semi-rigid backbone like phthalic acid (PA). We envisioned that benzene ring in PA will provide rigidity along with pi-pi stacking interaction with hydrophobic region of the toxic pathogen which in turn could provide good binding efficacy. In addition to that the ester functionality may help in making hydrogen bonding interaction with hydrophilic part of the pathogen. The remaining butane tail may provide flexibility to the core structure and along with that it may enhance the possibility of cell permeability nature of the molecule. Consequently, synthesizing this kind of molecule may inhibit pathogens. Having these anticipations in mind, we wanted to install the ester moiety into a rigid system, Phthalic acid (PA). Therefore, we persuaded to synthesize compound, PA-P-ester (Scheme 1) with an intention that this compound may show better inhibition property towards pathogen.

In the beginning, the precursor compound, phthalic acid (PA) converted to corresponding acid chloride, PA-Cl by treating with thionyl chloride under room temperature for 3 hours (Table 1). The mixture of water

and tetrahydrofuran was chosen as the solvent system to carry out the reaction. Minimum amount of water was taken to solubilize the precursor compound, phthalic acid. The resulted acid chloride, PA-Cl was further treated with n-propanol in the presence of triethyl amine as a base to get the desired product, PA-P-ester. Reaction kinetics was completely monitored by thin layer chromatography (TLC) techniques. But surprisingly, TLC result analysis showed no new product formation, rater mostly precursor, PA was observed on TLC (Figure 1). We anticipated that the phthalic acid does not convert to corresponding acid chloride due to high transition state energy barrier which may require high temperature rather than room temperature.

Therefore, to overcome this challenge we further executed the reaction in the reflux condition keeping all other parameters unaltered. TLC result shows very mild product formation (Figure 1) which is negligible. This experiment clearly indicating that, not only temperature but also protic solvent like water and methanol play a role in inhibiting the reaction. In presence of methanol we observed almost the similar results like water. Therefore, we turn our attention to start the reaction using only aprotic solvent like THF. To overcome this problem, we designed two experiment in parallel by using THF as solvent. In the first experiment, the reaction was carried out at room temperature in presence of THF as a solvent and kept all other parameter constant. However, in the second experiment the reaction was carried out in reflux condition in presence of THF as a solvent without altering any other parameters. TLC analysis clearly shows new product formation (Figure 1, TLC-4) in both cases.

The intensity of product spot observed for refluxed reaction is much brighter than room temperature reaction. These results clearly reveal that the reaction must be carry out in aprotic solvent at refluxed condition to get the optimum product formation. With this optimized reaction condition, we observed up to 91 % of product yield (Table-2). The products were further purified by column chromatography in 1% methanol and chloroform system. The desired product, PA-B-ester formation was further confirmed by UV-Visible spectroscopy study (Figure 3) by comparing with commercially available starting material. This data clearly showed the formation of product. Elemental analysis data also support the product formation. Again, to cross verify the product formation we carried out the base hydrolysis reaction (Scheme 2) with sodium hydroxide solution in water as a solvent and isolated the hydrolyzed products using column chromatography techniques. After column purification of crude product, we observed two kinds of material. One material is solid and another one is liquid. The solid material expected to be phthalic acid and liquid material may be n-propanol.

The solid material obtained after hydrolysis was analyzed by comparing the TLC with commercially available starting material. The TLC result clearly shows, the hydrolyzed product is a starting material (Figure 2). It was further confirmed by comparing melting point with the starting material. The melting point of the starting material (207 °C) was found to be same as that of isolated product (207 °C). This indicated that the hydrolyzed product is PA (Scheme 1). The liquid material obtained after hydrolysis was analyzed using TLC with reference to commercially available n-propanol compound. TLC result clearly showed both have the same retention time. This result indicated the second hydrolysis product may be n-propanol. It further confirmed by determining the boiling point of the liquid material which was similar to commercially available n-propanol. This result confirmed that the resulted hydrolysis product is a n-propanol. Therefore, these results strongly support the formation ester derivative, PA-P-ester.

Figure 1: TLC-1-Reaction carried in water+ THF solvent mixture at room temperature; TLC-2-Reaction carried in water as solvent at reflux condition; TLC-3-Reaction carried in methanol as solvent at reflux condition; TLC-4-Reaction carried out in THF at reflux condition

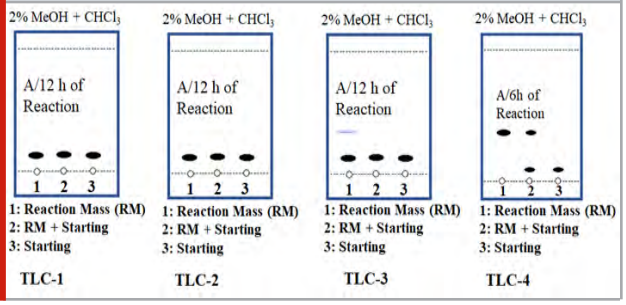


Figure 2: Monitoring the reaction kinetics of PA-P-ester hydrolysis reaction (scheme-2): after 1, 6, and 12 hours of reaction time using TLC.

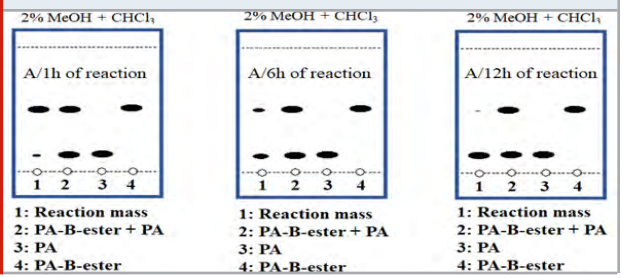
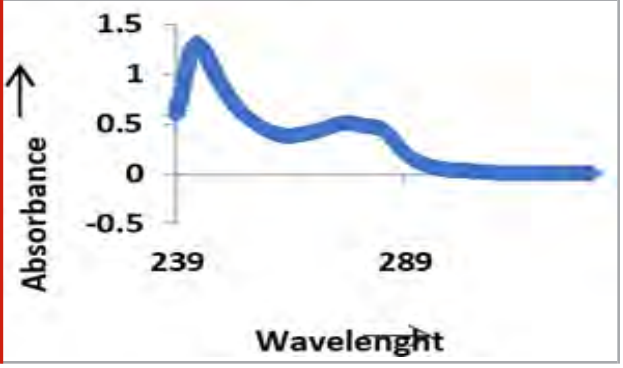


Figure 3: UV-VIS Spectra of the compound, PA-B-ester



The antibacterial activities of PA-P-ester against pathogenic bacteria i.e. *Salmonella typhimurium* and *Escherichia coli* were checked. A standardized concentration of both the inoculums were evenly spread on the surface of two different agar plates. A 500 µM concentration of PA-P-ester was loaded into both the bore wells and incubated for 24h at 30±2oC temperature. The results clearly showed a very strong inhibition zone of diameter 30.2 mm size against *Salmonella typhimurium* after incubation with PA-P-ester for 24 hours (Figure 4a). This result clearly reveals that, PA-P-ester has potential antibacterial activity against *Salmonella typhimurium*. However, the antibacterial activities of PA-P-ester was found to be relatively lesser for *Escherichia coli* than *Salmonella typhimurium* which is evident from the inhibition or clearance zones of both the pathogenic strains (Figure 4 a & b).

Table 1. Chemicals used for the reaction

Chemicals	Molecular Weight in g/mol	Quantity	Moles
Phthalic acid $\{C_6H_4(COOH)_2\}$	166.13	5 gm	0.03
Thionyl Chloride ($SOCl_2$)	118.97	6.6 ml.	0.075
THF (C_4H_8O)	72.11	100 ml.	0.09
n-Butanol ($C_4H_{10}O$)	74.121	6.04 ml.	0.81

Table 2. Reaction analysis in different solvent system

Solvents	Temperature	TLC Analysis	Yield	Remark
Water + THF	Room Temp. (RT)	No new spot observed	0%	The reaction was not progress in aqueous medium.
	Reflux Condition	No new spot observed	1%	
Methanol +THF	RT	No new spot observed	2-3%	The acid was not converted into acid chloride in methanol solvent.
	Reflux Condition	No new spot observed	12%	
Tetrahydrofuran	RT	New spot observed (not clear)	43%	In RT the rection progress very slowly
	Reflux Condition (70-80° C)	New clear spot observed	91%	and is taking long time. Yield is less. But in refluxed condition the rection progress very fast and observed quantitative yield.

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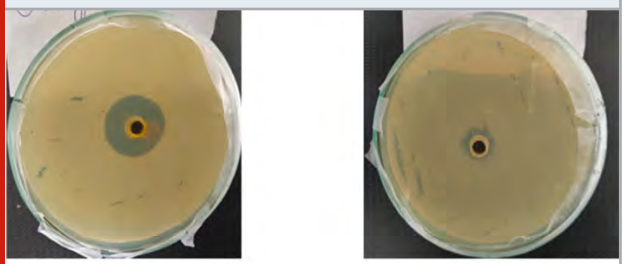

(Salmonella Typhimurium) (a) (Escherichia Coli) (b)

This antibacterial activity could be due to the presence of well-balanced hydrophobic, hydrophilic and long chain hydrocarbon molecules. Owing to these well-balanced properties of PA-P-ester, might be proficient to cross the bacterial cell membrane resulting in the inhibition of the bacterial strain growth.

Experimental section
MATERIAL AND METHODS

The chemicals and solvents were purchased from Spectro chem Ltd and Sigma Aldrich. All the chemicals were directly used without further purification. Normal phase column chromatography purification was carried out by using MERCK silica gel 60 (particle size: 100-200 mesh). Reactions were monitored wherever possible by thin layer chromatography (TLC). Silica gel G (Merck) was used for TLC and column chromatography was undertaken on silica gel (100–200 mesh) in hexane, hexane–ethyl acetate or chloroform. UV and visible peaks of synthesized organic compound was measured in chloroform as a solvent in the range of 200-400 nm. The wavelength (in nm) was taken in X-axis and absorbance in the Y-axis. It shows maximum absorbance at 243.6 nm wavelength. Melting points were recorded in a Fisher-Johns melting point apparatus.

Test organisms: The test bacterial cultures including *Escherichia coli* (MTCC No.- 614), *Salmonella typhimurium* (MTCC No.-3224) were collected from IMTECH Chandigarh. All the bacterial cultures were maintained in

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CONCLUSION

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A Simple Anatomical Method for Identification and Authentication of Medicinally Important Herbal Drug 'Chitrak' (*Plumbago* species)

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ABSTRACT

Plumbago zeylanica L. is used in Ayurveda to treat various diseases and is often called as Chitraka in Sanskrit. The root of this plant is used in Ayurvedic formulations that stimulates digestion and improves appetite. The raw materials or the plant parts are collected by untrained workers and supplied in dry condition to the drug manufacturing industries. Therefore, there is a chance of adulteration because of vested interest or ignorance as many plants are known by common names or in vernacular names. Three species of *Plumbago* viz. *Plumbago* auriculata Lam., *P. indica* L. syn. *P. rosea* L. and *P. zeylanica* L. are found in Odisha and amongst them *P. auriculata* Lam. is rare. All the species are known as Chitraka, but for commercial preparation of Ayurveda, Siddha and Unani (ASU) drugs in Indian System of Medicines, *P. zeylanica* L. is widely used because of its therapeutic activities. The present study deals on the anatomical characteristics of roots of these three *Plumbago* species for proper identification and authentication of the desired species in drug manufacturing industries before formulating the compound drugs. Root specimen of *Plumbago auriculata* Lam., was collected from the authentically identified plant specimens maintained in the nursery of Regional Plant Resource Center, Bhubaneswar and the roots of *P. indica* L. Syn. *P. rosea* L., and *P. zeylanica* L. were collected from authentically identified plant specimens maintained in the nursery of Silviculture office, Ghatikia, Bhubaneswar. Transverse sections were made manually for each specimen separately and were cleared by using 2% chloral hydrate. The sections were then subjected to staining with safranin and fast green solutions and then mounted over the glass slides using DPX (Qualigens, India) mountant and observed under the microscope. Results: Transverse section of the root of *P. auriculata* Lam. revealed the presence of a group of stone cells arranged in a ring occasionally interspersed with cortex surrounding the large stellar region. Transverse section of the root of *P. indica* L. revealed a wide cortical region comprising many layered rounded or oval shaped compactly arranged parenchymatous cells without any intercellular spaces. Stone cells are absent in the cortical region and also the cortical region do not show the presence of fibers. Microscopic observations of transverse section of the root of *P. zeylanica* L. showed small groups of stone cells with wide lumen present irregularly at cortical region which does not form a ring around the stellar region and also the cortical cells were found to be filled with starch grains of about 6 to 10µ in size. Conclusion: All the three species revealed distinct anatomical features. Microscopic observations of transverse section of the root of *P. zeylanica* L. showed small groups of stone cells with wide lumen present irregularly at cortical region which does not form a ring around the stellar region and also the cortical cells were found to be filled with starch grains of about 6 to 10µ in size which are the identifying features. This study will certainly help to authenticate the crude drug and ensure the quality.

KEY WORDS: ANATOMY, AUTHENTICATION, ASU FORMULATIONS, COMPOUND DRUGS, *PLUMBAGO ZEYLANICA* L.

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INTRODUCTION

Plants are an important source of medicine and play a key role in world health. Medicinal herbs have been known to be an important potential source of therapeutics or curative aids. The use of medicinal plants has attained a commanding role in health systems all over the world because of their less toxicity. The genus *Plumbago* of family Plumbaginaceae comprises 10 species, extensively used for various purposes. *Plumbago* zeylanica L. is known as 'Chitraka' in Ayurveda has been used in alternative systems of medicine as component formulations for the treatment of multiple disorders such as arthritis, anemia, cardiovascular disorders, metabolic disorders, chronic rhinitis, sinusitis, dysponea, anorexia and dyspepsia (Patel R.V. et al. 2011; Sharma, P.C.2001). The entire plant is used in traditional systems of medicine. This plant is used in treating leucoderma, piles, bronchitis, liver diseases and intestinal trouble.

