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Bioscience Biotechnology Research Communications

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Editors Communique

Have we tamed the coronavirus? May be yes,
as pandemics do not die, they can only be faded !

Science and technology has made it possible, in the shortest span of time, it has shown that with firm determination and international cooperation, we can win over the onslaughts of even the worst of the pandemics. COVID-19 is perhaps fading over now, due to our coordinated efforts worldwide. Though we have lost millions, in the two year period, partly due to the mishandling of the viral attacks and somewhat by our own follies and carelessness. Anyway lessons learnt from the past, always make us more stronger and determined. Let us now not relax and work on a better mode, as all is still not well yet. The almost taming of the virus and its cousins have indicated some of the concealed failures, on which we have to focus now. We have to be more vigilant, and even a bit of laxity can spoil the good work done. On societal and governmental parts, utmost care and caution is required on a long term basis.

On behalf of Bioscience Biotechnology Research Communications, we falter at words to express our deep sense of solitude and grief on the catastrophic events of the world wide pandemic, spanning over two years now. We pray for the strength to bear this universal calamity and come up with long lasting fortitude to eradicate it soon.

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Sharique A. Ali, PhD
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Biotechnological Communication

Effects of Salt Stress on the Growth and Nodulation of the Chickpea, *Cicer Arietinum*

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ABSTRACT

The chickpea *(Cicer arietinum L.)* is the most significant legume crop and good source of protein. The biggest obstacle currently facing agriculture is salt stress which damage soil fertility and constantly shifting abiotic stressors result in low chickpea yields. Therefore, the purpose of this work was to identify and characterize the *Rhizobium* isolates and its effect on salt (NaCl) stress. Total 281 isolates were isolated from rhizospheric soil samples fromTaif agriculture filed of Kingdom of Saudi Arabia. All isolates were showing same phenotypically shape size and morphology on YEMA. Only 7.47 % (21) of isolates were showing the biochemical characterization of *Rhizobium*. Only 10 (47.61%) of the 21 isolates exhibited nodulation in the chickpea under controlled condition After the symbiosis establishment at 25 days the data clearly indicate that the dry weight of plant was increase at 50 and 75mM NaCl stress at four successive harvesting stages while at 100 mM decreases the accumulation of dry mass at the rate of 16.21%, 26.15%,10.97% and 13.04% in first, second, third and fourth harvesting stage respectively. With the salinity the root to shoot ratio increased at 0, 50 and 75mM at first, second and third harvesting. But decrease at 100mM NaCl at every harvesting stage. Nodule dry weight remains decrease under the salt stress conditions. Few isolates of *Rhizobium* exhibited good growth at high temperature and high pH. More research is needed to understand the *Rhizobium* isolates under abiotic stress conditions including salt stress to raise the better nitrogen fixers in the form of biofertilizer in harsh environment.

KEY WORDS: ABIOTIC STRESS, RHIZOBIUM, SALTS STRESS, CICER ARIETINUM, AGRICULTURE.

INTRODUCTION

The most important legume crop is the chickpea (*Cicer arietinum* L.). It is well known for its nutritional advantages and is a member of the Fabaceae (Leguminosae) family. Currently, salt stress is the main barrier to the agricultural sector, which has a significant impact on the development, survival, and metabolic activity of bacteria and plants that fix nitrogen (Shilev, S., 2020). In many nations, including Saudi Arabia, agriculture is at the forefront of economic development. However, one of the key barriers preventing the growth of the agricultural area or the rise in agricultural productivity for many crops is salinity, which affects the majority of the country (Sunita et al., 2020).

One of the main causes of dryness and salinity is high temperatures. Due to the significant amount of soluble salts present in irrigation water and the high rate of evaporation

brought on by Saudi Arabia's high temperatures, ineffective drainage, or soil type. In light of the aforementioned information, the goal of our research is to identify salt-tolerant *Rhizobium* species in Saudi agricultural soil that promote chickpea growth under adverse and salt-stress conditions.

Numerous living creatures, including bacteria, fungi, nematodes, worms, and others, can be found in soil in their natural habitat. According to (Sunita et al., 2020) Rhizobia are a special class of bacteria that live as symbionts with legumes and fix atmospheric nitrogen. The most important legume crop is chickpea (*Cicer arietinum* L.). One of the main obstacles in the agricultural sector that has a significant impact on plant growth is salt stress. In desert habitats, the Rhizobium-legume symbiosis is particularly crucial in areas where the prevalence of saline soils is rising and posing a danger to plant productivity. Legumes, which are typically found in dry settings, may be better adapted than legumes grown in other habitats to fix more nitrogen dioxide in saline environments (Etesami, H. and Adl, S.M., 2020).

Toxic ion accumulations in various plant tissues, which disrupt some enzyme activity, can be blamed for the salt sensitivity. According to Lauter and Munns (1986), the chickpea (*Cicer arietinum*) is particularly sensitive to soil salt. Failure of the infection process resulting from salinity's effect on rhizobia's establishment may be the cause of unsuccessful symbiosis under salt stress. Legumes with salt stress have less nodulation because they block the symbiotic interactions (Chakraborty and Harris, 2022). Salinity levels that prevent the growth of each individual symbiont differ from those that prevent the symbiosis between legumes and rhizobia. Some legumes perform poorly in symbiotic relationships when exposed to salt, but this is not because salt limits rhizobial growth. In saline environments, rhizobial colonization and invasion of the rhizosphere, root-hair infection, and the development of efficient salt-tolerant nodules are necessary conditions (Ma et al., 2020).

Arid and semi-arid climates affect about one-third of the planet's geographical area (Skujins, 1991). One-third of the world's irrigated areas and around 15% of dry and semi-arid regions are affected by salinity, according to Pitman et al. (2002). Lack of nitrogen frequently restricts plant productivity on many semi-arid lands, particularly saline regions. Legumes that grow in arid environments may be treated with N fertilizers to help them tolerate salt better (El at al., 2020; Shevyakova 1984). The use of Nitrogen in agriculture land is likely to be further constrained by rising N fertilizer costs and the risk of growing soil salinity (Mohammad et al. 1989). As a result, biological nitrogen fixation has become more significant. Legumes, which are typically found in dry ecosystems, may significantly contribute to the global nitrogen economy, and their capacity to fix nitrogen could raise soil productivity (Bhat et al., 2020).

Legumes may have evolved to adverse climatic conditions in part as a result of the host plant's interaction with Rhizobium species. These modifications may make *Rhizobium*-legume relationships more capable of fixing N2 under stress situations than associations that have developed in other contexts (such as salt stress), according to Zehran (1999) it has long been known that legumes are either susceptible to salinity or only moderately resistant to it. Most legumes experience a growth reduction in response to moderate salt (Jamil et al., 2012). In many nations agriculture is at the forefront of economic development. Salinity is one of the key barriers which preventing the growth of the agricultural area or the rise in agricultural productivity for many crops. One of the main causes of dryness and salinity is high temperatures (Sindhu et al., 2020).

The high rate of evaporation brought on by Saudi Arabia's high temperatures, the high concentration of soluble salts in irrigation fluids, ineffective drainage, or soil type are the causes of excessive salinity. We tryed to estaiblish as relation of salt-tolerant Rhizobium species with the chickpea seedlings which help to promote the growth of chickpeas in a controlled manner in light of the aforementioned facts in Saudi agricultural soil. Weimberg and Shanon (1988); Cordovilla et al., 1996) found that there is generally little

association between salt concentration and the concentration of these chemicals (Chakraborty and Harris, 2022). The present study looked at how the well-established symbiosis between Cicer *arietinum* and Rzobium responded to salt stress during the vegetative phase. The purpose is to determine whether the adverse effect of salt stress influence the vegetative growth of Rhizobium and its association of nodulation.

MATERIAL AND METHODS

Collection of Soil Samples: The study site of research comprises five different Rhizospheric soil samples was collected from agriculture field of Taif, Saudi Arabia. Samples were kept in clean sterile bottles sealed and transferred to the Microbiology and Biochemistry laboratory at university of Jeddah and stored at 4° C.

*Isolation of Rhizobium isolates***:** By vigorously vertexing, the soil samples were suspended in distilled water and prepared for serial dilutions up to 10-6 in pure distilled water. After the proper dilution, put the solution to the YEM Agar plate in a petri dish with the correct pH calibration (6.8 to 7) and cover for 72 hours at 32° C. Colony was obtained and was streaking on YEMA media. Sub-cultures of the cultures were developed and be used often. The biochemical characteristics of Rhizobium will be determined using Berge's Manual of Bacteriology (Bergeys et al., 1939).

*Purification of Isolates***:** Using a sterile inoculating loop, one well-separated rhizobial colony was chosen and added to 6 mL of sterilized yeast extract mannitol broth (Sindhu et al., 2020). The test tubes were then vortexed and swirled on a rotary shaker for 48 hours at room temperature. A loop of the culture suspensions from each test tube was removed after two days and streaked on sterile yeast extract mannitol agar (YEMA), where they were cultivated for three to four days at 28 °C. By repeatedly re-streaking, the colony's purity and consistency were thoroughly examined.

Biochemical characterization of isolates: *Rhizobium* isolates were biochemically characterized using a variety of biochemical tests, including the Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Catalase test. Tests for nitrate reduction, starch hydrolysis, gelatine hydrolysis, and oxidase also done to determine the biochemical characterization of *Rhizobium* isolates (Rafique et al., 2021).

*Citrate utilization test***:** The only carbon source accessible to the bacteria in this medium is citrate, but the *Rhizobium* cannot grow on citrate, no change in colour takes place. A loop filled with Rhizobium culture was used to inoculate the slant. The stab and streak method were utilized, and the slant was then inspected after a 24-hour incubation period at 37°C.

*Starch utilization test***:** The test was run to see if microorganisms might use starch as a source of carbon (Datta et al., 2015). Rhizobium was inoculated into starch agar media, which was then incubated and examined. Extracellular enzymes are produced when starch is present,

showing that the organism could exploit starch as a carbon source. The capacity of bacteria to consume starch was evaluated using an iodine test. Iodine solution drops were applied to Petri-plate-grown cultures that had been cultured for 24 hours. No starch utilization was indicated by the formation of blue, and vice versa.

Gelatine test: This test was run to see if bacteria could produce the enzyme gelatinase and use gelatine as a media source. Gelatine degradation is a sign that the gelatinase enzyme is present. The actively growing cultures underwent nutritional gelatine medium inoculation and 48-hour growth. The cultures that produce gelatinase stay liquefied after being exposed to a low-temperature treatment at 40 °C for 30 min, but the cultures that do not produce gelatinase the media was remain solidify (Deka and Azad 2006).

Catalase test: This test was run to investigate whether the catalase enzyme was present in bacterial colonies. On glass slides, 24 hour-old Rhizobium colonies were collected, and one drop of 30% H_2O_2 was applied. The presence of the catalase enzyme was shown by the appearance of a gas bubble (Rafique et al., 2021).

Physiological Tolerance Test of Rhizobia: Physiology of the isolated rhizobia were done by the determination of the following parameters

Temperature, pH and Salt tolerance of the Isolates

Antibiotic tolerance.

Temperature, pH and Salt tolerance of the Isolates: By allowing the isolates to grow on YEMA plates with various salt concentrations, the isolates' salt tolerances were evaluated (Chakraborty and Harris, 2022). Different NaCl concentrations, including 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0% NaCl, were used to make YEMA. Growing the isolates on YEMA modified with 0.1N HCl or NaOH at various pH values of 4, 4.5, 5, 5.5, 6, 8, 8.5, 9, 9.5, and 10 allowed researchers to establish the isolates' pH tolerance (Maatallah et al., 2002). By inoculating and incubating the media at 5, 10, 15, 20, 35, 40, 45, and 50°C, YEMA medium was used to study the growth response of rhizobia isolates to various temperatures (Kulkarni and Nautiyal, 2000). The presence and lack of growth on the plates was noted after 4 days of incubation at 28 2°C.

Antibiotic sensitivity test: Antibiotic sensitivity tests were conducted using antimicrobial discs. The following antibiotic disc was employed: Nalidixic acid (Na), Cloxacillin (Cx), Chloramphenicol (C), Tetracycline (Tc), Streptomycin (S), Ciprofloxacin (Cf), Ampicillin (Am) and Penicillin (PEN) are some examples of antibacterial drugs. Every isolate was individually inoculated in YEM broth and incubated at 37 °C for an overnight period. A glass spreader was used to evenly distribute the culture broth of each isolate on the nutrient agar medium (NA). Different isolates' inoculation plates were set with antimicrobial discs at about 2.5 cm, and they were then incubated at 37°C overnight. The zone of inhibition surrounding the discs was used to assess the sensitivity and resistance of Rhizobium isolates. All those isolates inhibit by antibiotic disc in the form zone consider as sensitive isolates while those perform the growth near to antibiotic disc is consider as resistance isolates (Warsi et al., 2017).

Surface sterilization and germination of chickpea seeds: Seed Preparation: Seeds are cleaned with tap water before being sterilized in 98% alcohol for 5 minutes, followed by 5 minutes of distilled water and another 2 minutes of sterile distilled water. For a short period of time, seeds were dried in laminar air flow (Sarwar et al., 2006; Yadav et al., 2010). After being surface sterilized, seeds germinated for 48 hours at 26 °C in moist autoclaved water. The immature plants were placed in freshly prepared nutrient solutions in autoclaved sand pots. Every 24 hours, the nutritional solution is replaced. Each seedling is injected with a suspension of 10–8 Rhizobium cells per millilitre. Plants that had been inoculated were placed in a control chamber with a 16/8-hour light/dark cycle operating at 25/17 C during the day and night.

In order to stress the plant with salt at different concentrations, we were to wait at least 20 to 25 days until the establishment of the symbiosis as per Banik et al., 2018 with bacteria. The development of chickpea seedlings under various salt stress conditions and its impact on nodulation in roots were studied. The seedlings were exposed to different concentrations of NaCl salt (0, 50, 75, and 100 mM NaCl). After inoculating chickpeas with Rhizobium isolates, we use methods from Elsheikh & Wood (1990) to assess the length of the root and shoot as well as the fresh and dry biomass. Salt stress (0, 50, 75, and 100 mM NaCl) was applied. There will be no salt concentration. Zero salt concentration will be considered as control plants. The harvesting was carried out at 4 different stages @ 4, 8, 12, & 16 days.

RESULT AND DISCUSSION

Total 281 isolates were isolated from rhizospheric soil samples of given sites fromTaif agriculture filed of Kingdom of Saudi Arabia. In the first site of soil sample we got 57 isolates in which only 5.2% of isolates were showing the rhizobium biochemichal charecterstics while sampling site 2 were showing only 3.9% of rhizobium isolates among 51. On the other hand in the third soil samples the identified rhizobium isolates was only 8.9% among 56 isolates while in fourt and fifth soil samples it was 8.6% and 10.6% among 58 and 56 isolates respectively.All isolates were showing same phenotypically shape size and morphology on YEMA (Yeast extract Mannitol Agar) while on NA (Nutrient agar) were showing different morphology of bacterial isolates. Only 7.47 % (21) of isolates were showing the biochemical characterization of Rhizobium among all 281 isolates according to Bergey's manual.

In first soil sample only 3 isolates were showing Rhizobium on YEMA plates among 57 isolates. While in secound, third, fouth and five soil sample only 2, 5, 5 and 6 isolates were showing Rhizobium charecterstics among 51, 56, 58, and 59 isolates respectively.

Before the pot experiment we studied the seed germination of chick pea under different salt stress. Salinity has a negative impact on chickpea development and germination, as seen in the table 2. Particularly in stressful environments, the germination and seedling stages are crucial to plant survival and proper seedling establishment. The results of this investigation showed that rising salinity stress gradually inhibited chickpea seed germination and establishment. Seed germination was completely inhibited at a high salinity level of 100 mM NaCl.

Table 1. Different collection of soil samples and number of isolated Rhizobacterial isolates

	Different rhizosphere soil Samples					
	Site 1	**Site 2**	**Site3**	**Site 4**	**Site 5**	
No of isolates (YEMA)	57 (3)5.2%	51(2)3.9 %	56 (5)8.9%	58(5)8.6 %	59 (6)10.6%	T=281 (21)7.47%
Total No of Isolates	57	51	56	58	59	

Table 2. Biochemical characterization of Rhizobium isolates

	No of isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	Gram's staining	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Morphology	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Biochemical Test	Indole test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Methyl red test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Voges Proskauer	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Catalase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Citrate utilization	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Starch Hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrate utilization	Gelatine Hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Oxidase test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Dextrose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

According to numerous studies, increasing salinity levels decreased germination percentage and speed in the field for various legumes, including peas (Wolde and Adamu, 2018), chickpeas (Ashraf and Waheed, 1992), wheat (Majid et al., 2013), and other legumes (Esechie, 1995; Morais et al., 2012; Piwowarczyk et al, 2016). This fact demonstrates that the salinity increased slowly. This result demonstrates that salinity inhibits, and delays seed germination through a variety of mechanisms, including a reduction in water absorption, adjustments in the mobilisation of stored food, and disruptions in the structural organisation of proteins (Ibrahim, 2016).

Table 3. Effect of growth under salt stress (NaCl) at four different harvesting stages. PDW; Plant dry weight, RSR; Root to Shoot Ratio and NDW; Nodule dry weight

Days of treatment	NaCl (mM) Concentrations (mM)	Plant dry weight/PDW (gm)	Nodule Dry weight/NDW (mg)	Root to shoot ratio/RSR (mg)
4	0	0.37	50	0.54
	50	0.52	41	1.12
	75	0.48	15	1.15
	100	0.31 (16.21%)	05	0.6
8	0	0.65	80	1.06
	50	0.61	85	1.12
	75	0.58	95	0.89
	100	0.42 (26.15%)	45	0.75
12	0	0.82	150	1.15
	50	0.87	122	0.98
	75	0.95	105	0.75
	100	0.73 (10.97%)	98	0.72
16	0	0.92	225	1.20
	50	0.91	120	1.08
	75	0.81	111	1.07
	100	0.80 (13.04%)	91	0.60

Table 4. Antibiotic tolerance of isolated Rhizobial isolates under different antibiotic concentrations. Parentheses indicate percent tolerant isolates.

Antibiotics	Concentration /disc	Tolerant isolates (%)
Nalidixic acid (Na)	30μg	4 (19.04)
Cloxacillin (Cx)	10μg	5(23.80)
Chloramphenicol (C)	30μg	7 (33.33)
Tetracycline (Tc)	30μg	8 (38.09)
Streptomycin (S)	10μg	11 (52.08)
Ciprofloxacin (Cf)	5μg	6 (28.57)
Ampicillin (Am)	10μg	3 (14.28)
Penicillin (PEN)	10μg	7 (33.33)

The current study showed that saline levels dramatically affected plant leaf area, branches, height, plant biomass (fresh & dry), and plant mortality. The death of plants was observed at the high salinity concentration (100 mM NaCl) after four weeks of plant establishment. These findings agreed with earlier studies that had been published (Grozeva et al., 2019). The study's findings clearly show that the toxicity of the salinity treatments manifests itself more visibly in dry weight. This result confirms earlier research that showed dry biomass production was more susceptible to growth inhibition by NaCl treatments (Rasool et al., 2013; Yousef et al., 2020). Additionally, the lack of water, ion toxicity, and nutrient imbalance brought on by the

blocking of other nutrients, including N, P, K, Ca, and NO3, could all be contributing factors to the growth suppression (Hasegawa et al., 2000).

When cultivated in salt, other research has demonstrated comparable results in other legume plants, including sesbania (Mahmood et al., 2008), chickpea (Ashraf and Waheed, 1992), and pea plant (Hernandez et al., 1999). The outcome indicated that the number of leaves per plant was decreasing. Physiologically, salt stress has a detrimental impact on several processes, but the most notable effect is a reduction in cell division and expansion, which led to a decrease in leaf number. The results also showed that a high saline level reduced leaf area, which may have been caused by a decrease in cell division and cell expansion. These findings are consistent with another finding that indicated salt stress reduces the leaf surface expansion ratio, which leads to the stoppage of expansion (Kordrostami and Rabiei, 2019).

The purpose of this study was to look into how salt stress affected chickpea growth and nodulation. High salt stress hindered and postponed the germination and growth of chickpea plants, according to the study. The study found that whereas chickpeas are unaffected up to a salinity of 50 and can endure salinity at a level of 75 mM NaCl, this cultivar is extremely susceptible to 100 mM NaCl, and considerable salt-stress-related impairments were noted. However, compared to germination, plant development was more responsive to salt stress. The molecular, physiological, and metabolic alterations brought on by salinity stress must be the focus of future study. Additionally, comprehensive

knowledge is necessary to comprehend this plant's physiological responses to environmental factors in the field.

Figure 1; a, b, c and d represent percent isolates of Rhizobium under the condition of antibiotics, pH, Temperature and salt (NaCl) stress.

Figure 2: Figure a, b and c represents nodule dry weight, root to shoot ratio and plant dry weight respectively under different salt (NaCl) stress conditions at four different harvesting stage

After the biochemical characterization of isolates we were tested the isolates for physical (Antibiotics, Ph, Temperature & salt) tolerance in which all the selected isolates were showing both sensitivity and resistance at various concentration (Figure 1 a). Streptomycin was effective against the isolates at doses of 10 µg/ml and were showing 52.08% resistance isolates and remain sensitive while 23.80%, 14.28% and 33.33% isolates were showing resistance to cloxacillin, ampicillin and penicillin respectively. 28.57% of isolates were showing resistance

to ciprofloxacin at 5µg concentration of antibiotics disc. 33.33% and 38.09% isolates were resistance to Chloramphenicol and tetracycline respectively. The isolates varied in their resistance to and sensitivity to various antibiotics at various concentrations.

Only 10 (47.61%) of the 21 isolates exhibited nodulation in the chickpea under controlled condition. Chickpea plants were inoculated with Rhizobium isolates, grown in a control environment, exposed to various NaCl salt stress conditions (0, 50, 75, and 100 mM NaCl), and the growth parameters were examined up to four harvesting stages (4, 8, 12, and 16 days). The purpose of Wark was to determine whether salt stress affected chickpea development and nodulation. Data shows that high salt stress conditions affect plant growth and nodulation both during germination and nodulation.

Figure 3: (A-d) represents the nodulation in chickpea using isolated isolates (RTf-7, RTf-15, and RTf-17) while the figure (K-N) showing an un inoculated check pea.

The data clearly indicate that the dry weight of plant was increase at 50 and 75mM NaCl stress at three sucsessive harvesting stages while at 100 mM decrease the accumulation of dry mass at the rate of 16.21%, 26.15%,10.97% and 13.04% in first , secound, three and fourth harvesting stage respectively. With the salinity the root to shoot ratio increased at 0, 50 and 75mM at first, secound and third harvesting. But decrease at 100mM NaCl at every

harvesting stage. Although nodule dry weight remain decrease under the salt stress conditions except at the secound harvesting stage that is 8 day of treatment boost nodulation under the 50 & 75 mM NaCl salt stress conditions.