The crude root paste is used by local people for the management of arthritis and other inflammatory disorders. A tincture of the root bark is used as anti-periodic. The root is used in Ayurvedic formulations that stimulates digestion and improves appetite (API, Part-II (Formulations), 2007). *Plumbago auriculata* Lam., P. indica L. Syn. *P. rosea* L., and *P. zeylanica* L. are the three species commonly found in India. *Plumbago auriculata* Lam. is known as blue *Plumbago*, Cape leadwort and Cape *Plumbago* and in Odia it is commonly known as 'Nila chita' and is native to South Africa (Botanica, 2004; Nico Vermeulen, 1998). *P. indica* L. Syn. *P. rosea* L is known as Indian leadwort, scarlet leadwort, whorled plantain and in Odia it is called as 'Rakta chita' or 'Lal chita' and is native to Southeast Asia. *P. zeylanica* L. is known as white leadwort, Ceylone leadwort and in Odia it is commonly called as 'Agni' or 'Sweta chita' and is widely grown in India and has been used by rural and tribal communities for hundreds of years as traditional system of medicines. In Ayurveda, Siddha and Unani (ASU) drugs in Indian System of Medicines, *P. zeylanica* L is used in treatment of intestinal troubles, leucoderma, dysentery, piles and bronchitis and roots are used to improve digestion by stimulating appetite.

All the plant parts are used in the Indian system of medicine. Plumbagin, a naphthoquinone well distributed among *Plumbago* species, specially found in their roots (Van der Vijver, 1972) with various pharmacological activities i.e., antimalarial (Likhitwitayawuid et al., 1998) antimicrobial (Didry et al., 1994), anticancer (Parimal and Sachadanandam, 1993), cardiotonic (Itoigawa et al., 1991) and antifertility action (Premkumari, P, 1977; Bhargava, 1984). Morphologically the roots of these three species look similar and difficult to distinguish (Figure 1 a-c). Also, most of the roots in dried form are procured for making formulations by the manufacturing industries and the procured roots look similar which leads to confusion and results in adulteration (intentional or un-intentional). In such circumstances, application of plant anatomy is an effective tool in identifying the plant samples. Although, a few reports on anatomy of leaves of *P. zeylanica* L. reported earlier but report on root anatomy of *Plumbago* species is lacking. The purpose of the present study is to describe the anatomical features of the three species to distinguish from each other based on anatomical studies.

MATERIAL AND METHODS

Root specimen of *Plumbago auriculata* Lam., was collected from the authentically identified plant specimens maintained in the nursery of Regional Plant Resource Center, Bhubaneswar. The roots of *P. indica* L. Syn. *P. rosea* L., and *P. zeylanica* L. were collected from authentically identified plant specimens maintained in the nursery of Silviculture office, Ghatikia, Bhubaneswar. Transverse sections were made manually for each specimen separately. The transverse section of the root was cleared by using 2% chloral hydrate for greater tissue transparency and light transmission. Chloral hydrate is known to preserve cellular features than many other clearing agents (Rost and Oldfield, 2000). The sections were then subjected to staining with safranin and fast green solutions (Johansen, 1940) and then permanently mounted over the glass slides using DPX (Qualigens, India) mountant and observed under the microscope.

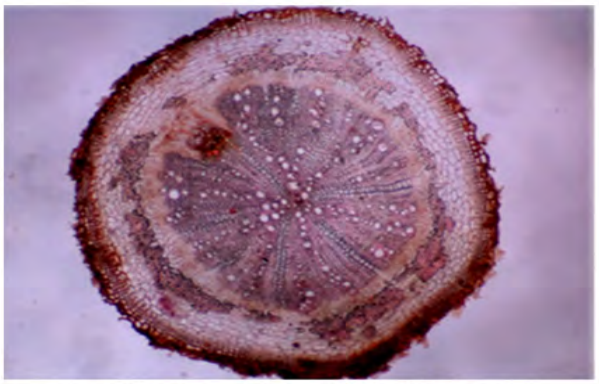

Fig. 1. T.S. of root of P. *auriculata* L. (Whole mount)

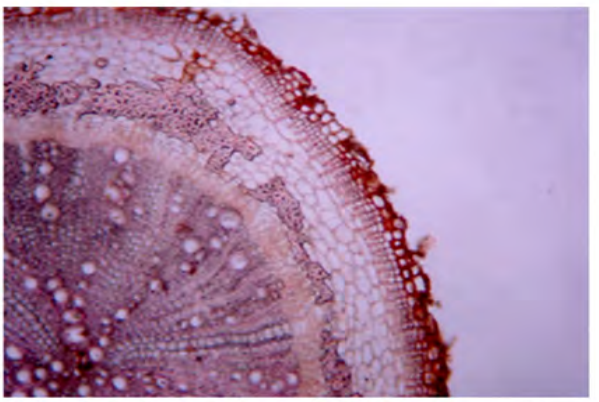

Fig. 2. T.S. of root of P. *auriculata* L. (A portion magnified showing group of stone cells arranged in a ring above the stellar region)

RESULTS

Anatomical studies on the roots of *P. auriculata* Lam., *P. indica* L. Syn. *P. rosea* L. and *P. zeylanica* L. clearly showed distinguishing features and were described in the following heading for each species separately. *P. auriculata* Lam. Transverse section of the root of *P. auriculata* Lam. revealed 4-6 layered cork on the outer surface of the root consisting of flattened parenchymatous cells. Group of stone cells arranged in a ring occasionally interspersed with cortex surrounding the large stellar region. The innermost layer of cortex that surrounds the stele is called endodermis, which is very distinct. Cells of endodermis have special thickenings viz. casparian strips. Central stellar region occupies a larger area than the cortex. Xylem is exarch. Xylem vessels, medullary rays are prominent. Pith is absent (Fig. 1 & 2).

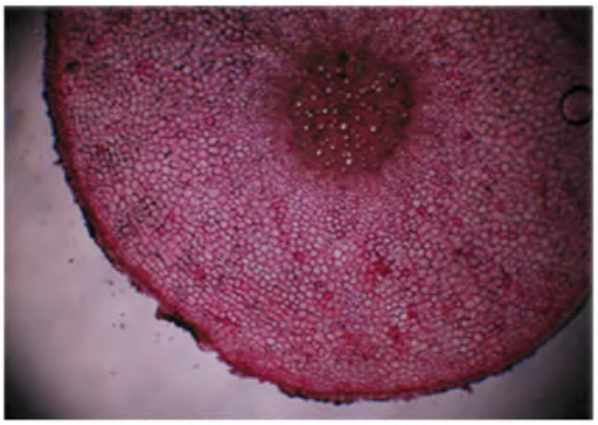

Fig. 3. T.S. of root of *P. indica* L.

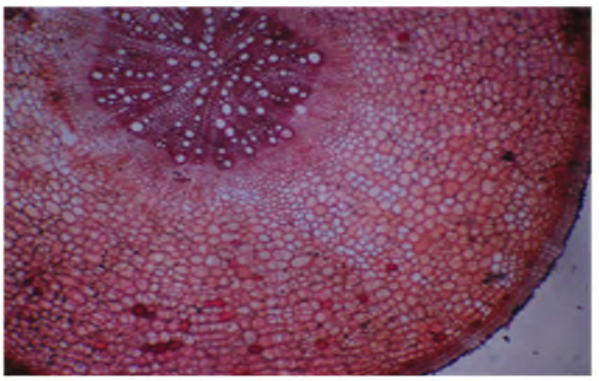

Fig. 4. T.S. of root of *P. indica* L. (A portion magnified)

P. indica L. Syn. *P. rosea* L. Transverse section of the root of *P. indica* L. revealed the presence of 4 or 5 layers of cork consisting rectangular cells followed by a layer of cork cambium; cortex showed wide cortical region comprising many layered rounded or oval shaped parenchymatous cells, compactly arranged without any intercellular spaces. Stone cells are absent in the cortical region, also the cortical region does not show the presence of fibers. The stellar region was found to be narrow in comparison to *P. auriculata* Lam. (Fig. 3& 4).

P. zeylanica L. Microscopic observations of the transverse section of the root of *P. zeylanica* L. showed the presence of cork consisting of 4 to5 layers of rectangular parenchymatous cells with broad cortex made up of parenchymatous cells without any intercellular spaces. The cortical cells were found to be filled with starch grains of about 6 to 10µ in size; small groups of stone cells with wide lumen present irregularly at cortical region which does not form a ring as in *P. auriculata* Lam.; endodermis present; pericycle a single layer of thin walled parenchymatous cells; usually polyarch xylem ring, surrounded by phloem (Fig. 5, 6 & 7). The cortex region of P. indica L. was broader than the other two species. *P. zeylanica* L. shows groups of stone cells haphazardly present in the cortical zone with large lumen, presence of starch grains in the cortical cells and narrow stellar region are the identifying characters from the above two species.

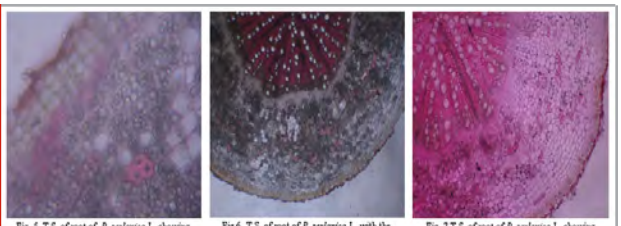

Fig. 5. T.S. of root of *P. zeylanica* L. showing starch filled cortical cells

Fig.6. T.S. of root of *P. zeylanica* L. with the stellar region

Fig. 7 T.S. of root of *P. zeylanica* L. showing cortical and stellar region after clearing with Chloral hydrate solution

DISCUSSION

Transverse section of the root of *P. auriculata* Lam. showed groups of stone cells arranged in a ring surrounding the centrally occupied larger stellar region which is larger than the cortex and occasionally found interspersed with cortex and is the unique identifying features of this species. The anatomical observations of the root of *P. indica* L. revealed a wide cortical region comprising many layered rounded or oval shaped compactly arranged parenchymatous cells without any intercellular spaces. Stone cells are absent in the cortical region and also the cortical region does not show the presence of fibers.

Microscopic observations of transverse section of the root of *P. zeylanica* L. showed small groups of stone cells with wide lumen present irregularly at cortical region which does not form a ring around the stellar region and also the cortical cells were found to be filled with starch grains of about 6 to 10µ in size. Literature data revealed the identification of six *Ocimum* species namely *O. tenuiflorum* L. (both green and purple variety) *O. sanctum* L., *O. basilicum* L. (both green and purple variety), *O. canum* Sims. and *O. gratissimum* L. on the basis of their anatomical features (Parida et al, 2020).

Microscopic observations of roots of *P. auriculata* Lam. and *P. zeylanica* L. showed the presence of lignified pericyclic fibers whereas it is absent in *P. indica* L. (Galal et al., 2013)and the cortical region of *P. indica* L. is broader than the other two species.

CONCLUSION

One of the major challenges that the pharmaceutical industries face is the genuinity of the raw materials due to its morphological resemblances. This ends in adulteration and substitution for genuine drugs. Such adulteration and substitution of genuine raw material is the main cause of degradation of the therapeutic effect of particular drugs used in Indian System of Medicine that lead to poor quality of herbal products. Developing suitable anatomical markers would help in clear identity of the raw materials. Present anatomical investigations on the roots of *Plumbago auriculata* Lam., *P. indica* L. Syn. *P. rosea* L. and *P. zeylanica* L. clearly showed distinguishing features among them and proved as an authentication tool to identify the crude drugs.

Conflict of Interests: There is no conflict of interest.

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Antibacterial Activity of Kernal Extracts from Three Mango Cultivar s 'Totapuri', 'Langra' and 'Sundri'

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ABSTRACT

Antibacterial activity of two extracts aqueous and methanol extract of three varieties of mango seeds was done against gram negative E coli bacterial strain. Antibacterial activity was studied by "agar disc diffusion" method. The methanolic extract of langra and aqueous extract of sindhri showed highest antibacterial activity against *E coli* suggesting that the phytochemicals present in these three varieties of mango can be used as an alternate source of medicine for treatment of bacterial infections. Comparative analysis has been done against three commercial antibiotics Rifampicin, Gentamicin and *Apoxicillin* The present study suggests that the kernel of Mangifera indica can be used as an effective potential candidate for the development of new strategies to treat bacterial infections.

KEY WORDS: MANGIFERA INDICA, E COLI, METHANOLIC EXTRACT, AQUEOUS EXTRACT

INTRODUCTION

Mangifera indica L. (Eng. Mango; Beng: Aam) of the family Anacardiaceae, is the king of fruit in India and has also been adopted almost all over the tropical and subtropical countries. There are about 500 known mango varieties available some of them which evolved from different geographical region of the world. Almost 69 species of Mangifera mostly restricted to tropical regions of Asian continent (Jeeva, 2009). The infectious disease continues to be a major health problem nowadays worldwide though a wide range of synthetic antibiotics is available in the market. Synthetic drug shows adverse side effects at continued use (Rajan et al., 2011; Sahu et al., 2006).This increasing trend of undesirable side effects of certain antimicrobial chemical agents, and quick development of drug resistance pathogens triggers to search for better agents that will be cheaper and less side effects for treating infectious diseases. Antibiotics can be defined as "a chemical substance derived from living micro-organism which has power to kill or inhibit the growth of other microorganisms". *Mangifera indica*. Verna. Aam, Eng (mango tree) is a large evergreen tree with widely spreading branches and dark coloured bark.