The growth rate didn't noticeably slow down until the highest salt dosage was applied (Table 1). Only at the limited level of salt stress i.e is 50 & 75mM RSR increased. This reveals due to short-term response to salinity and increases RSR in the above samplings were hardly significant. Although on average nodule dry weight remain decrease under the salt stress conditions at high concentration. The above results justified by the other researcher those who work on effects of NaCl on the growth of legumes (Egamberdieva et al., 2016).

CONCLUSION

The current work focuses on the isolation and biochemical characterization of Rhizobium isolates from Saudi desert soil in the Taif region, as well as their impact on chickpea growth and nodulation under various salt stress conditions. The physiological tolerance of each Rhizobium isolate was examined (temperature, salt, pH, and antibiotic resistance). According to the study, rhizobial isolates are very susceptible to abiotic variables such as salt, pH, and temperature. Further, we investigate the impact of NaCl administered during the vegetative growth and nodulation of chickpea plants. We conclude that abiotic stress, such as salt stress, poses major hazards to plants.

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Analyzing the Bivalve Species of Genus Parreysia Along with Water Quality of Kuadhas and Pangoli Rivers from Gondia Maharashtra, India.

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ABSTRACT

Aquatic environments benefit greatly from the contribution of freshwater bivalves. They are crucial for ancient remedies, and a variety of industrial uses, primarily the manufacturing of pearls, slacked lime, and use as a food source in various regions Malacofauna was assessed from Kuadhas river and Pangoli river of Gondia Maharashtra for six months. The physicochemical parameters comprising pH, turbidity, color, nitrate, sulfate, phosphate, silica, free carbon dioxide, alkalinity, dissolved oxygen, and total hardness were evaluated since aquatic body water quality has an impact on the occurrence and survival of flora and fauna. This paper deals particularly with different species and varieties from the genus *Parreysia* among freshwater bivalves. From study sites genus Parreysia represents total 8 species as *Parreysia favidens, Parreysia favidens marcens, Parreysia corrugata, Parreysia corrugata nagpoorensis, Parreysia cylindrica, Parreysia Radiatula shurtleffiana, Parreysia (Radiatula) khadakvaslaensis, Parreysia Radiatula caerulea gaudichaudi.* Bivalve occurrence peaked in the post-monsoon season and declined during the monsoon. *Parreysia favidens and Parreysia corrugata* are widely distributed in the Kuadhas River. The two major species in the *Pangoli River are Parreysia (Radiatula) khadakvaslaensis* and *Parreysia favidens*. In both rivers *Parreysia favidens marcens, Parreysia corrugata nagpoorensis*, and *Parreysia Radiatula shurtleffiana* are listed as being rarer species.

INTRODUCTION

Freshwater mollusks contribute significantly to the biogeochemical cycle and are an essential component of the aquatic ecosystem (Dey, 2007). Among molluscs, bivalves don't have head like that of gastropods. Externally structure consisting of two valves made up of calcium carbonate and joined together by soft ligament at a hinge. Inside the shell have mantle secreted by shell enclosing visceral mass, two pairs of gills and foot (Rao, 1989). In rivers and lakes, freshwater bivalves serve as filters. Numerous species can be found in dense clusters and filter out a lot of silt, bacteria, diatoms, blue-green algae, and other microscopic organisms as well as heavy metals and big organic compounds. Genus *Parreysia* of class Bivalvia, comes under subclass

Palaeoheterodonta and family Unionidae of phylum Mollusca (Rao, 1989).

Some species of *Parreysia* are used as food sources such as *Parreysia favidens*, P*arreysia caerulea*. Soup made from *Parreysia favidens*, intended to treat blood pressure and heart issues also shell lime water is used to cure intestinal parasites (Tripathy and Mukhopadhayay, 2015). Molluscs distribution and abundance in water bodies are influenced by physicochemical parameters and the vegetation of the water body (Choubisa, 1992). Bad water quality and other human activities, leading to habitat destruction, effects on survival and diminishing diversity of sensitive malacofauna.

In India molluscan diversity and relation to physicochemical properties studied recently by number of researchers including, Chutia and Kardong (2021), Kumar et al., (2019), Padghane et al., (2017), Kamble (2018). Sarwade et al., (2015). Gondia situated north-eastern site of Maharashtra

state most of the land of district is covered with forest and lakes and rivers. It is in the northeastern region of Maharashtra state, and most of the district's territory is covered in rivers, lakes, and forests. In this area little or no work is done on malacofaunal diversity hence This paper particularly enlists species of genus *Parreysia* from Kuadhas and Pangoli rivers of Gondia Maharashtra along with information on some physicochemical parameters of these water bodies.

Figure 1: Location of study area Kuadhas river (A) and Pangoli river (B)

MATERIAL AND METHOS

Gondia district in the Indian state of Maharashtra. It is located between latitudes 20.39 and 21.38 North and 79.27 and 80.42 East. As most of the territory is covered by forests, the Gondia district of Maharashtra is home to a wide variety of plants and animals. Kuadhas river originates from Darekasa hills of the district and is a sub-tributary of Bagh River of the region. Pangoli river has it's beginning from Tumsar hills of the district and is tributary of Bagh River (Figure 1).

Kuadhas and Pangoli rivers were visited from November 2022 to April 2023. The quadrate sampling method was used to perform the quantification (Christian and Harris 2005). Samples were collected from bank of rivers by hand picking and with the help of hand net and scoop net. (Rao et al., 1989). (Only molluscan shells are used for study and no live molluscs were brought to laboratory for analysis). After being transported to the lab, the shells were cleaned with a soft bristle brush to avoid breaking the shells and to properly clean the mud for shell character identification. Identification of malacofauna was done by following keys by Rao (1989), Ramkrishna and Dey (2005).

Table 1. Physicochemical analysis of Kuadhas river and Pangoli river in Gondia district Maharashtra, India

Study Sites ➜	Kuadhas River		Pangoli River	
Water Parameters	Minima	Maxima	Minima	Maxima
Temperature (^{0}C)	24	31	23.6	32
pH	7.0	7.52	7.33	7.67
Color (Hazen)	------	BQL	BQL	3
Turbidity (NTU)	1.5	2.61	3.8	23.1
Phosphate (mg/L)	BQL	0.29	BQL	4.43
Sulphate (mg/L)	8.73	10.38	2.94	17.26
Nitrate (mg/L)	1.14	3.86	1.50	16.16
Total Dissolved Solids (mg/L)	82	224	106	322
Alkalinity (mg/L)	42	104	55	124
Total Hardness (mg/L)	45	130	55	130
Dissolved Oxygen (mg/L)	5.50	6.80	5.8	6.7
Silica (mg/L)	BQL	4.24	0.31	4.12
Free CO_2 (mg/L)	3.17	7.4	3.0	5.28

Water samples were collected from both rivers at the same time and analysed for thirteen water parameters as temperature, pH, color, turbidity, total dissolved solids, total hardness, alkalinity, free carbon dioxide, dissolved oxygen, silica, phosphate, sulphate and nitrate of water sample. The physicochemical analysis of water was performed as per standard methods, (APHA, 1998; Trivedi and Goel 1984).

RESULTS AND DISCUSSION

The studied physicochemical parameters of water samples from Kuadhas river and Pangoli river have been given

in Table 1. The physicochemical parameters of Kuadhas river were recorded as: water temperature (24 - 31^0 C), pH (7.0-7.52), Color (BQL), Turbidity (1.5- 2.61 NTU), Phosphate (BQL- 0.29 mg/L), Sulphate (8.73-10.38 mg/L), Nitrate (1.14- 3.86 mg/L), Total Dissolved Solids (82- 224 mg/L), Alkalinity (42-104 mg/L), Total Hardness (45-130 mg/L), Dissolved Oxygen (5.50-6.80 mg/L), Silica (BQL – 4.24mg/L), Free CO_2 (3.17-7.4 mg/L).

As compared to Kuadhas river, the upper and lower limits of water parameters of Pangoli river had higher: temperature (23.6 - 32^0 C), pH (7.33-7.67), Color (BQL-3 Hazen), Turbidity (3.8- 23.1 NTU), Phosphate (BQL- 4.43

mg/L), Sulphate (2.94-17.26 mg/L), Nitrate (1.5- 16.16 mg/L), Total Dissolved Solids (106-322 mg/L), Alkalinity (55-124mg/L) Total Hardness (55-130 mg/L), Dissolved Oxygen (5.8-6.7 mg/L), except Silica (0.31 – 4.12mg/L) and Free CO_2 (3.0-5.28 mg/L). Positive correlation between molluscan population and total hardness, alkalinity, chlorides, phosphates, nitrate- nitrogen by many researchers (Garg et al 2009; Dorlikar et al., 2014; Sarwade et al., 2015).

In the present work, we have reported species from genus Parreysia of family Unionidae of bivalves from Kuadhas and Pangoli rivers of Gondia district Maharashtra India (Figure 2). All eight species of Genus Parreysia were found at Kuadhas river from November 2022 to April 2023 includes,

Figure 2: Collected and identified Parreysia species from Kuadhas river and Pangoli river of Gondia district Maharashtra, India

Parreysia favidens, Parreysia favidens marcens, Parreysia corrugata, Parreysia corrugata nagpoorensis, Parreysia cylindrica, Parreysia Radiatula shurtleffiana, Parreysia (Radiatula) khadakvaslaensis, Parreysia Radiatula caerulea gaudichaudi. Parreysia favidens followed by Parreysia corrugata are most abundant species found at Kuadhas river and Pangoli river. Parreysia corrugata nagpoorensis and Parreysia favidens marcens as rare species at study area. Only four species of Parreysia were reported from Pangoli river includes Parreysia favidens, Parreysia corrugata, Parreysia (Radiatula) khadakvaslaensis and Parreysia cylindrica (Figure 3 and 4).

Kuadhas river showed maximum diversity of molluscs due to present of phytoplankton vegetation serve as food for molluscs, less anthropogenic activity found at this river. Least diversity and molluscan assemblage found at Pangoli river due to habitat destruction as number of small dams built across the river and other anthropogenic activities.

Figure 3: Percentages of Parreysia species from Kuadhas river.
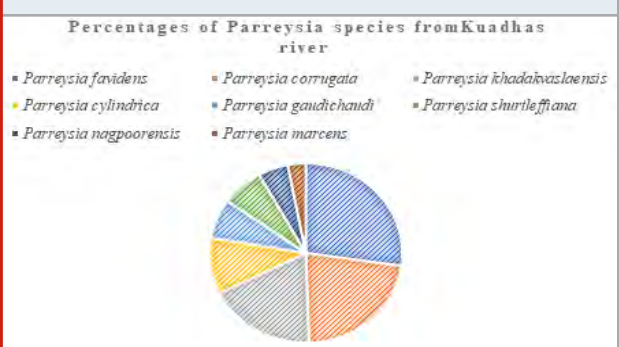
Figure 4: Percentage of Parreysia species from Pangoli river
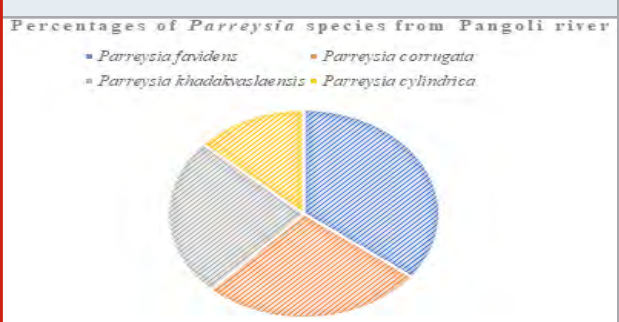
CONCLUSION

Eight species of genus *Parreysia were* recorded from two rivers Kuadhas and Pangoli rivers from Gondia district Maharashtra during six months of the survey. This is the first attempt to study malacofauna in this region and will help to explore malacofauna and development of conservation policies to protect the species in the near future. Further study will be continued for a longer period and from different water bodies of the region to enlist molluscan diversity and their seasonal variations.

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Biotechnological Communication

Multimedia Education Program on Knowledge and Attitude Regarding Management, Prevention of Complication and Side-Effects of Drugs on Hepatitis-B Among Hepatitis Patients – A Pre-Experimental Study

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ABSTRACT

Hepatitis is considered a public health challenge on the Asian continent and also in India. But due to the paucity of the data, the exact burden of the condition has not been established. Better disease-related knowledge will help to improve attitudes toward managing the complications and side effects and also prevent the further spread of infection. This study aimed to evaluate the knowledge and attitude regarding management, prevention, complications, and side effects of drugs among hepatitis B patients in Bengaluru, India. A hospital-based pre-experimental study was undertaken with 60 hepatitis-B patients who were admitted to KCG Hospital in Bengaluru. Knowledge and attitude regarding hepatitis-B were assessed using a validated questionnaire containing demographic data, a 30-item knowledge questionnaire, and 16-item Likert scale responses, respectively. SPSS V20.0 was used for data analysis and interpretation. The descriptive statistics were presented with the mean, standard deviation, frequency, and percentage. The data were analyzed using a paired t-test. Further, to check the association, chi-square analysis was used. In the pre-test, 41 (68.3%) of the participants had inadequate knowledge, 19 (31.7%) had moderate knowledge, and none of the study participants had adequate knowledge. Furthermore, 60 (100%) participants had a neutral attitude, and none of the participants had an unfavorable and favorable attitude towards a hepatitis B patient. The findings of the study indicate that hepatitis B patients have a lack of knowledge and attitude regarding the prevention of complications, management, and side effects of drugs, which may lead to the further spread of infection in the community and hospital itself. Extensive health education, community reach, and health workers participation in campaigns related to hepatitis can have a rational control over the management of the disease.

KEY WORDS: MULTIMEDIA EDUCATION PROGRAM, KNOWLEDGE, ATTITUDE, PREVENTION OF COMPLICATION, SIDE EFFECTS OF DRUGS, HEPATITIS-B PATIENTS.

INTRODUCTION

Hepatitis B is a viral infection of the liver that is transmitted from an infected person to another through direct blood-to-blood contact, semen, or vaginal fluid. Although the route of transmission is similar to that of the HIV virus (Human Immunodeficiency Virus), the virus is 100 times more virulent and may live outside the body for up to a week. During this time, a virus can infect a person who is not protected from the virus. 1 HBV is present in blood, semen, and vaginal fluids and is transmitted primarily through sexual activity. Another major transmission route is the sharing of injection drug equipment (including needles, cookers, and tourniquets) and, to a lesser extent, non-injection drugs (cocaine straws and crack pipes) due to the possibility of exposure to blood. Pregnant mothers with hepatitis B may spread the virus to their offspring, most likely after childbirth (Ray 20217).

According to the World Hepatitis Alliance report, Africa has some of the highest prevalence rates in the world—over 8% for hepatitis B and 10% for hepatitis C in some areas. In America, it has been found that hepatitis B prevalence ranges from 2–8%. Areas with a high prevalence are those populated by indigenous communities. Hepatitis C prevalence among injecting drug users is 90%; 16–33% are co-infected with hepatitis C and HIV/AIDS. In Europe, it has been seen that 14 million people are chronically infected with hepatitis B and 9 million are chronically infected with hepatitis C, compared with 1.5 million HIV infections. In southeast Asia, about 65% of those with hepatitis B and 75% of those with hepatitis C don't know they are infected (Puri 2014).

HBV is part of the Hepadnavirdae family in the genus Orthohepana virus. It is the leading cause of hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) worldwide, resulting in 500,000 to 1.2 million deaths per year. The prevalence of HBV infection varies widely, so the risk of HBV infection to travellers will alter depending on destination 2. HBV infection has been associated with travel. Nine percent of all HBV cases reported in the Netherlands between 1992 and 2003 were travel-related, with an estimated incidence of HBV infection of 4.5 per 100,000 travellers. Fifty-one cases of HBV infection were identified from a cohort of ill travellers presenting to geosentinel clinics between 1997 and 2007, with HBV acquisition independently associated with older age and male sex (41–51 cases), possibly reflecting risk-taking behaviour including unsafe sex while abroad. However, given the long intubation period, we were unable to exclude the acquisition of acute HBV infection cases prior to travel. Studies of travellers have demonstrated that new sexual partners and unprotected intercourse are relatively common, particularly in the setting of excessive alcohol intake.

A study reported that India has over 40 million HBV carriers and accounts for 10–15% of the entire pool of HBV carriers in the world. Of the 25 million infants born every year in India, it is estimated that over 1 million run the lifeline risk of developing chronic HBV infection. Every year, over 100,000 Indians die due to illnesses related to HBV infection. There are varying reports of an overall rate of HB positivity ranging between 2-4.7%. Two agents are currently available for prophylaxis against hepatitis B viral infection. The first is hepatitis B immune globulin (HBIG), which provides temporary protection from HBV, (Ahmed et al., 2023).

The second is the hepatitis B vaccine, which has had a significant impact on health care workers to date. Recently, the FDA approved a synthetic nucleoside analog, lamivudine (Epivir-HBV™), for use in patients with chronic hepatitis B viral infection associated with evidence of viral replication and active liver inflammation. But Despite the introduction of hepatitis B virus (HBV) vaccination programs, chronic hepatitis B (CHB) remains an important disease burden worldwide. Currently, HBV treatment has improved in India. However, the effort is limited due to a lack of a hepatitis registry, good community-based epidemiology, and serological epidemiological studies (Ray 2017).

Therefore, this study was planned to assess the knowledge and attitude regarding management, prevention of complications, and side effects of drugs on hepatitis B among the hepatitis B patients. The findings of this study will help the medical and nursing fraternity come up with new ideas to tackle hepatitis B-related drug complications and side effects and also improve patients knowledge regarding hepatitis B (Jarju et al., 2022).

MATERIAL AND METHODS

Study design and population: An hospital-based pre-experimental study with a one-group pre-test and post-test design was undertaken on hepatitis B patients at KCG Hospital, Malleswaram, Bengaluru, in the years 2022–2023. A total of 60 hepatitis-B patients have participated in the study.

Study setting: Bangalore (also known as Bengaluru) is the capital of Karnataka state, India. This study was conducted at KCG Hospital, which is a public hospital situated at Malleswaram Circle, Bengaluru.

Sample size and sampling procedures: 60 patients who were diagnosed with hepatitis B and who were also admitted to the hospital were selected using the non-probability convenience sampling technique based on the inclusion criteria. The data was collected in person from patients at KCG Hospital who were willing to give informed consent and were included in the study. Patients who had developed a hepatitis complication, had an altered level of consciousness, and were not available at the time of data collection were excluded from the study (Ul Haq et al.,2013).

Data collection instrument: The data collection instrument was developed by the investigator. The validity and reliability of the tools were checked with the help of nursing experts and a biostatistician. The data collection instrument was divided into three sections. Section A included demographic characteristics such as age in years, gender, religion, education, occupation, monthly income, and family history of hepatitis. Section B included the structured knowledge assessment questionnaire. The questions were formulated based on general information regarding hepatitis (9 items), management of hepatitis B (7 items), prevention of complications regarding hepatitis B (8 items), and the side effects of drugs regarding hepatitis B (5 items). Furthermore, Section C included Likert's attitude scale and the responses (strongly agree, agree, uncertain, disagree, strongly disagree).

Further, for the scoring interpretation, '1' was awarded to the correct response and '0' for the wrong response in all items for Section B, and a total score of 30 was allotted to interpret the level of knowledge. For the Likert's attitude scale, the positive question score of 5' was awarded for strongly agree, '4' for agree, '3' for being uncertain, '2' for disagree and '1' for strongly disagree. Furthermore, for the negative question, strongly agree was awarded to '1', '2' for agree, '3' for uncertain, '4' for disagree, and '5' for strongly disagree. Thus, a total score of 80 was allotted. In order to establish the reliability of the tool, the split-half method was used. The calculated 'r' value was 0.92 for knowledge and 0.82 for Likert's attitude scale, and the tool was found to be reliable (Mohamed et al., 2012).

Data collection technique: Formal permission was obtained from the concerned authority and the ethical committee of KCG Hospital. The data were collected from February 2 to March 31, 2023, and were collected by the researcher itself. The patients were requested to respond to the questionnaires through pen and paper with a consent form attached. The instructions were given to the auto drivers on the front page and also orally, not to use any materials for reference purposes and not to discuss with other patients to find out

the correct answer. Around 15 minutes were taken for each subject to complete the questionnaires (Das & Shil 2022).

Data quality control: Data quality was assured by proper pre-testing and designing of the questionnaires, which were done at a rural community health center far from the original test site on 10% of the total participants to ensure that the questions were clear and easily understood by the respondents, and further questionnaires were refined based on the results of the pilot study (Mohamed et al., 2012).

Data processing and analysis: After the data collection, the data were exported from an Excel sheet to the statistical package for social sciences (SPSS) version 20.0 for analysis and interpretation. The descriptive statistics were presented with the mean, standard deviation, frequency, and percentage. The data were analyzed using a paired t-test.

Further, to check the association, chi-square analysis was used (Dahl et al., 2012).

RESULTS

A total of 60 hepatitis B patients participated in this study. Out of the study participants, the majority, 43 (71.7%), were male, and 24 (40%) were in the age group of 21 to 30 years. 28 (46.7%) of the participants were Hindu, and the majority of 29 (48.3%) of them work as private employees. With regards to education, 24 (40%) of the participants had higher secondary education and had an income of 5000 to 10000 rupees per month 22 (36.7%). Furthermore, every participant 60 (100%) heard about hepatitis, and the sources of information were health care professionals and family members 18 (30%). However, none of the 60 (100%) of the study participants had any family history of hepatitis (Das & Shil 2022).