The mango fruit is one of the most prized desert fruit of the tropics and is known as "king of all fruits". The use of seed kernel for the treatment of diarhhoea and dysentery has been reported since ancient period (Sharma, 2003; Kiruba et al., 2006; Rajan et al., 2011). The antibacterial activity of the seed kernel may be due to the phytochemical constituents such as polyphenols, phenolic acids present in the mango seed kernel (Sandhu and Lim, 2007; Barreto et al., 2008). It has been reported that antimicrobial activity may be due to the presence of active metabolites like tannin (Rajan et al., 2011; Singh, 1986; Ponce et al., 1994). Different Extracts of mango seed kernel have been reported as antidiarrhoeal (Kabuki et al., 2000; Kaur et al., 2010), antibacterial (Sairam et al., 2003), and anti-inflammatory activity (Bussmann and

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Sharon, 2006). From the ancient past mango seed kernel powder are usually used as a home remedy by indigenous peoples against gastrointestinal disorders and also for the treatment of various diseases throughout the world (Parvez, 2016; Bekoe et al., 2017; Khadare, 2016).

The mango variety Langra is the great pride in Northern Indians. The variety totapuri resembles a parrot, hence its name (as "tota" means "parrot" in Hindi) has small green appearance and beak at the end of the fruit. The 'Sindhri' mango is a mango cultivar grown in Sindhri, a town in Sindh, Pakistan. *E. coli* are natural inhabitants of the human large intestine as well as intestine of warm-blooded animals. Most of the strains are beneficial, but some strains are pathogenic and are responsible for diarrheal infections, neonatal meningitis, septicemia, and urinary tract infections (UTIs) etc., (Makvana and Krilov, 2015). In this study an attempt has been taken to study the antimicrobial activity of the aqueous and methanolic kernel extract against pathogenic *E. coli* bacteria.

MATERIAL AND METHODS

Plant material: To study the antimicrobial activity of mango kernels, three varieties of mango namely, Langra, Sindhri, Totapuri were taken from market of different locations at Balangir District.

Preparation of mango seed kernels: The seed kernels of different mango were collected, dried and grinded separately. By using grinder, the kernels were sliced and cut into small piecesand kept at -80°C for 3days. Excessive moisture has been removed using freeze dryer. The samples were stored at 4°C for future use.

Culturing of the test organism: In this study the test organisms was taken as *E. coli*–(*Escherichia coli*). The test organisms was grown on Luria Bertani (LB) media containing Yeast extract(10g/L), Peptone (10g/L), NaCl (5g/L), Agar 10g. pH was adjusted to 7.0 before sterilization. Then, it was stored at 4°C for further studies (Sambrook and Russell, 2001). E. coli cultures were preactivated using LB broth medium before antimicrobial assay. For the preparation of agar media, LB top agar and 1 liter of LB broth were mixed together (3:1). pH was adjusted to 7.0 before sterilization.

Extraction Procedure: The dried powder was used for the study of antimicrobial activity against *E.coli*.Two extraction solvent used was used i.e., methanol and distilled water (separately). 100 gm of dry seeds kernels of *Mangifera indica* were used for extraction with methanol and distilled water in Soxhlet apparatus for eight hour till colorless solvent appeared. Colorless solvent of the extract was evaporatedusing rotary evaporator. Finally, extract was dried in air at room temperature.

Antibacterial assay: *E. coli* strains were cultured and maintained in Luria Bertani (LB) nutrient broth at 37oC. Agar Slant culture were kept 4oC for future use. The antimicrobial study was performed by using disc diffusion technique. An overnight suspension culture of *E.coli* were inoculated on the Mueller-Hinton agar media. The sterile discs were soaked with 10 ml of both the extract i.e., methanolic and aqueous extracts of mango seed kernel of three varieties individually respectively for langra (a), sindhri (b) and totapuri (c). Methanol and water was taken as negative control and three commercial antibiotics Rifampicin, Gentamycin and Apoxicillin were taken as positive control. Plates were kept at 37oC for 24 h. Diameter of inhibition zone around the disc was measured to evaluate the antimicrobial activity. Here the three varieties are labeled as 'a'–for Langra variety, 'b'–for Sindhri variety – for Totapuri variety (Genus Mangifera) 'met'–for Methanolic extracts, and 'aq'–for Aqueous extracts.

Determination of relative percentage inhibition: The "relative percentage inhibition" with respect to positive control was calculated as follows.

$$\text{Relative Percentage of inhibition of Test extract} = \frac{100 * (a-b)}{(c-b)}$$

Where,
a: "Total area of inhibition of the text extract"
b: "Total area of inhibition of the solvent" (control)
c: "Total area of inhibition of the standard drug"

Figure 1: Shows zone of inhibition of methanolic and aqueous extract of three kernel varieties

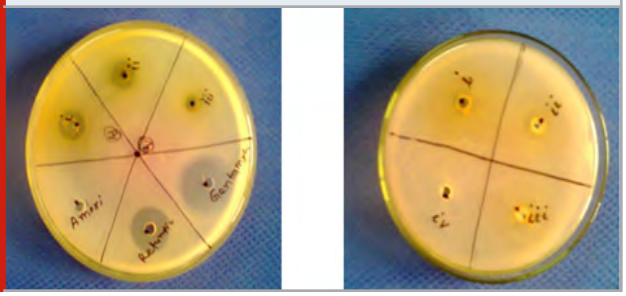


Table 1. Zone of inhibition exhibited by different kernel extracts and antibiotics

Amoxicillin	0
Rifampicin	17.67±0.33
Gentamicin	26±1
Aqueous a	9.67±0.67
Methanolic a	13.67±0.67
Aqueous b	12.33±0.33
Methanolic b	10.33±0.33
Aqueous c	4.23±0.22
Methanolic c	6.57±0.33

RESULTS AND DISCUSSION

In the present study,100gm each of *Mangifera indica* air-dried kernel of three local varieties langra, sindhri and totapuri and two solvents, methanol and water were

used for the extraction to study the antibacterial activity of *E. coli*. After incubation of the extracts inoculated bacterial plates at 37°C for 24 h, they were evaluated for the antibacterial activity using the diameter of inhibition zone (mm) formed around the disc (Fig. 1).

In this study, the antibacterial activity of different extract of *M.indica* was tested against E. coli bacteria. In this antimicrobial assay it was observed that the methanolic extract of sample 'a' showed maximum zone of inhibition against *E. coli* (13.67 mm) followed by the aqueous extract of sample 'b' (12.33 mm). Methanolic extract of sample 'b' has shown fair antimicrobial activity (10.33 mm) against the bacteria. Methanolic extract of sample 'c' exhibited low (6.57 mm) microbial activity while the aqueous extract exhibited no antimicrobial activity against the *E coli*. It was clear from the present results, that methanolic kernel extract of the variety langra exhibited pronounced activity against the tested bacteria (Table 1).

Figure 2: Comparison of zone of inhibition of different extracts of langra and sindhri varieties mango kernel

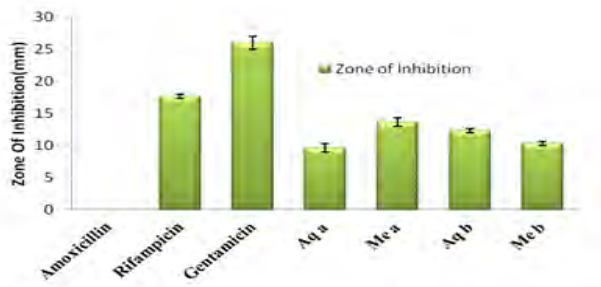
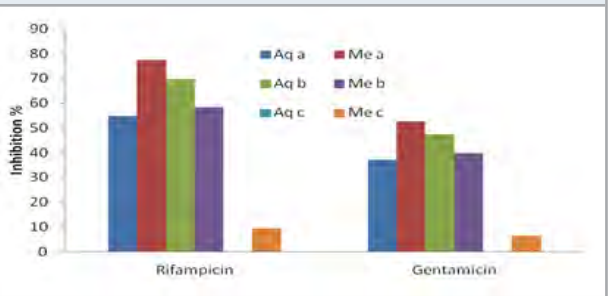
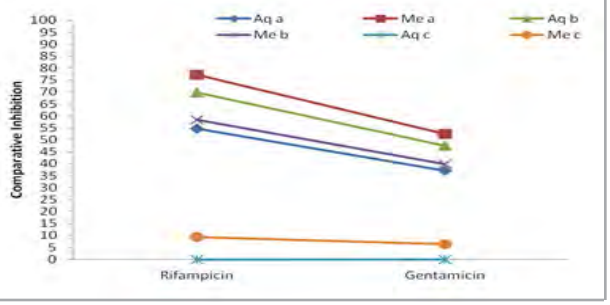


Figure 3: Antibacterial activity of sindhri and totapuri Mango kernel of methanolic and aqueous extract



This study confirms that the methanolic extract of kernel of different mango varieties have high activities against the tested microorganism i.e. *E. coli*. The aqueous extract of the variety Langra and Totapuri exhibited lower inhibitions as compared with the other methanolic extracts (Fig.2) whereas aqueous extracts of variety Sindhri shows relative higher zone of inhibition as compare to its methanolic extracts (Fig 3). The present results contradict the findings of Abdalla et al., (2016), who showed that "aqueous extracts are generally less potent in their bioactivity than organic extracts". It was found that aqueous extract of variety Sindhri shows little more inhibitory effect than its methanolic extract, whereas organic extracts of variety Langra shows more effective inhibition zone compare to other extracts (Fig 4).

Figure 4: Comparative inhibition of three methanolic and aqueous mango kernel extract against different antibiotics.



Among the 3 different antibiotics used as control, Rifampicin and Gentamicin shows inhibition zone but amoxicillin cannot inhibit the growth of *E. coli*. It seems that the target *E. coli* strains is Amox® (Table.1) resistant. Crude methanolic extract has been found to exhibit more inhibitory effect against *E. coli*. organic solvents provide more efficiency in extracting antimicrobial agents from kernel (Kaur et al., 2010). These mango varieties exhibit antibacterial activity which makes them interesting wastes for screening natural products. So study should be taken in this field so that natural antibiotics can be made from mango seed wastes. Therefore it is of highly important to pay more attention to develop antibiotics which would fight against causative disease of many dreadful diseases (Sahu et al., 2006).

Methanolic extracts of variety Sindhri has more antibacterial properties to Rifampicin than Gentamycin, against the *E.coli* Amox®. This experiment shows a antibacterial activity of mango kernel extracts varies with solvent type and variety dependent manner. The capacity of inhibition of different extracts is met Langra > aq Sindhri > met Sindhri > aq Langra > met Totapuri. Aqueous extracts of variety 'c' (Totapuri) does not show any antimicrobial properties against *E.coli*.

CONCLUSION

In the present investigation from the results obtained it can be concluded that the production of pharmacologically products from variety 'a' and variety 'b' could represent a viable and environmentally friendly alternative to reduce the use of synthetic chemicals because of their unintended side effects for the control of pathogenic microorganisms. As the results showed beneficial assessment, so the phytochemical compounds found in kernel extracts of *M.indica* can be used as an alternative antibacterial agent in the treatment of diarrhoea caused by the *E coli*. The presence of bioactive compounds in the kernel extracts might have antimicrobial activity which may be used as drug formulation. In future this organic

waste can be used as a better agent in the medical and pharmaceutical research.

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3-Epi-Betulinic Acid Acetate as A Drug Candidate for Tuberculosis

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ABSTRACT

Tuberculosis continues to worry mankind. Because of the sharp increase in multi and total drug resistant cases the scientific community is looking for new drugs for *Tuberculosis*. The presence of *Rhamnosyl* residue in the cell wall of *Mycobacterium tuberculosis* pathogen and absence in human host projects the enzymes for its biosynthesis as possible drug targets. The presented work focuses on finding out possible plant based inhibitors for *Rhamnose* biosynthetic enzyme RmlC. 126 plant based anticancer compounds were obtained from NPACT database. The compounds were then evaluated for their binding affinity to RmlC through molecular docking procedure. 3-epi-betulinic acid acetate was found to be the best candidate inhibitor for RmlC and thus can work as a putative drug for Tuberculosis.

KEY WORDS: DOCKING, DRUG, NPACT, PLANT, TUBERCULOSIS.

INTRODUCTION

Tuberculosis continues to pose a threat to mankind (WHO's Global Tuberculosis Report, 2019). The disease is caused by *Mycobacterium tuberculosis* (Mtb) (WHO's Global Tuberculosis Report, 2019, Barberis et al., 2017). Last thirty years has seen a steady increase in Tuberculosis cases largely because of a concomitant increase in HIV-AIDS (WHO's Global Tuberculosis Report, 2019, Barberis et al., 2017). India carries the largest burden of Tuberculosis cases (WHO's Global Tuberculosis Report, 2019). Moreover, multidrug and total drug resistant cases magnify the problem manifold (WHO's Global Tuberculosis Report, 2019, Barberis et al., 2017). Therefore, there is a growing appeal to find out new drugs for this disease. The cell wall of Mtb is one of the validated drug targets (Babaoglu et al., 2003). α-L-rhamnosyl-(1→3)-α-D-N-acetyl-glucosaminosyl-1-phosphate, a disaccharide linker is present in Mtb cell wall (Babaoglu et al., 2003, Dong et al., 2007). The outer mycolyl arabinogalactan layer is connected to the peptidoglycan layer by this linker (Babaoglu et al., 2003, Dong et al., 2007, Ma Y et al., 2002). Therefore, this linker is crucial for structural integrity of the cell wall (Ma Y et al., 2002). As the rhamnosyl residue present in the linker is unique to *Mycobacterium tuberculosis* and absent in human host therefore the enzymes responsible for its biosynthesis can work as possible drug targets (Ma Y et al., 2002). The biosynthesis of the L-rhamnosyl residue is being carried out by four enzymes i.e. RmlA (glucose-1-phosphate thymidyl transferase), RmlB (dTDP-D-glucose 4, 6-dehydratase), RmlC (dTDP-6-deoxy-D-xylo-4-hexulose 3, 5-epimerase) and RmlD.

(dTDP-6-deoxy-D-xylo-4-hexulose reductase) those work in sequential manner (Nikaido H et al., 1965). However, as structure is available for RmlC and it is much specific to its substrate, therefore it gives an edge to RmlC as the drug target over other enzymes (Ma Y et al., 2002). As finding a new drug is a time consuming and a cumbersome process. Therefore, we thought of adopting

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a drug repositioning approach i.e. evaluating existing drugs for new therapeutic values. This study focuses on evaluating some known plant derived anticancer agents present in the NPACT database for their efficacy in inhibiting RmlC (Mangal et al., 2013).

MATERIAL AND METHODS

Molecular docking software used: ArgusLab 4.0.1. (Thompson et al., 2004) was used for molecular docking of compounds against RmlC. The target protein RmlC (PDB ID: 2IXC) with bound substrate analogue was retrieved from the Protein Data Bank (www.rcsb.org/pdb). Amino acid residues within 5 A0 radius of the bound substrate analogue were considered as the active site. Water molecules and the substrate analogue were then removed. As the protein is a functional dimer therefore two polypeptide chains were retained removing the other two.

Figure 1: NPACT00219 (3-epi-Betulinic acid acetate) with highest binding energy docked to the active site of RmlC.

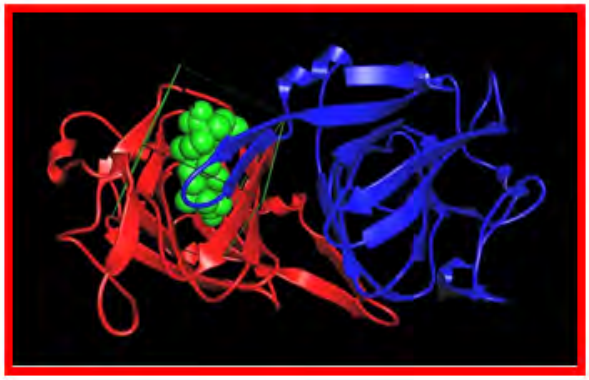

Table 1. Top ten NPACT molecules with high binding energy.

Sl. No.	NPACT ID	Name of the molecule	Binding energy (Kcal/mol)
1	NPACT00219	3-epi-betulinic acid acetate	-15.78
2	NPACT00585	Friedelan-1,3-dione	-14.32
3	NPACT00057	1-O-formyl-4'-demethoxy-3',4'-methylenedioxy-methyl rocaglate	-13.12
4	NPACT00959	Squamostatin-D	-12.94
5	NPACT00332	Betulin	-12.42
6	NPACT00051	18-beta-Glycyrrhetic acid	-12.35
7	NPACT00390	Caracasine	-12.19
8	NPACT00201	candenatenin B	-12.06
9	NPACT01390	7-hydroxycadallin	-12.04
10	NPACT00556	Erlangerins B	-11.88

The anti cancer compounds listed for cervix cancer in Naturally Occurring Plant-based Anti-cancer Compound-Activity-Target database (NPACT, http://crdd.osdd.net/raghava/npact/) were considered for docking (Mangal et al., 2013). All 116 molecules listed as anti cervix cancer NPACT database were retrieved. These compounds were then docked to the active site of RmlC one by one and the resultant binding energy was recorded. Top ten molecules with high binding energy were then recorded (Table 1).

RESULTS AND DISCUSSION

The top ten ligands appear to bind to RmlC properly (Table 1). However, as 3-epi-betulinic acid acetate, NPACT00219 having highest interaction energy (-15.78 Kcal/mol) binds to the active site efficiently thus it is supposed to be a lead drug for Tuberculosis (Figure 1).

CONCLUSION

Since 3-epi-betulinic acid acetate (NPACT00219) is found to have highest interaction energy and it is interacting with the active site efficiently therefore it is supposed to be a lead drug for Tuberculosis. However, in vivo experimental study will garner more support for the work.

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Impact of Mulching in Improving Soil Properties and Crop Performance- An Introspect

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ABSTRACT

The Green revolution is responsible for paradigm shift in Agriculture in the World ensuring food security. This led to heavy use of synthetic inputs to fulfil the demand of high yielding fertilizer responsive crops which over a long run questioned sustainability of the Agricultural system. Recently, resource conservation technology is gaining popularity due to its potential to manage ill effects of green revolution thereby minimizing the threat to the environment. Among several RCT's mulching is one such practice that is efficient, cheap and easily adoptable. Mulching is a practice of covering the soil with organic or inorganic loose materials. In general, it acts as a barrier to entry of light and suppresses the weed growth. Moreover, this also minimizes the exchange of energy resulting in a significant role in moisture retention and conservation. This impact on soil properties has found to have a significant impact on growth, yield, and quality of many crops under field conditions. However, growth, yield and quality of any crop majorly depends on micro-climate of the crop and significant influence of mulching in microclimatic modification widened the scope of mulching favouring new integrations towards profitable crop production.

KEY WORDS: MULCHING, RCT, SOIL PROPERTIES, GROWTH, YIELD, MICRO-ORGANISMS

INTRODUCTION

Globally, rapid increase in population raised the demand for food. Although the green revolution enhanced production during the initial years, due to unprecedented use of external inputs resulted in poisoning the soil in due course (Singh, 2011). To overcome these negative impacts of the green revolution and to sustain food production to meet the demand of expanding population, inclusion of resource conserving technologies in farming can be viewed as a promising alternative (Pingali, 2012). Among the various resource conserving technologies mulching was found to be a cheap and popular alternative (Hussain et al., 2015).

Mulching is a process of covering the soil surface with certain loose materials either organic or inorganic in nature derived from a german word "Molsch" means "easy to decay" which usually focuses on improving infiltration, minimized fertilizer leaching, restricted weed infestation and thereby resulting in profitable crop production (Chakraborty et al., 2008 ; Aragues et al., 2004). The materials that are used for mulching are called mulches. Keeping the above facts in view, to identify the ultimate potentiality of mulching on Agro-ecosystems through over-viewing the past investigations to help the

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researchers understand the pros and cons of mulching this comprehensive review entitled "Impact of mulching in improving soil properties and crop performance- An Introspect" has been initiated.

Types of Mulches

Organic mulching: Organic mulching is the process of using easily decomposable organic materials viz. straw, dry clips, husks, paper, animal wastes, cover crops, etc. for mulching (Kumar et al., 2014 ; Kwambe et al., 2015). Besides soil and water conservation; organic mulches also add up organic matter and some nutrients due to its decomposable nature (Gurjar et al., 2019). Organic mulches can be classified into two types, living (eg: Smothering crops) and non-living (eg: Straw) organic mulches. Increased food grain production subsequently increased the availability of straw besides ensuring food security (Thakur et al., 2018). Deploying straw as a mulching material rather than burning ensued restoration of degraded soils (Jain et al., 2014 ; Singh et al., 2018) due to its role in reducing absorption and transfer of energy within the soil. Consequently, this role is attributed to lower evapotranspiration losses, natural resource conservation, and ultimately in improving crop production (Sharma et al., 2019 ; Raghavendra et al., 2017). Besides, the biodegradable nature of this mulching material contributes towards improving soil fertility (Castillo et al., 2012).

Inorganic Mulching: Inorganic mulching is a process of using slowly decomposable or completely decomposition resistant material such as rocks, gravels, plastic material etc as mulching material (Patil et al., 2013). Although, due to its no decomposition nature of the mulching material used inorganic mulches are unable to add nutrients to the soil but still due to efficient weed suppression character helps in overcoming this deficit (Singh et al., 2018 ; Gerasimova and Yordanova 2015). Commercially, these mulches are of great utility for profitable crop production (Rao et al., 2016). Among different inorganic mulching materials polyethylene based materials are of great use through these mulching materials reduces the evapo-transpiration losses and further helps in weed control (Verma et al., 2017). Further, black plastic mulch is more efficient in controlling weeds due to complete prevention of light entering the soil (Vetter et al., 2017). Similarly, Black coloured polythene increases the soil temperature while white coloured reflects the radiation thereby help in cooling the soil temperature (Laulina and Hasan 2018) and at the same time continuous poly sheet is considered to be more efficient than using pieces of poly mulching materials (Anzalone et al., 2014).

Effect of Mulching on soil properties

Soil organic matter: Soil organic matter was significantly influenced by mulching which in turn resulting in improvement of soil physical, chemical and biological properties upon decomposition (Ampofo 2018 ; Kumar et al., 2015). High organic matter content in the organic mulches attributes to the improvement of soil organic matter upon application while inorganic mulches consists of very low percentage of organic matter but accelerates organic matter decomposition resulting in soil moisture conservation and enhancing microbial gro (Hossen et al., 2017 ; Aragues et al., 2014) Besides, organic mulches act as a nutrient reserve to the soil which upon mineralization adds up to the soil fertility in turn resulting in higher crop yields (Fang et al., 2011). However, rise in temperature due to mulching leads to loss of carbon stocks in the soil under plastic mulching (Swiatkiewicz and Siwek 2018); while under organic mulching this loss of organic matter is moreover balanced by crop residual input (Chowdhury et al., 2015). This clearly highlights its role in improving phyco-chemical and biological properties of the soil upon decomposition resulting in loose, friable and easily malleable soil favouring easy root penetration.

Soil Temperature: The mulches play a significant role in regulating the soil temperature (Donk et al., 2011). Usually, the change in soil temperature depends on duration and intensity of direct exposure of soil surface to solar radiation and exchange of heat between the soil and atmosphere (Pramanik et al., 2015 ; Tongchuan et al., 2017). However, the role of mulches in microclimatic modification is mainly attributed due to its improved insulation from direct solar radiation and increased albedo (Stigter et al., 2018 ; Yi et al., 2011). Depending on the potential of mulching materials in reflecting and transmission of solar radiation determines its efficiency in soil thermal regulation (Haapala et al., 2018). The reflectivity of a mulching material depends on the color of the mulching material (Yordanov and Nikolov 2017) such that dark coloured mulches absorb much of the radiation and keep the underlying soil warm during winters while cool during summer (Mahadeen 2014). Similarly, Aktan et al. 2018 observed that mulching with rice straw efficiently helps to mitigate high temperature losses during grain filling. Mulching regulates soil temperature such that minimizes in summer and rises in winter. In general, the effect of mulching on the temperature regime of the soil varies according to the capacity of the mulching material to reflect and transmit solar energy. White mulches decrease soil temperature while clear plastic mulches increase soil temperature.

Soil Moisture: The shortage of water resources and undependable rainfall patterns have stressed on adoption of water-saving technology for successful crop production (Patle et al., 2019). In this context, mulching is widely acknowledged as an efficient water-saving option due to its role in protecting the soil from direct exposure to sunlight which reduces surface evapo-transpiration owing to the maintenance of plant water status besides influencing temperature regulation and soil water conservation (Tongchuan et al., 2017 ; Sharma and Bhardwaj 2017). In general, mulched soils store more soil moisture than bare soils (Taparauskiene and Miseckaite 2014) and the amount of moisture conserved varies with the type and thickness of mulching (Dalorima et al., 2014). On the other hand, the role of mulching in minimizing evaporation losses was widely attributed due to checking the flow of energy between the soil and the atmosphere (Biswas et al., 2015 ; Vashisht et al., 2013).

Consequently, retains soil moisture for the growth of plants attributing to the rise in transpiration leading towards maintenance of high plant water status and cooler canopy (Hamerlynck et al., 2011 ; Yu et al., 2015). In an experiment comparing the efficiency of different mulching materials on wheat showed that performance of rice husk mulch to be significantly superior than plastic mulch in terms of water saving potential under sub-tropical soils (Li et al., 2013 ; Inusah et al., 2013). Furthermore, mulching checks surface runoff by retaining the rainwater for a longer period of time thus enhancing the rate of infiltration (Montenegro et al., 2013).

Soil Micro-organisms: In an Agro-ecosystem, soil microbial diversity plays an important role in nutrient cycling and in imparting soil structural stability (Bach et al., 2018). Influence of mulching on physio-chemical properties of soil is significant with the organic mulching practices. In general, soil microbial communities determine the sustainability of soil ecosystems (Manna et al., 2017 ; Ni et al., 2016). Organic mulches being the rich sources of carbon satisfies the dietary requirement of microbes and thus stimulates its growth attributing to rapid multiplication and break down of organic matter resulting in release of essential plant nutrients in available forms through mineralization, enriching the soil quality (Mehraj et al., 2016). On the other hand, cumulative influence of moisture conservation and thermo regulatory impact of mulching further help in providing a conducive environment for microbial development in the soil (Song et al., 2018). Moreover, enhanced microbial development plays an important role in biological nitrogen fixation and on soil reaction owing to increased soil nutrient status upon decomposition (Hamza et al., 2017). However, plastic mulches cannot get decomposed but facilitates rapid decomposition by promoting microbial activity due to its role in raising the soil temperature (Kasirajan and Ngouajio 2012).

Effect of Mulching on Plants

Growth and development: In general, mulching provides a most favourable environment for the growth and development of different crops. Mulching results in more uniform and vigorous growth of the crop owing to improved competitive ability (Das et al., 2018 ; Pramanik et al., 2015). This might be due to its direct influence on efficient suppression of weed population which would otherwise compete for inputs, space and light with crops in turn resulting in poor growth and dry matter distribution Besides this direct role of mulching on other favourable conditions like its in moisture conservation and erosion control further paces up the process of growth. Moisture conservation and erosion control effect of mulching is of prime importance in Agriculture (Ramesh et al., 2017 ; Vashish et al., 2013). Balance of temperature is very essential for the plant development and yield improvement thus due to the positive role in temperature modification, evaporation control etc (Raza et al., 2019). All these factors cumulatively influence the growth of the crops under field conditions.

Grain Yield: Yield of the crop in arid and semi arid areas is usually influenced by limited availability of moisture and nutrients in the soil. In this context, mulching is one of the most popular resource conserving technologies which upon application improves soil water content in the soil (Bana et al., 2016 ; Patil et al., 2013). At the same time mulching also plays an important role in enhancing transpiration rate due to reduced evaporation which in turn attribute to efficient translocation of water and nutrients (Lordan et al., 2015). Further, it also establishes better source-sink relationship and involves improved translocation of photosynthates owing to increased grain filling contributing to higher grain yield (Ali et al., 2010). At same time boosting of grain yield is also indirectly influenced by increased nutrient use efficiency augmented due to reduced leaching of fertilizers (Ghosh et al., 2019).