Sl.no	Demographic variables	Categories	Frequency (f)	Percentage (%)
1	Age	21-30	24	40.0
		31-40	19	31.7
		41-50	17	28.3
2	Gender	Male	43	71.7
		Female	17	28.3
3	Religion	Hindu	28	46.7
		Muslim	12	20.0
		Christian	18	30.0
		Others	2	3.3
4	Educational status	No formal education	2	3.3
		Primary	13	21.7
		Secondary	17	28.3
		Hr. Secondary	24	40.0
		Graduate and above	4	6.7
5	Occupation	Unemployed	2	3.3
		Agriculture	7	11.7
		Govt. Employee	13	21.7
		Pvt. Employee	29	48.3
		Housewife	5	8.3
6	Family income per month(Rs)	<5000	10	16.7
		5001-10000	22	36.7
		10001-15000	19	31.7
		>15000	9	15.0
7	Family history hepatitis	Yes	-	-
		No	60	100.0
8	Heard about hepatitis	Yes	60	100.0
		No	-	-
9	Source of information	Magazine	12	20.0
		Health care professional	18	30.0
		Family members	18	30.0
		Friends	12	20.0

Table 1. Description of socio-demographic variables of patients with hepatitis B N=60

Table 2. Frequency and percentage distribution of patients with hepatitis B according to pre and post-test level of knowledge   N = 60

Sl.no	Level of knowledge	Pre test		Post test	
		No. (60)	Percentage (%)	No. (60)	Percentage (%)
1	Inadequate knowledge (<50%)	41	68.3	-	-
2	Moderate knowledge (50-75%)	19	31.7	60	100.0
3	Adequate knowledge (>75%)	-	-	-	-
	Total	60	100	60	100

Figure 1: Distribution of patients with hepatitis B according to pre-test and post-test level of knowledge

Figure 2: Percentage distribution of hepatitis B patients according to pre-test and post-test level of attitude
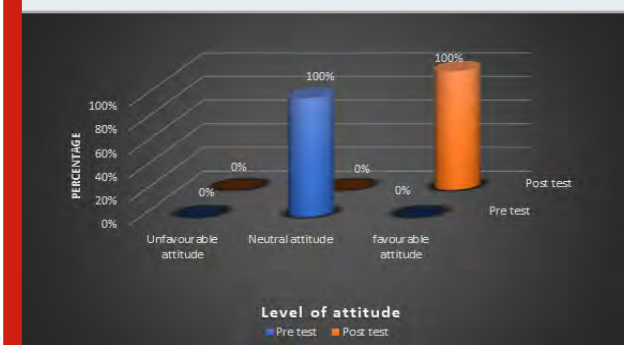
Table 3. Frequency and percentage distribution of patients with hepatitis B according to the pre and post-test level of attitude N = 60

Sl.no	Level of knowledge	Pre test		Post test	
		No. (60)	Percentage (%)	No. (60)	Percentage (%)
1	Unfavorable attitude (<50%)	-	-	-	-
2	Neutral attitude (50-75%)	60	100.0	-	-
3	Favourable attitude (>75%)	-	-	60	100.0
	Total	60	100	60	100

Table 4. Mean, SD and range of pre-test and post-test level of attitude on hepatitis among patients with hepatitis B N = 60

Sl.no	Attitude	Max. Score	Range	Mean	SD	Mean%
1	Pre test	80	44-49	47.02	1.44	58.7
2	Post test	80	65-71	67.80	1.86	84.7

Table 2 depicts the pre-test and post-test frequency and percentage distribution of knowledge about hepatitis B. The result shows that in the pre-test, 41 (68.3%) of the participants had inadequate knowledge, 19 (31.7%) had moderate knowledge, and none of the study participants had adequate knowledge. Whereas, in the post-test, the result shows that all 60 participants (100%) had moderate knowledge, and none of them had inadequate or adequate knowledge. It shows that after the multimedia education program, the subject knowledge has improved, and the program is found to be effective (Dahl et al., 2012).

Table 3 depicts the pre-test and post-test frequency and percentage distribution of attitudes towards hepatitis B. The result shows that in the pre-test, 60 (100%) of the participants had a neutral attitude, and none of the participants had unfavourable and favourable attitude towards hepatitis B patient. Whereas after the multimedia education program,

all 60 participants (100%) had a favorable attitude towards hepatitis B. Therefore, it shows that the overall attitude has improved and the program has been found to be effective (Jarju et al., 2022).

Table 4 depicts the mean, SD, and range of the pre- and post-test attitude levels. The table shows that in the pre-test, the range was 44–49, the mean was 47.02, the SD was 1.44, and the mean percentage was 58.7%. Whereas in the post-test, the range was 65–71, the mean was 67.80, the SD was 1.86, and the mean percentage was 84.7%. and it can be

clearly seen that the mean percentage improved after the post-test (Mohamed et al., 2012).

Table 5 depicts the paired t-test analysis for the pre-test and post-test analysis. The table shows the mean difference, SD of difference, mean difference percentage, and statistical significance of knowledge regarding management, prevention of complications, and side effects of drugs on hepatitis among patients with hepatitis. The mean difference was 4.96, the SD was 0.75, the mean difference percentage was 16.5, and the t-value was found to be 18.67, which was highly significant at p<0.05 level (Das & Shil 2022).

Table 5. Paired t-test analysis for the significance of pre and post-test level of knowledge hepatitis among patients with hepatitis B N = 60

Sl.no	Knowledge	Max. Score	Mean difference	SD of difference	% of mean difference	Paired t-value	p-value
1	General information regarding hepatitis B	8	1.06	1.26	13.2	6.55	p<,0.05
2	Management of hepatitis B	10	1.78	1.54	17.8	8.96	p<,0.05
3	Prevention of complication of hepatitis B	8	1.13	1.39	14.1	6.29	p<,0.05
4	Side effects of drugs	4	0.98	1.33	24.5	5.71	p<,0.05
	Over all	30	4.96	0.75	16.5	18.67	p<,0.05

Note: *- denotes significant at 0.05 level at 59df (i.e. p<0.05)

Table 6. Paired t-test analysis for the significance of pre and post-test level of attitude on hepatitis patients with hepatitis B N = 60

Attitude	Max. Score	Mean difference	SD of difference	% of mean difference	Paired t-value	p-value
Over all	80	20.78	2.00	25.9	24.25	P<0.05

Note: *- denotes significant at 0.05 level at 59df (i.e. p<0.05)

Table 7. Correlation between knowledge and attitude on hepatitis among patients with hepatitis B N = 60

Variables	Mean	SD	R	p-value
Knowledge	14.02	0.79	0.741*	p<0.05
Attitude	18.98	0..83		

Note: *- Significant at 5% level (i.e., p<0.05).

A paired "t" test was performed to determine the significance of the pre- and post-test levels of attitude regarding hepatitis. The table shows that the mean, standard deviation, and mean percentage of improvement in attitude regarding hepatitis B among hepatitis B patients where the maximum score was

Figure 3: Scatter graph of correlation between knowledge and attitude
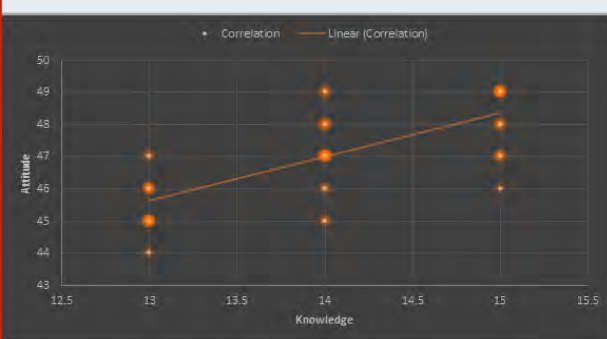
80, the mean difference was 20.78, the SD of the difference was 2.00, the mean difference percentage was 25.9, and the

t-value was found to be 24.25, which was highly significant at p<0.05 level (Mohamed et al., 2012).

Table 7 depicts the correlation between knowledge and attitude toward hepatitis among patients with hepatitis. The result shows that there was a strong positive correlation between knowledge and attitude, and the calculated r value is 0.741*. Table 8 depicts the knowledge level and demographic variables. The result shows that the association between educational status ($\chi 2$ = 28.69), occupation ($\chi 2$ = 19.09), family income ($\chi 2$ = 9.98), and the source of information ($\chi 2$ = 7.868) was found to be statistically significant. Other demographic variables such as age, gender, religion, family history of hepatitis, and heard about hepatitis were found to be statistically non-significant.

Table 8. Association between mean difference of knowledge on hepatitis among patient with hepatitis B with selected demographic variables N = 60

Sl.no	Demographic variables	Categories	Sample (60)		Knowledge				χ2-value	p-value
					≤ median		>median			
			No.	%	No.	%	No.	%		
1	Age	21-30	24	40.0	18	43.9	6	31.6	4.652, df=2, NS	p>0.05
		31-40	19	31.7	13	31.7	6	31.6		
		41-50	17	28.3	10	24.4	7	36.8		
2	Gender	Male	43	71.7	29	70.7	14	73.7	0.056, df=1, NS	p>0.05
		Female	17	28.3	12	29.3	5	26.3		
3	Religion	Hindu	28	46.7	19	46.3	9	47.4	3.56, df=3, NS	p>0.05
		Muslim	12	20.0	10	24.4	2	10.5		
		Christian	18	30.0	10	24.4	8	42.1		
		Others	2	3.3	2	4.9	0	0		
4	Educational status	No formal education	2	3.3	2	4.9	0	0	28.69, df=4, S	p<0.05
		Primary	13	21.7	13	31.7	0	0		
		Secondary	17	28.3	16	39.0	1	5.3		
		Hr. Secondary	24	40.0	10	24.4	14	73.7		
		Graduate and above	4	6.7	0	0	4	21.1		
5	Occupation	Unemployed	2	3.3	2	4.9	0	0	19.09, df=5, S	p<0.05
		Agriculture	7	11.7	7	17.1	0	0		
		Govt. Employee	13	21.7	3	7.3	10	52.6		
		Pvt. Employee	29	48.3	21	51.2	8	42.1		
		Housewife	5	8.3	5	12.2	0	0		
		Coolie / Daily wages	4	6.7	3	7.3	1	5.3		
6	Family income per month (Rs)	<5000	10	16.7	8	19.5	2	10.5	9.98, df=3, S	p<0.05
		5001-10000	22	36.7	19	46.3	3	15.3		
		10001-15000	19	31.7	8	19.5	11	57.9		
		>15000	9	15.0	6	14.6	3	10.5		
7	Family history hepatitis	Yes	-	-	-	-	-	-	Invalid	
		No	60	100	41	100	19	100		
8	Heard about hepatitis	Yes	60	100	41	100	19	100	Invalid	
		No	-	-	-	-	-	-		

Continue Table 8

9	Source information	Magazine	12	20.0	8	19.5	4	21.1	7.868, df=3, S	p<0.05
		Health care professional	18	30.0	10	24.4	8	42.1		
		Family members	18	30.0	11	26.8	7	36.8		
		Friends	12	20.0	12	29.3	0	0		

Table 9 depicts the association between attitude level and demographic variables. The result shows that the association between educational status ($\chi2 = 14.96$), family income ($\chi2 = 9.950$), and occupation ($\chi2 = 19.064$) was found to be statistically significant. Furthermore, the rest of the variables, such as age, gender, religion, and source of information, were found to be statistically non-significant.

DISCUSSION

India has an approximately 3.0% HBV career rate, with a high prevalence rate in the tribal population. Due to the lack of basic medical facilities in the remote areas, many of the tribal populations don't get the required treatment, which can be fatal for the patients and also for the people nearby. Due to this, with a population of more than 1.35 billion, India has more than 37 million HBV carriers, which contributes a large proportion of this HBV burden. 9 The purpose of the present study was to assess the knowledge and attitude regarding management, prevention of complications, and side effects of drugs on hepatitis B among hepatitis B patients. Currently, there is little research data available related to this statement.

The findings of the present study suggest that in the pre-test, 41 (68.3%) of the participants had inadequate knowledge, 19 (31.7%) of them had moderate knowledge, and none of the study participants had adequate knowledge. Whereas, in the post-test, the result shows that all 60 participants (100%) had moderate knowledge, and none of them had inadequate or adequate knowledge. However, the findings were inconsistent when compared with a similar study done in Gambia 10 where the result shows that the majority of the participants have an adequate level of knowledge regarding hepatitis B. This discrepancy might be due to the study setting and large sample size. However, some study findings done in Pakistan 11,12 Malaysia13 and India 14 were consistent with our result.

One of the objectives of our research was to find out the attitude towards hepatitis B. The result shows that in the pre-test, 60 (100%) of the participants had a neutral attitude, and none of the participants had unfavourable and favourable attitude towards hepatitis B patient. However, after the education, all the participants 60 (100%) developed a favorable attitude. The similar negative attitude 309 (79.2%) was found in the study done in Pakistan 11,12 and Malaysia. 13 Although a higher attitude was found in a study done in Gambia 10 where two-third 107 (70%) of the participants had a positive attitude towards hepatitis B infection. This difference in the result could also be due to the fact that African nations have one of the highest numbers of hepatitis B patients. So the health information and higher education

the people are receiving could also be more. Due to medical advancement, the ratio of hepatitis B is decreasing in India, which is also impacting the knowledge and attitude towards hepatitis B.

Our research has found multiple associations between knowledge level and demographics that were found to be statistically significant, such as educational status ($\chi2 = 28.69$), occupation ($\chi2 = 19.09$), family income ($\chi2 = 9.98$), and the source of information ($\chi2 = 7.868$). Some of the similar associations were also found in some studies. 11,12,13 Furthermore, a significant association was also found between attitude level and demographic variables such as educational status ($\chi2 = 14.96$), family income ($\chi2 = 9.950$), and occupation ($\chi2 = 19.064$). A positive correlation between knowledge and attitude was found in this study, which reaffirms the relationship between knowledge and attitude. It can be concluded that adequate knowledge can lead to a positive attitude that can manage and improve the management of complications and side effects of drugs in hepatitis B. The findings are not in line with the result of one of the previous studies, where the study showed no significant predictor of attitude among the sociodemographic and clinical characteristics variables. However, such differentiations in the results could be a representation of people's education, the governmental approach to tackling hepatitis B, and mass health education to the general public.

To our knowledge, a small number of studies have been conducted regarding hepatitis B in western countries compared to Asian countries, as the majority of hepatitis B cases are found in Asian countries. A study was done in Melbourne, Australia 15 regarding the health literacy of chronic hepatitis B patients, which claimed to be the largest study done in Australia to investigate the knowledge of chronic hepatitis B patients who are attending a special outpatient clinic. The result suggested that most of the participants had a higher level of knowledge (7.5 out of 12 points) compared to previous studies.

Also, the study found a statistically significant association between knowledge score and demographic variables such as gender (P = 0.0268**), English literacy (P = 0.045**), educational level (P = 0.05**), having seen clinician previously (P = 0.010**), knowing anyone else with HBV (P = 0.007**), Friend comfortable (P = 0.049**). However, we have observed that the majority of the study has a significant association between knowledge and educational level, so in conclusion, we can say that patients with a higher educational level have higher knowledge regarding hepatitis B, its treatment, side effects, etc. The higher knowledge score in the study might be due to the higher literacy level and better sources of information in Australia. But different

cultural factors and linguistically salient information from different countries can also play a role in the results of different research studies.

Education plays a vital role and has a definite effect on improving knowledge and also shaping negative views and attitudes about this disease. Early education regarding hepatitis B can help improve the knowledge of urban and rural populations. A better understanding of hepatitis B will improve the treatment outcome, reduce negative societal image, and reduce morbidity, stress, and anxiety regarding hepatitis B.

Table 9. Association between mean difference of attitude on hepatitis among patients with hepatitis B with their selected demographic variables N = 60

Sl.no	Demographic variables	Categories	Sample (60)		Attitude				χ2-value	p-value
					≤ median		>median			
			No.	%	No.	%	No.	%		
1	Age	21-30	24	40.0	16	42.1	8	36.4	1.58, df=2, NS	p>0.05
		31-40	19	31.7	11	28.9	8	36.4		
		41-50	17	28.3	11	29.0	6	27.3		
2	Gender	Male	43	71.7	26	68.4	17	77.3	0.053, df=1, NS	p>0.05
		Female	17	28.3	12	31.6	5	22.7		
3	Religion	Hindu	28	46.7	16	42.1	12	54.5	2.36, df=3, NS	p>0.05
		Muslim	12	20.0	9	23.7	3	13.6		
		Christian	18	30.0	11	28.9	7	31.8		
		Others	2	3.3	2	5.3	0	0		
4	Educational status	No formal education	2	3.3	2	5.3	0	0	14.96, df=4, S	p<0.05
		Primary	13	21.7	12	31.6	1	4.5		
		Secondary	24	28.3	12	31.6	12	54.5		
		Hr. Secondary	17	40.0	16	39.	1	15		
		Graduate and above	4	6.7	0	0	4	18.2		
5	Occupation	Unemployed	2	3.3	2	5.3	0	0	19.0.64, df=5, S	p<0.05
		Agriculture	7	11.7	6	15.8	1	4.5		
		Govt. Employee	13	21.7	2	5.3	11	50.0		
		Pvt. Employee	29	48.3	20	52.6	9	40.9		
		Housewife	5	8.3	5	13.2	0	0		
		Coolie / Daily wages	4	6.7	3	7.9	1	4.5		
6	Family income per month (Rs)	<5000	10	16.7	8	21.1	2	9.1	9.950, df=3, S	p<0.05
		5001-10000	22	36.7	18	47.4	4	18.2		
		10001-15000	19	31.7	8	21.1	11	50.0		
		>15000	9	15.0	4	10.5	5	22.7		
7	Source of information	Magazine	12	20.0	7	18.4	5	22.7		
		Health care professional	18	30.0	11	28.9	7	31.8	2.703 df=3, NS	p<0.05
		Family members	18	30.0	10	26.3	8	36.4		
		Friends	12	20.0	10	26.3	2	9.1		

Note: S-Significant at 5% level (i.e., p<0.05), NS-Not significant at 5% level (i.e., p>0.05).

Limitation: The study was conducted at one hospital, which led to a small sample size. Also, the sampling technique used was non-randomized purposive sampling. and the study was limited to only hepatitis B patients. Hence, the findings of the study cannot be generalized to a large population.

CONCLUSION

There are substantial burdens of HBV infection in Asia and the Pacific Islands, sub-Saharan Africa, the Amazon Basin, and Eastern Europe, despite the disease's frequency being unevenly distributed throughout the world. But still, a number of issues in hepatitis B management remain controversial or unresolved, such as identifying treatment candidates, managing partial or nonresponses, and predicting treatment response. The authors hope that the study findings will support the stakeholders teachers, doctors, and nurses in making a significant decision to handle hepatitis B patients more efficiently.

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Biotechnological Communication

Oncogenic Viruses: A Comparative Evolutionary Study

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ABSTRACT

Cancer is caused by carcinogens. Among these causative agents viruses are the traceable source. Oncoviruses have both DNA and RNA as genetic material. The constant evolution of these viruses allows them to survive and thrive. HPV, HCV,HBV, HH8, EBV, HTLV, MCP are a few known carcinogenic viruses. The pathway of establishment and proliferation of the virus follows the order of initiation, promotion, and progression. Various viral proteins play a mechanistic role in the conversion of a normal cell into a neoplasm. This study is a small attempt to learn the evolutionary patterns of oncoviruses. Two common pathways of cancer are the suppression of p53 and Rb genes. This study looks at the viral proteins that perform this activity and looks at the similarity through the evolution patterns of the viral proteins. The niche of this study lies in the identification of possible targets that can collectively prevent viral cancers. Also in multiplexing targets for diagnosis. However , a common target identification from a evolutionary point of view was not established. The study however establishes that among the evolved viruses onco- proteins have similarity i.e a common pathway of evolution.

KEY WORDS: VIRUS, CANCER, MECHANISM, PROTEIN.

INTRODUCTION

Cancer is the collective name for diseases caused by the uncontrolled division of abnormal cells which later proliferate and spread to other organs. The aggregation of such cells in one place leads to a 'tumor, '(National Institutes of Health 2007, Anderson and Simon 2020). The process by which a cell converts from a normal to a malignant cell is called carcinogenesis. The factor that causes this mutation that triggers a cell to become malignant is called as carcinogens. Carcinogens are of 3 major types' chemical carcinogens (including those from biological sources), physical carcinogens, and oncogenic (cancer-causing) viruses. Among these oncogenic carcinogens depend on persistence and take a longer time to cause malignancy, (Anderson and Simon, 2020).

This makes viral cancers the most detectable and preventable form of cancer. Viruses contain DNA or RNA as genetic material. Among these viruses with single-stranded DNA are known to evolve rapidly (Zur, 1991). Evolution is the mechanism employed by an organism to overcome a hostile environment.The year 1964 saw the discovery of viruses also as a cause of cancer. Viruses cause cancer either directly or by causing inflammation. The first of the oncogenic

viruses to be discovered was Epstein-Barr Virus (EBV). The expression of proteins is a key step in the infection process and hence making immunosuppressed patients more suitable for viral cancers, (Tonseilo et al 2018 Tempera & Lieberman, (2021).

The key 7 types of viruses are proven to be oncogenic in nature namely. Viruses associated with causing cancer are EBV (Epstein–Barr virus), HPV (human papillomavirus), HTLV-1 (human T-lymphotropic virus 1), KHSV (Kaposi sarcoma-associated herpesvirus)/ HHV-8 (human herpesvirus 8), HCV (hepatitis C virus), HBV (hepatitis B virus) and MCPyV (Merkel cell polyomavirus). Among the above-mentioned HTLV-1, HCV and HBV are retroviruses. Constant assault leads to malignant transformation.2.2 million infections were attributed to cancer cases in 2018 worldwide which amounted to 13 % of the total cancers. 90% of the cases are caused by HPV, HBV, and HCV (Liao.,2006). Among the various diseases cancer is the highest death causing disease, accounting for almost 10 million deaths in 2020, (WHO 2022).

Among the above-mentioned viruses, most of them are DNA viruses and two of them are RNA viruses. Most RNA viruses are retroviruses that replicate via reverse transcriptase. EBV and KSHV are large DNA viruses that often cause solid tumors and lymphoid malignancies, (Charostad et al., 2020). HPV and MCPyV are smaller DNA genomes

in size and cause warts. Where oncogenic HPVs establish persistent infections in mucosal epithelia whereas MCPyV infects and likely persists latently in dermal fibroblasts. These small DNA oncogenic viruses promote tumorigenesis using relatively few multifunctional oncoproteins. HCV a retroviral virus has genetic material in a positive sense, HBV, on the other hand, is single a stranded RNA virus, that infects hepatocytes and causes chronic liver inflammation, liver cirrhosis, and hepatocellular carcinoma (HCC). HTLV-1 has RNA as genetic material and causes T-Cell lymphoma.

Infection of the above viruses is common but with viral cancers, there is no thumb rule that cancer progression needs the presence of virus. The general process of oncogenic viruses to cause cancer is chronic inflammation, immunosuppression, or environmental mutagens individually or in combination cause assault and helps with the progression to cancer. Several studies have been undertaken to prove that viruses are the root cause of cancer. Studies on both human and animal models have ascertained the same.