Quality: Mulching involves covering of the surface with a material either crop residues or plastic mulches. This practice involves varied implications such that mulch materials act as a barrier on soil restricting the leaching of nutrients into the soil ascribing to higher nutrient use efficiency (Mehmood et al., 2015). Adequate nutrient and moisture availability for a sustained period of time provides favourable conditions for growth and yield of crop (Tapiwa 2019). Besides, this enhanced availability also helps in expecting the higher quality product from the same (Alex and Thomas 2011). Mulching materials prevents soil poisoning due to minimization in the application of synthetic herbicides and at the same time they also act as a barrier to these synthetic inputs to reach the soil (Vox et al., 2013). These implications clearly mark the significant role of mulching on the quality of the agricultural produce. In certain cases especially in short statured vegetables or fruits it prevents the fruits or vegetables from contacting the soil thus avoiding rotting and cracking of fruits (Lalitha et al., 2010 ; Ayyogari et al., 2014).

Weed management: Influence of mulching in suppressing weed establishment plays an important role in providing a favourable and less competitive environment to the crop (Matkovic et al., 2015). Several mulching materials like straw, green litter material, bark slices etc usually act as an effective weed suppressors (Li et al., 2013). These mulching materials act as a barrier and prevent the entry of light (red light) thereby inactivating phytochrome system of the seed resulting in poor germination and establishment of weed seedlings (Altland et al., 2016). Mulching materials like saw dust acts more efficiently in soil improvement, moisture conservation and reducing weed growth substantially (Kumar and Dey 2011 ; Ewere et al., 2017) while black polythene mulches effectively control weed growth than white and transparent polythene, comparatively. This might be due to the opacity of the black polythene and its ability to check the entry of light through it.

CONCLUSION

In the present scenario, adopting the mulching as a resource conserving technology is has been identified

to have great potential address vagaries in farming especially through viewing its impact on different soil properties and its subsequent influence on crop growth and development clearly seems to have an extended scope in rainfed ecosystem thus identified as a silver lining for sustainable crop production ensuring food security.

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Extraction and Characterization of Protein Hydrolysate and Trypsin from Fish viscera of Labeo rohita

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ABSTRACT

Acidic and antacid proteases from instinctive misuse of *Labeo rohita* (Hamilton et. al., 1822) were confined, incompletely sanitized by ammonium sulfate precipitation followed by dialysis, their energy and attributes considered. The purging fold expanded from 1.24 to 2.49 and 1.19 to 1.55 in acidic and soluble protease individually along with the cleaning steps. The atomic weight was found in the scope of 15-35 kDa and 25-63 kDa individually in acidic and basic proteases. The pH and temperature optima for acidic and basic proteases were 3 and 10, at 40°C and 60°C individually. The Protease action was diminished by 40% and 60% when hatched at 90°C for 30 min. Both the proteases demonstrated a diminished movement of over half after brooding with NaCl centralization of 0.5%. The level of hydrolysis (DH) of the proteases on muscle protein expanded with an increment of chemical fixations. Both soybean trypsin inhibitor and EDTA displayed a high level of hindrance when proteases were hatched with 50 mM of both the inhibitors. The investigation demonstrated that proteases from Rohu instinctive misuse could discover use in applications where the greatest movement at moderate temperature and low NaCl fixation is wanted.

KEY WORDS: ACIDIC AND ANTACID, AMMONIUM SULFATE PRECIPITATION, LABEO ROHITA

INTRODUCTION

Fish preparing tasks produce more than 60 % of the crude material. In a non-industrial nation like India, these squander are arranged or changed over into creature feed, fish feast, and manure. This training prompts underutilization of crude material and may influence the manageable usage of accessible assets. The removal of fish handling waste is under exacting guidelines because of natural issues and it adds to the operational expense of the fish industry (Elavarasan et. al., 2016).Hence, the successful use of fish preparing waste is picking up significance. A rough amount of waste created during the preparation of a significant sort of fish items is introduced in Table.1. There is no verified information on the side-effect age from the Indian fish handling area. The measure of waste created will differ with the size, style of item and species, nature of taking care of (machine/manual taking care of, the aptitude of working/ taking care of individual). The significant squander from shellfish is shell squander which is used somewhat as a crude material in the chitin industry. Squanders from balance fishes are containing an impressive amount of proteins which can be changed over/recuperated into protein hydrolysates for improved usage. From the fish protein hydrolysate industry perspective, the amount of blade fish squanders/side-effects are more significant.

An enormous amount of instinctive squanders is produced in the retail fish advertises due to pre-preparing. Such organic squanders, if not used something else, would represent an issue of their removal and ensuing natural contamination. Fish preparing squanders is about 30% of the entire fish and contained head, scales, skins, and viscera (Klomklao et. al., 2006) and is considered as a

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phenomenal wellspring of protein and bio-dynamic peptides (Arnesen et. al., 2007). Even though there is a degree to recoup proteins and chemicals from the instinctive squanders of fish, however,a colossal amount of such waste is disposed of with no such endeavor (Bhaskar and Mahendrakar, 2007). As indicated by (Bezerra et. al.,2005), the fish instinctive waste generally represents 5% of the complete mass and incorporates stomach, pyloric caeca, digestion tracts, liver, pancreas, etc and different organs like spleen and balls. The stomach related chemicals from the fish instinctive waste are exceptionally dynamic over a wide scope of pH and temperature conditions and consequently speak to a significant esteemed side-effect of the fishing industry (Castillo-Yanez et. al., 2004).

Among the hydrolytic proteins, proteases speak to a significant class of mechanical compounds; have been utilized in various applications, generally in food, cleanser, material, calfskin, and pharmaceutics just as in squandering the executives and bioremediation measure (Anwar et. al., 1998)(Gupta et. al., 2002). Notwithstanding, proteases require their purging and portrayal before any application. Proteases contribute about 60% of the world's all out catalyst creation and utilized around the world (Gupta et. al., 2002). As of now, the greater part of the proteolytic proteins are separated from microscopic organisms, and generally, barely any endeavors have been made on the application of fish proteases as mechanical handling helps. Normally, the fishery results are commonly utilized as feeds and manures. As of late, intrigue has developed to look through high-esteem useful biomolecules from the fishery squanders, prominently catalysts. All things considered, a few specialists researched proteases from the instinctive squander from marine fish (Nasri et. al., 2011). However, the portrayal of fish proteases particularly from the instinctive squanders of freshwater fish is only occasionally revealed.

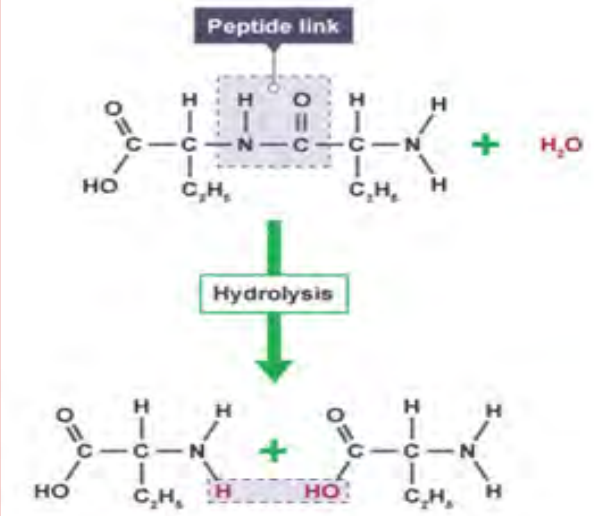
In light of the above reasoning, the current examination was done to describe mostly purged acidic and soluble proteases from the fish instinctive squanders for deciding their application in food handling activities just as to decrease garbage removal issues. Rohu (Labeo rohita), overwhelmingly a section feeder and feeds basically on filamentous green growth, disintegrated vegetation, and mud was chosen for concentrate as it is the most regularly devoured freshwater fish in India among the carps.

1.2. Worldwide protein market: Worldwide protein fixing market examination uncovered that 43.2 % of incomes in the worldwide wellbeing fixings market contributed from protein. Europe holds its enormous lead in the worldwide protein market. Key items under creature protein fixings incorporate, gelatin, collagen, egg, and dairy. There is a steady interest in creature protein fixings. The worldwide protein market is required to arrive at the estimation of US $ 40.88 Billion (2.7843618 trillion Indian rupees) by 2022. The utilization of protein fixings in newborn child equation diminished protein insufficiencies. The utilization of protein fixings in the drug and the restorative industry is expanding continuously.Global protein fixing a piece of the pie is reasonably solidified with DuPont, Bunge, ADM, Cargill, and Mead Johnson being the significant business players.

Raw material accessibility in China and India impact industry players to move to fabricate base in the region. The protein supplements market in India is developing at 6% and is presently esteemed at Rs. 252 crores every year. Fish protein hydrolysate Fish protein hydrolysate is an item set up from proteins sourced from fish meat/ fish handling by items using enzymatic or synthetic cycle. Enzymatically created hydrolysates are broadly acknowledged which contain a combination of peptides of changing sizes and free amino acids.

1.3. Cycle for creation of protein hydrolysate: Protein hydrolysates from fish handling dispose of can be readied utilizing four distinctive cycles in the particular corrosive cycle, antacid cycle, enzymatic cycle, and microbial maturation. The fundamental component of enzymatic hydrolysis and the impact of various elements is examined underneath.

Figure 1: Show the Hydrolysis of Peptide Bond



1.4. Enzymatic cycle: Enzymatic hydrolysis of the fishery by items uses either autolysis measure or by adding exogenous protein. Autolysis measure includes brooding ground fishery squander at ideal response states of endogenous chemicals and utilizes the fish instinctive waste (Kristinsson et. al., 2000). The endogenous catalysts trigger the stalling of biomolecules to more modest peptides through autolysis measures. The autolysis is generally directed at impartial or somewhat basic pH, abusing the presence of serine protease of the digestive tract in basic or the carboxyl favorable to a prod of gastric juice in acidic pH (Pastorizaet et. al., 2004).

Proteolysis is the enzymatic hydrolysis of the amide bond in peptides and proteins. The compounds are misused to perform wanted capacities in handling and examination

and to encourage the changes of crude materials into top-notch more alluring staples (Richardson et. al., 1984). Compounds utilized in the food business and exploration are dominatingly hydrolases. Proteolytic proteins are financially the main gathering of compounds and their utilization is entrenched in the food business (Godfrey et. al., 1983). The utilization of proteases in the planning of fish protein hydrolysates has gotten a wide consideration among scientists as it is more efficient and simple of cycle control. The idea of catalysts, substrate, and hydrolysis will decide the properties of (Fish Protein Hydrolysate) FPH.

The overall streamline for the creation of FPH by the utilization of chemicals is portrayed in Fig 1. The cycle includes the homogenization of fish meat or fish squanders with the expansion of water. The homogenate is brought to the ideal temperature and pH. The hydrolysis is started by the expansion of protein at the wanted focus. After a specific length of hatching, the hydrolysis is ended by applying heat or by changing the pH. The dissolvable part in the wake of eliminating the unhydrolyzed partition is concentrated by freeze-drying/ stove drying/shower drying. The dried protein powder alludesto protein hydrolysate.

- To deliver the FPH with various and wanted properties, it is critical to know the component of protein hydrolysis. A few proteases specially catalyze the hydrolysis of bonds nearby a specific amino corrosive build-up, while some are less explicit. The catalysis by proteases happens basically as three continuous responses (Krsitinsson et. al., 2000): the development of complex between the first peptide chain and the catalyst alluded as the Michaelis complex
- Cleavage of the peptide cling to free one of the two peptides
- Nucleophilic assault on the remaining parts of the complex to separate the other peptide and to reconstitute the free catalyst

The hydrolysis of peptide bonds prompts an expansion in the quantities of ionizable gatherings (NH3+ and COO-), with an associative expansion in hydrophobicity and net charge, the decline in the atomic size of the polypeptide chain, and an adjustment of the sub-atomic structure prompting the introduction of the covered hydrophobic deposits to the fluid climate (Phillips et. al., 198)(Kester et. al., 1984)(Mahmoud et. al., 1992). Endless supply of catalyst to the proteins, the chemical substrate complex will be framed. This complex alludestoMichaelis complex which may separate back to reactant substrate and free compound, or to free chemical and item atoms (Adler et. al., 1986). The by and large acknowledged component for proteases shows that the second step that is the separation of compound substrate complex into free protein and item is the rate-deciding advance, which decides the general pace of response. Enzymatic hydrolysis of proteins is a mind-boggling measure as a result of a few peptide bonds and their particular openness to enzymatic responses (Linder et. al., 1995).

The particularity of proteins isn't the main factor that influences the peptide profile of the eventual outcome and factors, for example, temperature and pH assume a significant job. The temperature and pH can extraordinarily influence the compound response energy and their effect is diverse for every chemical. By and large, there is an ideal mix of both pH and temperature, where the compound is generally dynamic. Temperature and pH limits deactivate the compounds by denaturing them. The elements engaged with the hydrolysis of proteins are most significant both as far as energy and nature of the finished result. The main components impacting the properties of FPH are the nature of the substrate, nature of protease, and level of hydrolysis (DH) and drying strategy.

1.5. Protein hydrolysates from various fish handling: Various squanders created during fish preparation like head, skin, roe, outline waste and bone have been utilized to deliver the hydrolysate. Then again, the proteins confined from the waste parts can likewise be utilized for this reason. The protein content in various fish squanders parts are introduced in Table 1. A large portion of the investigations has been completed regarding the hydrolysis cycle and their bioactive and practical properties.

Figure 2: Shows the Preparation of Fish Protein Hydrolysate

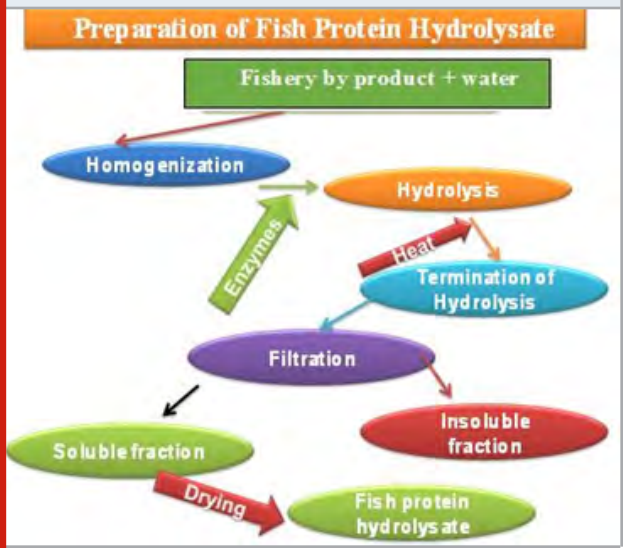


Table 1: Shows Protein Content In Significant Fish WasteParts

Waste Parts	Protein (%)
Head	11%-13%
Spine/outline	10-15
Cutoffs	12-22
Skin	8-12
Milt	14-27
Viscera	9-23

Logical investigations have been accounted for the planning of fish protein hydrolysate from the fish head, viscera, roe, skin, edge, and bone. The greater part of these examinations has zeroed in on their cancer prevention agent properties and different cell reinforcement peptide particles have been separated and portrayed. (Chalamaih et. al.,2012) has thoroughly evaluated the protein hydrolysates from different pieces of fish squander. The fish head is a significant fishery squander contains gills. Eyes, head casing, and shoulder muscle. It is hard to recuperate the protein because of its basic unpredictability. The enzymatic cycle will solubilize the protein by changing over into peptide shapes at that point encourage the simple recuperation of proteins. Protein hydrolysates from fish head side-effect squander have been set up from different species. The significant protein present in the fish head is collagen.

Thus the peptides produced will have ordinarily the arrangement from collagen which are known for their enemy of joint pain and hostile to corpulence properties. Fish skin is again a rich wellspring of collagen. Endeavors have been made to create the hydrolysate either legitimately from the fish skin or in the wake of disconnecting the collagen or gelatin. The fish liver is another result that generally goes for oil and dinner creation. The fish liver has been utilized to set up the hydrolysateusing protamex, flavorzyme, alacalase, and neutrase (Je et. al., 2009)(Ahn et. al., 2010). Fish viscera is also a potential source ofprotein that can fill in as a crude material for the readiness ofprotein hydrolysates. Normally, instinctive waste protein hydrolysate may exhibitunique properties. As of late, numerous attempts have been performed for the use of fish instinctive waste for protein hydrolysates creation (Batista et. al., 2010).

Fish roe contains a considerableamount of protein. To use this underutilized protein source from fish roe, protein hydrolysates have been readied. For instance, roe protein hydrolysate from Cirrhinusmrigalausing alcalase andpapain has been reported(Chalamaiah et. al., 2010). Fishbone, which is isolated after the evacuation of muscle proteins on the casing, is another important source in distinguishing well-being advancing parts. The natural segment of fishbone, which represents 30% of the material, is made out of collagen. Consequently, fishbone is considered as a hotspot for protein hydrolysate particularly collagen peptides and gelatin hydrolysates (Kim et. al., 2006).

1.7. Use of fish protein hydrolysates Nutritional application: The proximate organization of fish protein hydrolysate would shift with the crude material (head, bone, skin, viscera), sort of cycle, kind of drying, degree of hydrolysis, and some other pre-treatment of crude material. The substance structure of food materials has a significant part in human wellbeing in gracefully of basic supplements for keeping up prosperous wellbeing. The compound arrangement of fish protein hydrolysates is significant froma sustenance point of view of human wellbeing.

Amino corrosive creation of protein hydrolysates from various crude material delivered utilizing diverse chemical source under various hydrolysis conditions expected to have variety. All in all, required fundamental amino acids are bountiful in FPH with wealth in glutamic and aspartic corrosive substances. FPH do likewise have trivial amino acids. The presence of fragrant amino corrosive in fish outline protein hydrolysates has been accounted for. Studies have unmistakably indicated that FPH from fish meat/fish waste could be an ideal wellspring of basic amino acids (Chalamaiah et. al., 2010).

Table 2: Shows Proximate Structure Of Fish Protein Hydrolysate

Waste Part	Protein (%)
Moisture	< 10%
Protein	60-90%
Fat	<5%
Debris	0.45-27%

Nutraceutical applications: There are fish protein hydrolysate items/peptides explicitly showcased as well-being supplements in created nations (Table 3). These items are demonstrated to have an explicit well-being job other than the dietary advantage. Protein hyd5rolysates or peptides present in the hydrolysate have exhibited to have cell reinforcement, hostile to heftiness, invulnerable balance, against coagulation, hostile to microbial, anticancer and antihypertension and so forth (Elavarasan et. al., 2014)(Elavarasan et. al., 2016).

1.8. Fish protein hydrolysate as a practical ingredient: Fish protein hydrolysates are dissolvable in a wide scope of pH which is an ideal trademark assist with utilizing in a wide scope of items. Protein hydrolysates have improved water-holding, oil official, emulsifying, and frothing properties. Be that as it may, the key factor which decides the useful properties is the level of hydrolysis. When all is said in done, broad hydrolysis prompts loss of usefulness. There is a basic level of hydrolysis at which protein hydrolysates ought to be set up concerning specific capacity to be utilized as a practical fixing (Elavarasan et. al., 2016) (Gajanan et. al., 2017).

1.9. Fish protein hydrolysate as feed ingredients and different applications: Fish protein hydrolysates (FPHs) have been utilized in hydroponics to take care of to improve the development and endurance of fish. Studies have indicated that FPH has helped the development execution and immunological status of many culture species. The amino corrosive arrangement and the peptides present in hydrolysate are liable for the improved development and immunological status. FPH is additionally being utilized as a wellspring of protein in poultry feed detailing and in pet creature nourishments. Different applications incorporate FPH as a plant sponsor, fixing in microbiological media, and as a cryo-protectant in fish mince/surimi.

Table 3. Shows Monetarily Advertised Fish Protein Hydrolysate Items As Nutraceuticals

Item brand name	Points of interest	Nutraceutical applications	Nation
PROTIZEN®	Delivered by enzymatic hydrolysis of white fish proteins United Kingdom	It is "disposition food" and dietary enhancement to battle against pressure and its side effects (weight disorders, work pressure, rest troubles, fixation challenges, and mind-set inconveniences).	
Amizate®	Delivered from Atlantic salmon fish proteins via autolysis	Sports sustenance (bolsters the body's muscle anabolism and metabolic recuperation).	North America
Seacure®	Delivered by hydrolyzing profound sea white fish proteins	Dietary enhancement assists with supporting the cells in the gastrointestinal plot and direct gut capacities.	Canada & USA
Vasotensin®	Delivered from Bonito (Sardaorientalis) by thermolysin hydrolysis	It upholds solid vascular capacity for ideal bloodstream and sound pulse levels.	Japan & USA
LIQUAMEN®	Prepared from Molvamolva via autolysis	The dietary enhancement that helps in decreasing oxidative stress, bringing down the glycemic record and hostile to stretch.	United Kingdom
Stabilium® 200	Prepared from Molvadypterygia via autolysis	Supports the body's reaction to stretch and offers healthful help for memory and intellectual capacity.	United Kingdom
PEPTACE®	Delivered from Bonito (Sardaorientalis) by thermolysin hydrolysis	It brings down the circulatory strain by repressing the ACE compound.	Japan & USA
MOLVAL®	Delivered from North Atlantic fish Molvamolva by enzymatic hydrolysis	Dietary enhancement suggested for. cholesterol equilibrium, stress control, and advances great cardiovascular wellbeing	United Kingdom

Security of protein hydrolysates in human nourishment: By and large, food business administrators ought to guarantee the well-being of items. The wellbeing parts of any food fixing should be recorded before discharge on the lookout. Protein hydrolysates can be considered as protected when they are hydrolyzed from proteins having a past filled with alright for utilization and they are created utilizing proteases that are of food-grade and utilized regular food-handling techniques. The well-being of parts and bioactive peptides, gotten from safe hydrolysates, ought to be assessed by the assembling before the market presentation. A survey of the well-being evaluation of the organization by an outside free council and resulting endorsement by the skilled specialists as per novel food methodology is fundamental when the wellspring of protein and cycle is novel and under strange high admission of amino acids (Schaafsma et. al., 2009).

MATERIAL AND METHODS

2.1. Rohu viscera: Viscera of Rohu was gathered in polyethylene packs and shipped with ice. In the research center, viscera was washed with chilled water to eliminate the disciple blood, sludges, and soils, kept in plastic packs, and put away at - 20°C until utilized for chemical extraction.

2.2. Planning of unrefined acidic and soluble protease: The technique recommended by (Vannabun et. al., 2014) was followed for the readiness of unrefined acidic and antacid proteases. At first, the instinctive mass was defrosted and homogenization was accomplished for 2 min with various extraction supports, for example, citrate cushion (10mM Citrate/HCl pH 3.0) for corrosive protease and tris cradle (10mM Tris–HCl pH 8.0, 10mM CaCl2) for basic protease, in the proportion of 1:5(w/v). The homogenate was centrifuged at 10,000 x g for 10min at 4°C. After homogenization, the pellet was disposed of to gather the supernatant which was utilized as „crude chemical extract".

2.3. Enzyme cleansing: The rough chemical concentrate was exposed to two-venture (NH4)2SO4 precipitation. According to primer measure, (NH4)2SO4 grouping of 40-60% gave the most noteworthy purging fold and

explicit action. The rough chemical was encouraged with a 40-60% immersion of ammonium sulfate and afterward permitted to agree to 24h at 4°C. The supernatant was disposed of and the accelerate was broken up in 0.02 M acetic acid derivation cushion, pH 3.0 and 0.02 M Tris–HCl support, pH 8.0 for acidic and soluble proteases individually, by centrifugation at 10,000×g for 30 min at 4°C. The protein consequently acquired was dialyzed against similar support for 24 h at 4°C with the discontinuous difference in the cradle after 12 h. After dialysis, the unrefined chemical was alluded to as „partially filtered proteases".

2.4. Assurance of atomic weight: The atomic weight (MW) of the somewhat cleaned compound was completed by SDS-PAGE, following the technique proposed by Laemmli (1970). Example support was set up by blending 2.5 ml 0.5 M Tris-HCl (pH 6.8), 4 ml 10% SDS, 2 ml glycerol, 1 ml 1% b-mercaptoethanol, 0.03 ml 0.002% bromophenol blue and the last volume was made to 10 ml. Protein arrangements were blended at a 1:2 (v/v) proportion and bubbled for 10 min. Tests (10 µl) were stacked on the gel made of 4% stacking and 12.5 % isolating gels and fractionated for 90 min at a consistent current of 400 mA. After electrophoresis, the gels were recolored with 0.05g Coomassie splendid blue R-250 in 15% methanol and 5% acidic corrosive and destained with destaining arrangements [solution-1 (half methanol and 7.5% acidic corrosive) and arrangement 2 (5% methanol and 7.5% acidic acid)]. The atomic weight was assessed utilizing protein standard (10-245kDa) (HiMedia, India).

2.5.Protein content: The protein content was assessed following Lowry "s technique (Lowry et. al., 1951) by estimating test absorbance at 280 and 260 nm, utilizing ox-like serum egg whites as standard.

2.6.Test of protease action: The acidic protease action was resolved as recommended by (Natalia et. al., 2004) utilizing 2% cow-like hemoglobin arrangement containing 0.04M HCl (corrosive denatured) as substrate at pH 3.0 and 37°C, while, a technique for Rawdkuen et al., (2010) was followed to decide soluble protease movement utilizing casein as a substrate. The absorbance read at 280 nm and changed over into µmoles of tyrosine freed utilizing arrangements of 25-250 µg/ml centralization of tyrosine for alignment bend. Enzymatic action was communicated as one unit equal to the measure of chemical equipped for hydrolyzing ox-like hemoglobin to free 1 µmole tyrosine under standard examine conditions. All-out action and explicit action was communicated as units of enzymatic movement per ml protein (U/ml) and per mg protein (U/mg) individually.

2.7.Complete action: The complete enzymatic action was assessed utilizing the accompanying condition.

$$Total\ Activity\left(\frac{U}{mL}\right) = \frac{\mu\ mole\ of\ Tyrosine\ equivalent\ released\ \times\ Total\ Volume\ of\ reaction\ assay\ (mL)}{Volume\ of\ Enzyme\ used(mL) \times Time\ of\ Assay\ (min) \times Volume\ used\ in\ Cuvette(mL)}$$

2.8.Explicit action of protein: The particular action of both the proteins was resolved to utilize the condition as proposed by El-beltagy et al., (2005).

$$Specific\ Activity\left(\frac{U}{mg}\right) = \frac{Total\ activity\left(\frac{U}{mL}\right)}{Protein\ content\left(\frac{mg}{mL}\right)}$$

2.8.Protein decontamination fold: The degree of sanitization was assessed by deciding the purging fold following the condition given by El-beltagy et al., (2005).

$$Purification\ fold = \frac{Specific\ Activity}{Specific\ Activity\ of\ crude\ extract}$$

2.9.Ideal pH and pH stability: The ideal pH for enzymatic action was resolved after the strategy for (Vannabun et. al., 2014), by testing protease action at various pH conditions utilizing 100mM cradle arrangements going from pH 1.0 to 12.0 (Glycine-HCl cushion for pH (1.0-3.0); sodium acetic acid derivation support for pH (4.0-6.0); Tris-HCl support for pH (7.0–9.0); and Glycine–NaOH support for (9.0-12.0), at the ideal temperature for action recently decided.

The impact of pH on protein security was dictated by the technique for (Vannabun et. al., 2014). The compound was hatched at different pH (1.0–12.0) utilizing various cushions of 100mM Glycine–HCl (1.0–3.0), Na-acetic acid derivation (4.0–6.0), Tris–HCl (7.0–9.0), and Glycine–NaOH (10.0–12.0) for 30 min alongside the spaces arranged all the while. The leftover enzymatic action after brooding was assessed and contrasted and the condition that indicated the most elevated worth (100% movement).