Viral mechanisms of inducing infection are direct and indirect mechanisms. Direct mechanisms involve. Insertion of viral oncogenes into host cells followed by enhancement of the proto-oncogenes in the host genes (Coffin et al., 1997) Indirect viral oncogenes activation occurs to triggering non–specific inflammation. It happens due to prolonged periods of inflammation. The direct method of infection mandates the presence of at least one virus in every tumor cell, expressing a minimum of one protein or RNA to help the cell become cancerous. Indirect tumor viruses are hypothesized to be lost from the tumor after the tumor has the sufficient mutation and attains hyperplasia (Morales-Sánchez & Fuentes-Pananá 2014 and Gaglia & Munger 2018).

Viral cancers are characterized by long latent periods of incubation. Cancers induced by viruses are part of the natural viral life cycle. RNA viruses have 3 distinct methods of transformation i.e., acute transformation, v-oncogene replication, and non-transforming replication. Oncoviruses often use pathways common pathways to induce tumorigenesis, some common pathways used by viruses to induce cancer are tumor suppressor pathways such as p53 and tumor necrosis–associated pathways (TRAFs), telomerase reverse transcriptase (TERT) and other pathways used are P13K-AKT-mTOR, JAK /STAT, NF-κB, β-catenin, MHC-1.The host DNA damage response is catalyzed and influenced by the viral proteins.

In the study of the Kegg pathway, it was seen that the most common pathways of the above-mentioned p53 suppression, P13K-AKT-mTOR, and JAK /STAT pathways inhibit apoptosis (Ozaki & Nakagawara, 2011), Brooks & Putoczki, 2022). In this study, the general evolution of the oncogenic viruses is studied with the proteins involved in viral oncogenesis. Further, the evolution of the DNA viruses was explored, and finally the evolution of the proteins involved in the suppression of apoptosis through p53, P13k-AKT –mTOR, and JAK /STAT pathways. The study

of these mechanisms shows a common route of infection by the oncoviruses. This study aims at understanding the common mechanisms of neoplasm induction by viruses and the evolutionary patterns of viral oncogenes involved in common mechanisms causing cancer.

MATERIAL AND METHODS

Retrieval of whole genome structures from NCBI was carried out for - Epstein–Barr virus, Human T-lymphotrophic virus -1, Kaposi sarcoma-associated herpesvirus/ human herpesvirus8 , Human papillomaviruses, Merkel cell polyomavirus, Hepatitis B virus and Hepatitis C virus. Retrieval of protein and gene sequences of the main viral onco-proteins from NCBI. Kegg pathway analysis: and Pathways in cancer - Homo sapiens (human)- pathway number hsa 05200 was also studied.

Viral carcinogenesis- pathway number map05203 was analyzed to identify and select common pathways in cancer caused by Viruses and to identify the viral genes involved in the mechanism of carcinogenesis. Similarly, multiple sequence alignment of the chosen virus and viral proteins were aligned to see similarity using Clustal omega, Clustal W and Mega X. Construction of evolutionary tree was done by using Clustal omega, Clustal W and Mega X based on neighbor joining method.

RESULTS AND DISCUSSION

The phylogenetic analysis of the proteins that induce oncogenesis is depicted in the below phylogenetic tree. The analysis results from Clustal omega and Clustal W are given below. Cancer causing viruses have both RNA and DNA as genetic material. Hence to analyze a common pattern of infection and carcinogenesis using keg analysis the main viral proteins were identified and taken for study. The sequences taken for study are listed in the table below.

The above tree shows vast evolution of the viral proteins. HTLV is the earliest known of the carcinogenic viruses. The evolution of the viruses based on the viral proteins takes place as two branches. The evolution takes place in clusters with the proteins of one virus most closely related to the other onco- proteins of the same virus. Although it is seen the EBV and HPV have common points of evolution. HBV and HPV viral protiens also are seen to have common point of origin. One marked point of similarity is between the protein of HHV 8 and HPV 31, both owing to be cancer causing viruses of the epithelium. Hence from this analysis it can be concluded that no common primers or multiplexing technique can be employed to identify the cancer causing viruses. Since viruses are both of DNA and RNA in nature , the next phase of screening was based on the DNA viruses.

Phylogenetic analysis of the DNA viruses: The results from the phylogenetic analysis are depicted from Mega X.

The interpretation of this tree was done by neighbor joining method.Evolutionary analyses were conducted in MEGA

X .The Maximum likelihood method infers the topology of the tree. HPV is the most diverged of the viruses in studied group.1,2,3 denotes the divergence patterns of the taxa .Order of evolution is from the tree is HTLV-1 , the next divergence in the codons evolution is EBV. The next evolution is KSHV and the arm length . The most divergent is HPV which comes next in the series . The last of the evolution is MCV.

Table 1. List of Viral proteins taken for the study.

ENTRY	ENTRY NAME	PROTEIN NAME	GENE NAME	ORGANISM	LENGTH
P03211	EBNA_EBVB9	EPSTEIN-BARR NUCLEAR ANTIGEN 1	EBNA, BKRF 1	EPSTEIN-BARR VIRUS (STRAIN B95-8)	641AA
P12977	EBNA3_EBVB9	EPSTEIN-BARR NUCLEAR ANTIGEN 1	EBNA3	EPSTEIN-BARR VIRUS (STRAIN B95-8)	944AA
A0A3R5XA97	A0A3R5XA97_EBVG	EBNA-3A	EBNA-3A	EPSTEIN BARR VIRUS (STRAIN GD1) (HHV-4)	925 AA
A0A075FDV6	A0A075FDV6_EBVG	EBNA-3B	EBNA-3B	EPSTEIN BARR VIRUS (STRAIN GD1) (HHV-4)	892AA
A0A0C7P4C5	A0A0C7P4C5_EBVG	EBNA-3C	3BNA-3C	EPSTEIN BARR VIRUS (STRAIN GD1) (HHV-4)	992AA
K9USE8	K9USE8_EBVG	EBNA-LP	EBNA-LP	EPSTEIN BARR VIRUS (STRAIN GD1) (HHV-4)	506AA
Q67978	Q67978_HBV	CAPSID PROTEIN	CORE	HEPATITIS B VIRUS (HBV)	212AA
Q89656	Q89656_HBV	CAPSID PROTEIN	PRE-C/CORE	HEPATITIS B VIRUS (HBV)	212AA
P03141	HBSAG_HBVA3	LARGE ENVELOPE PROTEIN	S	HEPATITIS B VIRUS GENOTYPE A2SUBUNIT ADW2(STRAIN RULTER 1979) (HBV-A)	400AA
Q9QMN6	Q9QMN6_HBV	LARGE ENVELOPE PROTEIN	S	HEPATITIS B VIRUS (HBV)	400AA
Q9QMN7	Q9QMN7_HBV	PROTEIN P	P	HEPATITIS B VIRUS (HBV)	843AA
P06463	VE6 HPV18	PROTEIN E6	E6	HPV TYPE 18	158AA
P03126	VE6 HPV16	PROTEIN E6	E6	HPV TYPE 16	158AA
P04014	VE1_HPV11	REPLICATION PROTEIN E1	E1	HPV TYPE 11	649AA
P27223	VE2_HPV42	REGULATORY PROTEIN E2	E2	HPV TYPE 31	398AA
Q705H5	VL2_HPV43	MICROCASPID PROTEIN L2	L2	HPV TYPE 45	463AA
Q80915	VE1_HPV44	REPLICATION PROTEIN E1	E1	HPV TYPE 45	643AA
P17387	VE7 HPV31	PROTEIN E7	E7	HPV TYPE 31	98AA
P06427	VE6 HPV33	PROTEIN E6	E6	HPV TYPE 18	149AA
A0A0P0ERM8	A0A0P0ERM8_HPV34	REGULATORY PROTEIN E2	E2	HPV TYPE 31	345AA
A0A6M3S242	A0A6M3S242_HPV35	REPLICATION PROTEIN E1	E1	HPV TYPE 16	637AA
T2A6T1	T2A6T1 HPV39	PROTEIN E6	E6	HPV TYPE 16	158AA
W5RS47	W5RS47 HPV45	PROTEIN E6	E6	HPV45	158AA
C9EF17	C9EF17_HPV51	MINOR CASPID PROTEIN L2	L2	HPV 51	469AA

The distances show evolution and variations in the codons. The HTLV-1 and EBV deal with lymphomas. The HH8 and MCV deal with skin cancers. Whereas, HPV deals with a group of specialized cells of the cervix, anus, penile etc., forming carcinomas in the epithelium and metastases into the squamous cells. The evolution and divergence shows the increased ability of the cancers to establish in the specialized regions.

are altered for cancer development. This makes the MAP-kinase pathway a reliable target for cancer therapy.

JAK –STAT pathway – plays a catalytic role in tumor progression .The pathway triggers tumor growth /metastasis or as an immune system modulator. The over stimulation of JAK/STAT pathway proteins leads to proliferation, progression, survival and metastasis of cancer cells.

Figure 1: Clustal omega based tree for viral evolution

Figure 2: Clustal W based phylogenetic tree
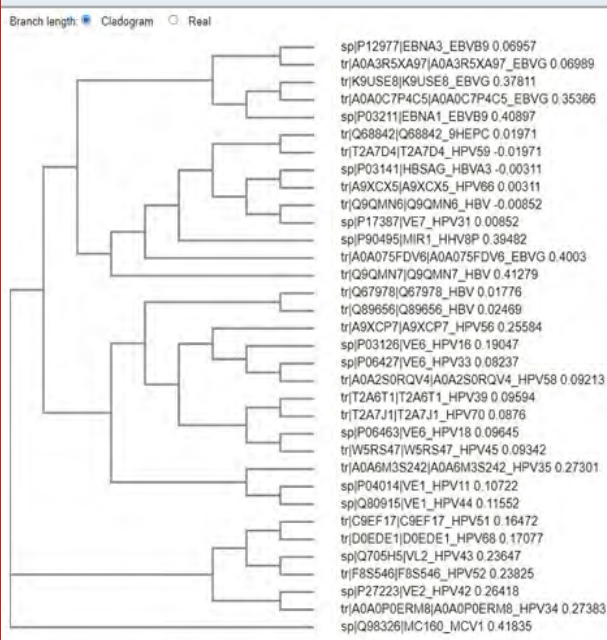
Figure 3: Phylogeny of DNA viruses using Mega X
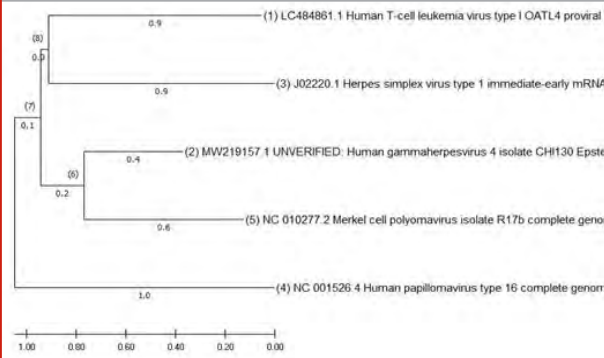
Figure 4: Phylogeny of MAP-Kinase and JAK –STAT pathway using Mega X
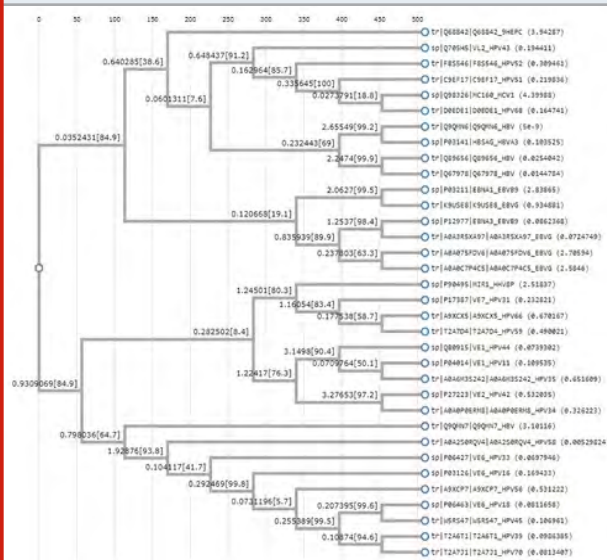
Figure 5: Phylogeny of proteins that block p53
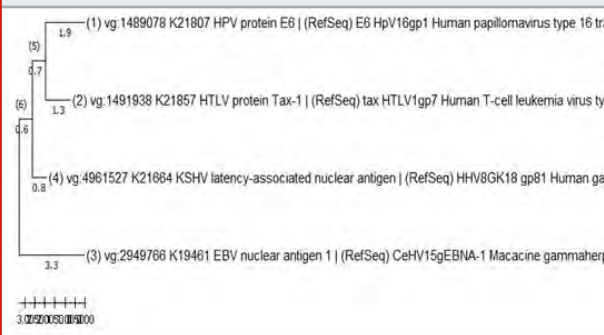
Phylogenetic analysis of proteins that trigger the MAP-Kinase and JAK –STAT pathway:MAP Kinase pathway –Mitogen activated protein kinase pathway is involved in several organized and linked signaling cascades which often involve oncogenesis , tumor progression and drug resistance. MAPK are a group of kinases whose activities