2.10.Ideal temperature and temperature stability: Protease movement at various temperatures (30–90°C) was performed by utilizing various cushions like Glycine–HCl (pH 3.0) and Tris–HCl (pH 8.0) for acidic and basic protease action separately as per the strategy given by Vannabun et al., (2014). To decide the warm steadiness of proteases, catalyst removal was brooded for different time lengths like 1,3,5,10,15,20,30,40,50, and 60 min at 90°C and the staying enzymatic movement was resolved. The control was not pre-hatched and considered as 100% action.

2.11.Impact of NaCl focus on enzyme movement: The response blend was made with various conc. of NaCl (0–2.5%, w/v) and protein was hatched trailed by the assurance of lingering action. The control was made without NaCl and its action was considered as 100% action (Vannabun et. al., 2014).

2.12.Impact of isolated enzymes on proteins hydrolysis: Separated acidic and antacid proteases were utilized to hydrolyze the groundfish muscle protein to decide the level of hydrolysis of catalyst on the fish muscle. The ground muscle (2g) was hatched with protein at various focuses (10-50 mL) for 30 minutes at 60°C. The response

was halted by adding 5 mL of 20% TCA followed by centrifugation at 3300 rpm for 10 minutes to gather the 10% TCA solvent material as the supernatant. The protein substance of the supernatant was assessed by the Biuret technique. The level of hydrolysis was dictated by the strategy (Hoyle et. al.,1994).

$$\%DH = \frac{10\%\ TCA\ soluble\ protein\ in\ the\ sample}{Total\ content\ of\ sample} \times 100$$

2.13.Chemical inhibitors and activators: Chemical inhibitors, for example, soybean trypsin inhibitor (STPI) and ethylene diamine tetra acetic corrosive (EDTA) were utilized to decide their consequences for the enzymatic movement. Pre-hatching of the substrates with those at various conc. like 10, 20, 30, 40, and 50 mM were accomplished for 10 min at the ideal temperature of compound followed by an assurance of catalyst movement. The outcomes were communicated as an overall level of the movement without modifiers.

2.14. Analysis of Statistics: Investigation of change (ANOVA) trailed by Duncan's different reach test was done to decide contrasts between implies. The measurable investigation was performed utilizing the Statistical Package for Social Sciences (SPSS for Windows adaptation 16.0, SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

3.1.Halfway filtration of proteases: The protein content, absolute movement, explicit action, and cleaning fold for acidic and antacid proteases of instinctive misuse of Rohu is introduced in Table 4. The normal protein content was discovered to be 6.31 mg/ml and 7.79 mg/ml in acidic and soluble rough proteases individually. After ammonium sulfate fractionation (40-60%), the protein content diminished in the unrefined proteases, and the qualities came to 3.72 mg/ml and 4.15 mg/ml in acidic and antacid rough proteases individually. The soaked ammonium sulfate arrangement specifically accelerates proteins from the rough catalyst separate by the salting-in and salting-out system to frame a mostly refined chemical concentrate (Klomklao et. al., 2006). This might be because of the pollutions present in the unrefined example which are taken out after ammonium sulfate precipitation. Dialysis, a stage in the refinement of proteases, displayed a further decrease of protein content in the (NH4)2SO4 accelerated proteases. In acidic and soluble proteases the normal protein content diminished to 1.68 mg/ml and 2.96 mg/ml separately. Such abatement of protein content after dialysis might be because of the additional evacuation of different proteins, not eliminated by ammonium sulfate fractionation.

3.2.The atomic weight of proteases: The electrophoretic example demonstrated a few clear groups showing the presence of various proteases of shifting sub-atomic mass if there should arise an occurrence of both basic and acidic protease tests (Fig. 3). If there should arise an occurrence of rough and somewhat sanitized acidic and antacid proteases 3-4 groups were watched going from 15-35 kDa and 25-63 kDa separately. A few creators revealed the sub-atomic loads of instinctive soluble and acidic proteases in the scope of 17-90 kDa. Atomic load of acidic protease from Tilapia nilotica after gel filtration on Sephadex G-100 was accounted for as 31.0 kDa (El-Beltagy et al., 2004). While, (Lopez-Liorca et. al.,1990) and (Liu et. al., 2008) revealed the sub-atomic load of the instinctive acidic protease of fish as around 32 kDa and 28.5 kDa individually.

Sub-atomic load of fish instinctive soluble proteases has been accounted for as 23.5 kDa (Bezerra et. al., 2005), 23-28 kDa (Balti et. al., 2009), 23 kDa (El-Beltagy et. al., 2004), 24-30 kDa (Sekizaki et. al., 2000). The current investigation uncovered that the atomic loads of soluble proteases are higher contrasted with acidic proteases. The presence of a few groups in the electrophoretic partition of stomach related proteases was disclosed as because of constituent chemicals like trypsin, chymotrypsin, collagenase, gastricin, pepsin, elastase, carboxypeptidase, and carboxylesterase (Barkia et. al., 2010), and due generally to the distinctive sub-atomic loads of individual catalyst. The current investigation supported the perceptions announced by before analysts with regards to atomic weight appropriation of stomach related proteases (Younes et. al., 2014)(Sila et. al., 2012).

3.3. Examine of proteolytic action: The normal all-out action of unrefined acidic and antacid Rohu viscera squander was resolved to be 18.33 U/ml and 34.11 U/ml individually. Complete proteolytic action diminished after (NH4)2SO4 fractionation (ASF) and further decrease occurred after dialysis. If there should arise an occurrence of basic proteases the recuperation rate was discovered to be 63.67 and 58.95 after ASF and dialysis separately (Table 1). Comparative was seen if there should arise an occurrence of acidic proteases, wherein, recuperation of absolute action after ASF and dialysis were discovered to be 72.76 and 66.28 separately (Table 4). Purging may have eliminated other catheptic compounds that were likely present in the instinctive waste, and came about the decline of the absolute action. Such abatement of protease movement after sanitization was additionally detailed (Subash et. al., 2011)(Kim et. al., 2012).

The normal explicit action after dialysis was discovered to be 6.79 and 7.23 if there should be an occurrence of soluble and acidic proteases separately. Such increment of explicit action along the cleansing advances might be clarified as the evacuation of meddling proteins during (NH4)2SO4 fractionation and further during dialysis, coming about improved action. Increment of explicit action with the advancement of the refinement was likewise detailed by (Liu et. al., 2008) (Bezerra et. al., 2005) (El-Beltagy et. al., 2004). This examination additionally uncovered that the particular movement of post-dialysis acidic proteases was more than the soluble proteases, even though, the later indicated more all-out action and pre-dialysis explicit action contrasted with the acidic one. Since the Rohu being missing of the genuine stomach, maybe the explanation behind the low measure

of acidic proteases discharge in the gut substance of viscera, as stomach establishes a significant wellspring of stomach related proteolytic catalysts (Simpson et. al., 2000).

The particular movement of the protein decides the sanitization overlay. In the two-venture purging framework, the cleansing fold encountered an expansion from stage two to stage three in the event of both acidic and basic proteases. If there should arise an occurrence of soluble proteases the outcome demonstrated that expansion in the purging fold was from 1.19 to 1.55, while, it was 1.24 to 2.49 in the event of acidic proteases. Increment of refinement crease following dialysis has likewise been accounted for by (El-Beltagy et. al., 2004) (Liu et. al., 2008). Immaculateness of the trypsin-like compound from anchovy stomach related plot was expanded by 2.7-overlap following ammonium sulfate precipitation (20-70%) (Martinez et. al., 1988).

3.4. Ideal pH and pH stability: Incompletely purged corrosive and soluble proteases were discovered to be dynamic over a scope of pH 1.0–12.0 utilizing casein and corrosive denatured ox-like hemoglobin as substrates for antacid and acidic proteases individually. The acidic protease showed high action in the pH range from 2-4 with an expected most extreme at 3.0 and afterward diminished essentially ($p<0.05$) with expanding pH (Fig. 4a). The relative movement of about over half was lost over pH 4. In other investigations, the ideal pH for hydrolysis of corrosive denatured cow-like hemoglobin by a halfway decontaminated acidic protease from Tilapia nilotica was discovered to be 2.5 (El-Beltagy et. al., 2004). Our outcomes substantiate well with the perception of (Bougatef et. al., 2009), who detailed pH optima for acidic proteases in the scope of 2-4. The basic protease displayed the most extreme action at pH 10 and afterward diminished fundamentally at higher pH levels (Fig. 4c). Ideal pH for most extreme movement of basic protease was accounted for in the scope of 8-10 (Nasri et. al., 2011). Assurance of pH optima of a protein is basic as this is viewed as a significant marker for its possible application for various purposes.

Both the acidic and basic proteases were exceptionally steady over a wide pH range, keeping up over 90% of its unique movement between pH 1.0-5.0 and pH 8.0-12.0 regarding corrosive and antacid proteases individually following 30 minutes brooding at 37°C (Fig. 4b, d). The pH steadiness of proteases relies upon the distinctions in atomic properties, which incorporates holding and soundness of the structure; adaptation of chemical in various anatomical areas among different species (Klomklao et. al., 2007). Comparative discoveries concerning pH dependability of acidic protease from fish have likewise been accounted for by (Castillo-Yanez et. al., 2004) for Monterey sardine. The pH solidness of antacid proteases in the scope of 6-12, has been accounted for by a few creators (Sila et. al., 2012)(Younes et. al., 2014). Acidic protease movement demonstrated a reduction of around 15-20% at pH over 6.0 though; a comparative diminishing was appeared by basic protease at pH beneath 7.0.

3.5. Ideal temperature and thermostability: In this examination, the ideal movement of acidic protease was found at 40°C (Fig. 5a) which is like the prior reports from other fish, viz., pepsins from Sardinelle by (Ben Kahled et. al., 2008) and smooth dog by (Bougatef et. al., 2009). The ideal temperature of basic protease movement was found as 60°C (Fig. 5c) and the comparative outcome was accounted for by (Klomklao et. al., 2011) (Cao et. al., 2000) for trypsin from the pyloric caeca of Chinook salmon (Oncorhynchus tshawytscha) and Japanese seabass (Lateolabrax japonicas) separately.

The outcome demonstrated that the chemical action of proteases expanded in a specific way followed by a reduction with increment in temperature framing a ringer molded bend. At temperature above ideal, the local adaptation of protein is changed because of the breakdown of feeble intramolecular bonds capable of adjustment of the three-dimensional structure of the catalyst dynamic site (Klomklao et. al., 2011). As thought by (Klomklao et. al., 2006), natural and hereditary variables among the various species may be answerable for the local adaptations of catalysts.

The investigation likewise uncovered that acidic and soluble proteases'' movement diminished by 40 and 60% individually when the hatching condition was 90°C for 30 min (Fig. 5b and d). This might be clarified as the inactivation of enzymatic action following loosening up of the enzymes local compliance during warm treatment (Klomklao et. al., 2011). As (Vannabun et. al., 2014) additionally announced comparable discoveries while portraying instinctive acidic and antacid proteases of cultivated monster feline fish. As proposed by (Sabtecha et. al., 2014), the soundness of a fish protein in various temperatures is affected by their natural surroundings, climate, and hereditary characters.

3.6. Impact of NaCl on catalyst action: As Fig. 6 shows the impact of NaCl on protein action of proteases. Relative protein action demonstrated a reduction of over half for both acidic and basic proteases at NaCl centralization of 0.5%. Further increment of NaCl focuses somewhat on diminished protease action. A 10% reduction in the general action of acidic protease from Sardinelle at 20% NaCl focus was accounted for by (Ben et. al., 2008). This demonstrates that the movement of proteases of freshwater fish contrasts from the marine fish, and this is essential because of salt centralization of the living space. (Klomklao et. al., 2011) explored trypsin action from crossover catfish and found that the compound action fundamentally diminished steadily with the expanding grouping of NaCl. This loss of compound movement maybe because of the denaturation of protein (Ben et. al., 2008) coming about the "salting out" impact.

The ionic quality is expanded with the expanding salt focus. In the high ionic quality, the compound movement

is decreased because of unrivaled hydrophobic–hydrophobic association between proteins of the chemical and upgraded liking of ionic salts for water consequently coming about precipitation of catalyst (Klomklao et. al., 2009).

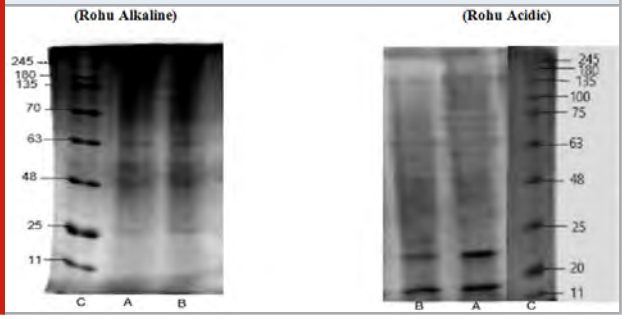
3.7. Impact of Isolated enzymes on proteins hydrolysis: As the level of hydrolysis (DH) is the demonstrative of the degree of peptide bonds separated (Adler-Nissen et. al., 1979), its assurance is significant since a few qualities of protein hydrolysates is DH subordinate. Utilizing ground muscle meat of fish as substrate, hydrolysis was directed at temperature 37°C and ideal pH for both the compounds. The level of hydrolysis (DH) as a component of the compound fixation is given in Fig. 7. The aftereffect of this examination connotes that higher measure of proteases in chemical division severed more peptide bonds and comparable perception was additionally announced by (Klompong et. al., 2008).

Table 4. Showa Purification Of Acidic And Basic Proteases From Instinctive Misuse Of Rohu

	Steps for Cleansing	Protein Content (mg/mL)	Complete Activity (U/mL)	Explicit Activity (U/mg)	Recuperation (%)	Purification Fold
Acidic Protease	Unrefined	6.31±0.08	18.33±0.06	2.90±0.05	100	01
	Ammonium Sulfate Fractionation (40-60%)	3.72±0.04	13.37±0.02	3.59±0.01	72.76	1.24
	Dialysis	1.68±0.05	12.15±0.28	7.23±0.15	66.28	2.49
Alkaline Protease	Unrefined	7.79±0.06	34.11±0.11	4.38±0.01	100	01
	Ammonium Sulfate Fractionation (40-60%)	4.15±0.03	21.72±0.76	5.23±0.04	63.67	1.19
	Dialysis	2.96±0.03	20.11±0.61	6.79±0.07	58.95	1.55

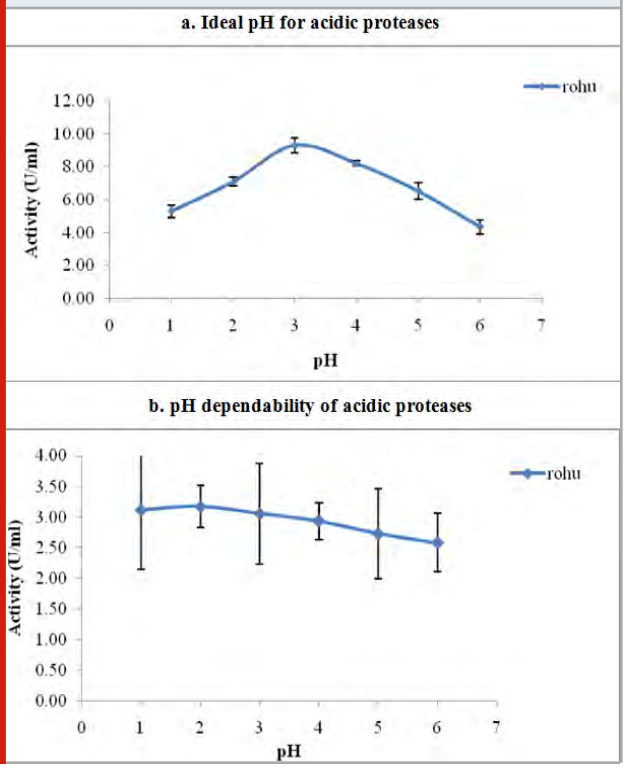
*Values given in the table are implied ± SD, n=3.

Figure 3: Shows Electrophoretic example of Rohu viscera squander (C=standard protein marker, A=crude catalyst concentrate, and B=purified chemical concentrate)



3.8. Impact of inhibitors on the catalyst action: High restraint rates of complete acidic protease action were gotten when the acidic protease was brooded with 50 mM of both soybean trypsin inhibitor or ethylenediaminetetraacetic corrosive (90.9% and 68.8%, separately), while they were 23.9% and 10.5% individually when 10 mM focus was utilized (Fig. 8). Practically comparative percent restraint was gotten in the event of basic protease. Our outcome is in concurrence with the discoveries of (Diaz-Lopez et. al., 1998) (El-Beltagy et. al., 2004) for the acidic protease. In an examination with tilapia stomach related proteases, a high hindrance of approx. 40% was accounted for utilizing low convergence of SBTI (Moyano et. al., 1999). Restraint of Rohu basic proteases at 250 µM centralization of SBTI was accounted for to be 78.1% (Kumar et. al., 2007).

Fig 4: Optimum pH and pH dependability for most extreme movement of proteases from Rohu viscera squander (a-ideal pH for acidic proteases, b-pH solidness of acidic proteases, c-ideal pH for antacid proteases, thed-pH steadiness of soluble proteases)



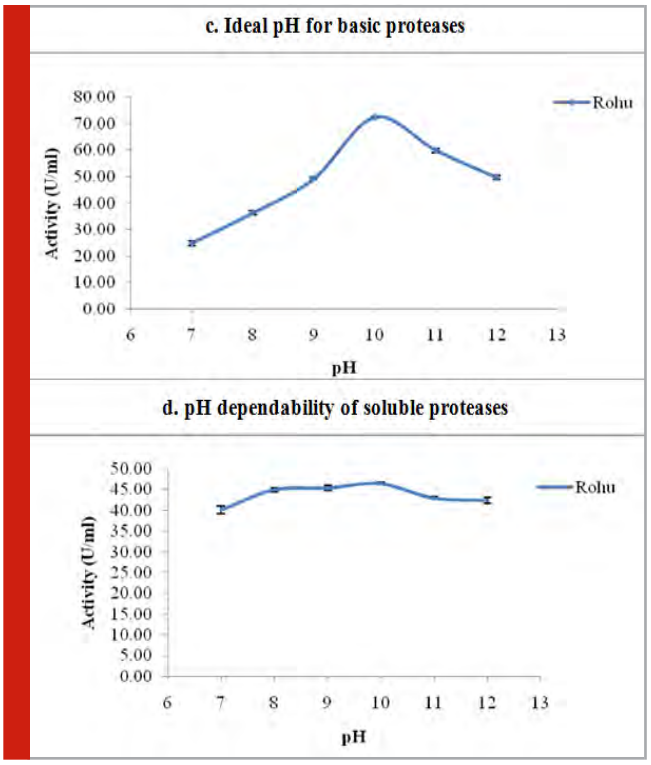


Fig 5: Optimum temperature and thermostability for most extreme action of proteases from Rohu viscera squander (a-ideal temp. a necessity for acidic proteases, b-thermostability of acidic proteases, c-ideal temp. a necessity for antacid proteases, and d-thermostability of basic proteases)

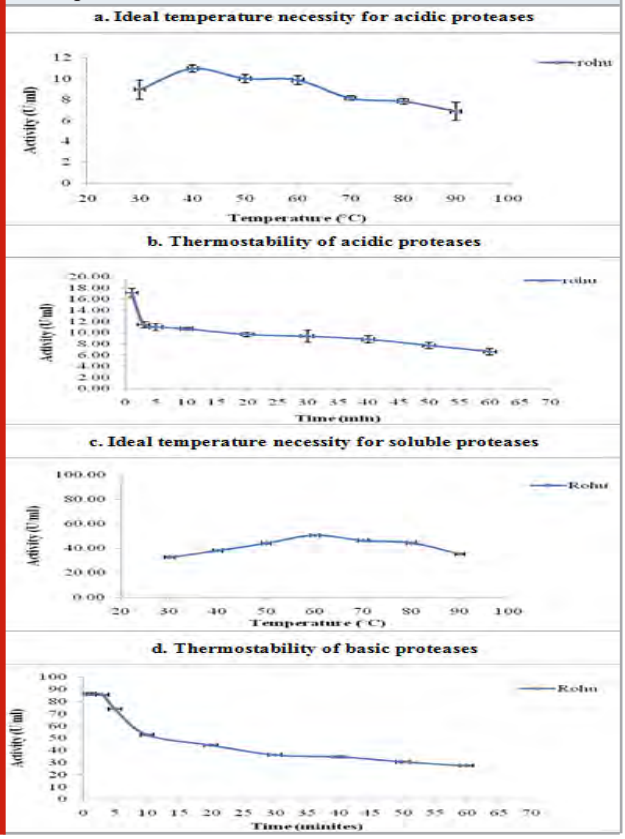


Figure 6: Effect of NaCl focus on the action of proteases from Rohu viscera squander (a= acidic proteases, and b= basic proteases)

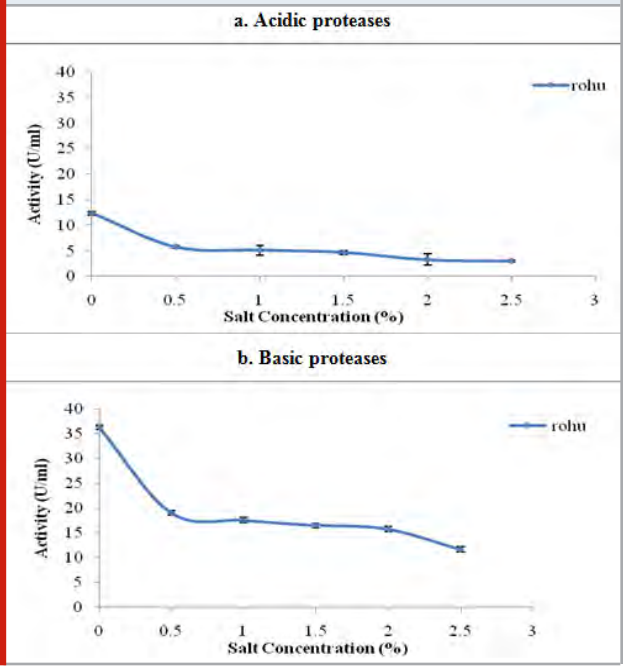


Figure 7: Effect of unrefined proteases from Rohu viscera squander on hydrolysis of muscle protein

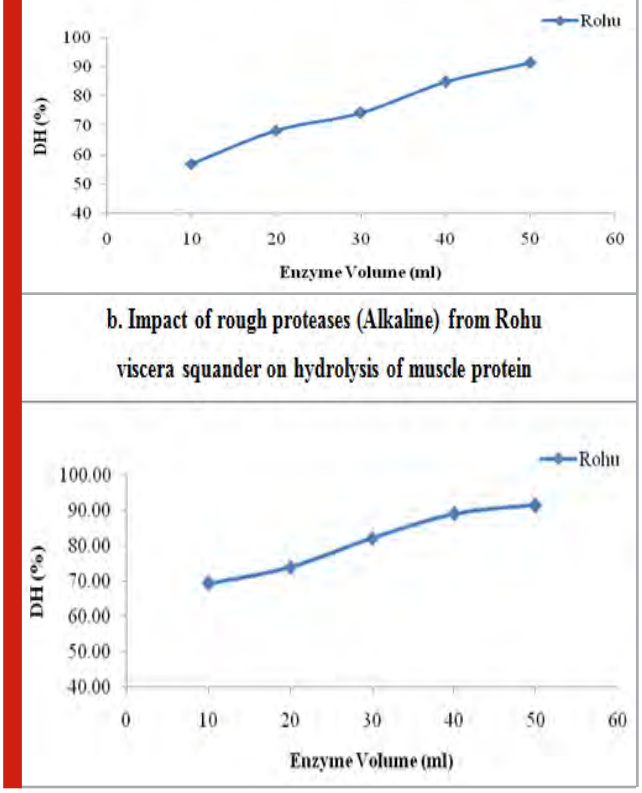
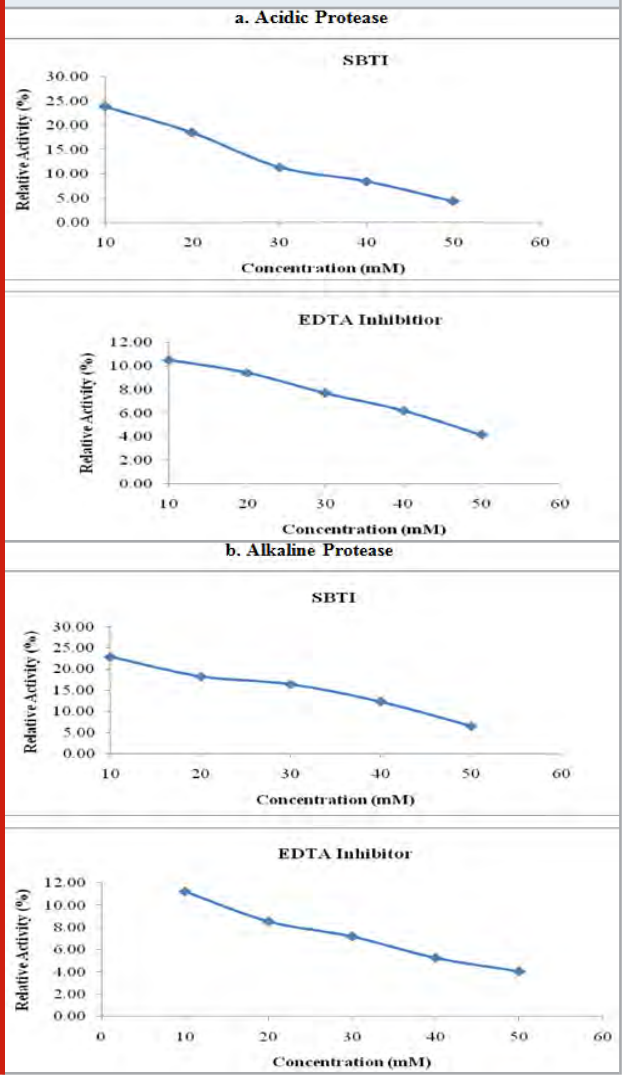


Figure 8: Influence of certain inhibitors on the movement of proteases from Rohu viscera squander (a= acidic proteases, and b= soluble proteases) Impact of inhibitors on the movement of proteases from Rohu instinctive waste



CONCLUSION

Taking everything into account, this examination has uncovered that impressive measures of acidic and antacid proteases are available in the instinctive misuse of Rohu fish, and those in sanitized structure have the potential for application as various food handling helps, and then again, would add to tackle bio-garbage removal issue generally. Both the proteases showed considerable action in both acidic and basic conditions. As a necessity for their application reason, the most extreme movement of acidic and basic protease was discovered to be at 40°C and 60°C individually. By the by, the solidness of these compounds at raised temperature and NaCl focus was not discovered to be good.

Because of the current investigation, the proteins from Rohu instinctive waste could discover use in applications where most extreme action at moderate temperature and low NaCl focus is wanted.The fish preparing industry in India produces enormous protein-rich material that is untapped and can be used by changing over into protein hydrolysate. Relies upon the properties and synthetic synthesis, further FPH discovers application in different ventures going from nutraceutical to plant development-boosting fixing. Ongoing enthusiasm of FPH as nutraceutical compound/bioactive peptide requests sterile taking care of and appropriate conservation of fish preparing waste. Be that as it may, the wellbeing of FPH when delivered from fishery squander, financial attainability, and business case are stays unaddressed around the world.

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