The evolutionary history was inferred by using the Maximum Likelihood method .This analysis involved 4 amino acid sequences. There were a total of 1133 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar., et al .2018).The MAP-kinase and JAK –Stat pathway promotes immortalization through a

series of pathways. The proteins in the viruses coding for the initiation of these pathways were compared to see the evolutionary pathway. Based on the distances the HPV E7 and TAX of HTLV1 are the most divergent. The LMP1 of the EBV and GPCR of KSHV are the intermediates in this evolutionary process. Phylogenetic analysis of proteins that block the p53 activity: P53 is attributed to promotion of apoptosis. p53 responds to factors such as DNA damage and promotes a cell cycle arrest by accumulation in the nucleus. If the DNA damage is irreparable then p53 promotes cell death. Neighbor joining method was used for this phylogenetic analysis.Evolutionary analyses were conducted in MEGA X (Kumar., et al .,2018).

The inactivation of p53 is one of the major steps in the establishment of carcinogenisis. The constant presence of the virus and multiplication helps in the establishment of carcinogenesis. This tree indicates EBNA-1 and E6 of HPV are most distantly related. KSHV, LANA and TAX1 of HTLV1. The evolution and mutation the species undergo is for the adaptation purpose. HPV is the most evolved of the studied viruses and this evolution has led to the many subtypes. Viral cancers take a long time to establish and convert to neoplasm and carcinogenesis. This is the key factor to curing the viral cancers. Viral cancer often manipulates pathways that coincide with the hall marks of cancer. The proteins that trigger and establish carcinogenesis have undergone evolution indicating them as the sources for detection / manipulation for treatment. Viral infections often provide the entropy that defines the genetic and phenotypic variation, hence manifesting itself as the driving force of cancer evolution (Tempera & Lieberman 2021).

Among the viruses EBV and KSHV are large DNA viruses who often cause solid tumours and lymphoid malignancies. HPV and MCPyV are smaller DNA genomes in size and cause wrats. Where oncogenic HPVs establish persistent infections in mucosal epithelia whereas MCPyV infects and likely persists latently in dermal fibroblasts. These small DNA oncogenic viruses promote tumorigenesis using relatively few multifunctional oncoproteins. HCV a reteroviral virus has genetic material as a positive-sense, HBV on the other hand are single stranded RNA virus,both infect hepatocytes and cause chronic liver inflammation, liver cirrhosis and hepatocellular carcinoma (HCC) (Zuckerman, et al., 1997).

HTLV-1 is a human oncogenic retrovirus that affects T cells and can initiates adult T cell lymphoma Deregulation of p53 and RB pathways is common mechanisms of most oncogenic viruses. The oncoproteins suppress p53 and RB by methods of degradation, inactivation, disassociation or repression (Tornesello, et al.,2018). The viruses have use different mechanisms for suppression, the proteins taken for the study are the main proteins that initiate the pathway. However the RB sequence is not part of all the viruses under study and mainly is only a part of the cancers involving epithelium.

CONCLUSION

The evolutionary study gives an insight to the mechanisms of evasion followed by the viruses and variations it undergoes over the years to thrive and hence providing an insight to treatment / prevention of the cancers. Form this study it can be concluded that a common target for viral cancers cannot be chosen. However, cancer causing viruses of similar organs (eg. Epithelium) seem to have a common point of origin. Further studies with the mechanisms of these proteins may give us an insight into preventable targets for viral cancers.

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Microbiology Communication

Characterization and Chemical Management of Cumin wilt Disease Caused by *Fusarium oxysporum*

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ABSTRACT

Cumin is the current leading currency earning crop in India, as per Agriwatch production estimate, Jeera production for 2021-22 marketing period is estimated at 391,291 MT, generating 8,600 crore rupees. Despite its importance, cumin is threatened by a serious, destructive disease called Cumin Wilt, which is caused by *Fusarium oxysporum*. The disease's impact has reduced income at both the household and national levels. The current study was carried out to look into chemical control strategies for the disease. The research was carried out from November to March (Rabi season) in Ravipura Kampa, Gujarat, India. Field observation of infected cumin wilt was followed by laboratory isolation, characterization of the causative agent, and assessment of the effects of various fungicides. In chemical management different fungicides were used. In laboratory tests, *F. oxysporum*, which is characterized by whitish mycelia growth and chlamydospores, was found to be the causative agent of the cumin wilting symptoms. There are two types of conidia: macro conidia and micro conidia. All the fungicides showed varying effects against *F. oxysporum*. This resulted in a complete stoppage of mycelial growth, followed by 5% EC formulation of Hexaconazole 2800 ppm, 50% WP CAPTAN3500ppm, Azoxystrobin 1600ppm, Azoxystrobin 11% + Tebuconazole 18.3% w/w SC 1000 ppm, Epoxyconazole + Flyxapyroxald 3500 ppm, Isopropanol azole 4000 ppm, Mancozeb 5000 ppm, Pyarclostrobin 13.3%+ Epoxyconazole 5% EC 4000 ppm, and Tebuconazole 25.9% EC 2000 ppm.

KEY WORDS: CHEMICAL FUNGICIDES, CUMIN, *FUSARIUM* WILT, *IN VITRO*.

INTRODUCTION

India has been a land of spices and the largest producer, consumer, and exporter of spices. Rajasthan and Gujarat are the important states to produce cumin. The crop suffers from several diseases. Among the major threats to the cumin is wilt. When stem was cut longitudinally, brownish discoloration is observed. Mathur and Mathura (1956) reported wilt of cumin from Rajasthan and identified the casual organism to be *Fusarium oxysporum*. Based on host specificity, Patel and Prasad finally named it as *Fusarium oxysporum* f. sp. *cumini. Fusarium* is largest genus of filamentous fungi widely distributed in agricultural soils. It contains large number of destructive plant pathogens such as *F. avenaceum, F. eumartii, F.oxysporum* and particularly *F. solani,* which is potential cause of vascular wilt, root rot and fruit rot as well as influence seed germination in

different host plants. The fungus is soil borne in nature and commonly found in all crop growing areas in world. The fungus belongs to ascomycetes and cause wilt disease of several important crops. Fungus produces micro conidia, macro conidia and chlamydospores, which persist in soil for several years. The pathogen is seed borne in nature too and transmits from one place to another through seed material (Watt, 2006, Akter *et al.,* 2021).

Crop plants are highly susceptible to attack of *Fusarium* spp. during the pre- and post-emergence stages (Nawar, 2007). Pathogens can quickly develop in light sandy soils, triggering root rot disease in a variety of crops in different parts of the world (Celar, 2000). The fungus produces toxic substances that are responsible for inducing wilt symptoms in susceptible cultivars. Brown blotches form on the wilted area. The disease causes withering signs in the seedling stage. Plants are damaged during every phase of its development. The infected plants turn yellowish and show characteristic dropping of leaves leading to mortality of plant. Brown

discolorations can also be seen on the vascular bundle, and the plants are easily pulled from the soil. Although there are a lot of approaches and procedures for controlling plant diseases, only a handful have been proven effective. The pathogen is managed using a combination of biological, cultural, and chemical methods. Varieties of systemic and non-systemic fungicides have been tested against the pathogen, having varied results. Several scientists have concluded that systemic fungicides are superior for disease management (Rafique *et al.,* 2016).

MATERIAL AND METHODS

Isolation of pathogen: The Pathogen of cumin wilt was isolated from wilted part of cumin plant (Ravipura kampa-Gujarat India). Small (5 by 5 mm) sections were excised from margins of browning stem bases and surface-sterilized in 75% ethanol for 10 sec, then sterilized in 0.5 % sodium hypochlorite for 1 min, followed by three rinses with sterile water. The fragments were then placed on to potato dextrose agar (PDA) and incubated at 27°C. Fungal growth was examined daily for up to7 days. Isolates were transferred to fresh PDA and purified by single-spore culturing. All fungal isolates were placed on fresh PDA slants and stored at 4°C Akter *et al,.*(2021).

Effect of temperature, carbon sources, different nutrient media, *pH and salt* on linear growth of pathogen: There were four temperatures selected for study 4°C, 28°C, 35°C and 40°C. Twelve sterile Petri-dishes with PDA medium were used (three plates for each treatment). With the help of 5 mm-diameter sterile cup borer a disc of inoculum of a 7- day old cultures, and then transferred to the center of each plate in close contact with the medium. The plates were incubated for 7 days at the following temperature range: 4°C, 28°C, 35°C and 40°C. The fungal growth was estimated daily by measuring the colony size and results were recorded for each degree of temperature, (Khilare and Ahmed, 2012).

Effect of carbon sources on linear growth of pathogen was investigated by using four different sources. Those carbon sources were dextrose, sucrose, maltose and starch. The amount of 2.0 gm of each sugar was added to the potato agar medium. The Petri-dishes were inoculated with 2 mm disc taken from 7 days old culture of the isolate and incubated in complete darkness at 28°C for 7 days. Readings were taken daily by measuring the growth diameters and the average was recorded.

Effect of different nutrient medium on linear growth of pathogen was assessed by taken five types of media those were potato dextrose agar (PDA), malt extract agar (MEA) and Czepek's Dox agar, Rose bangle agar, white bran medium. Four plates (9 cm diameter) were prepared from each medium and then the plates were inoculated with 5 mm disc from 7 days old culture of isolate. The plates were incubated in complete darkness for 7 days at 28°C and then the rate of fungal growth was estimated daily by measuring the colony size (Chittem and Srikant Kulkarni, 2008)

Effect of pH and salinity on linear growth of pathogen: 5.0, 6.0, 7.0, 8.0 and 9.0 pH were taken. The pH was adjusted in PD Amedium. Salinity was adjusted with NaCl in medium. Four concentrations of NaCl were taken for study 0.10,0.25,0.50and0.75percent were maintained. All Petri plates were incubated at 28°C for a week. Radial growth observations were taken at 24 hour intervals up to one week, radial growth was measured by scale Gordon *et al,* (2019).

Effect of different fungicides on pathogen at laboratory level: 5% EC formulation of Hexaconazolek, 50% WP CAPTAN, Azoxystrobin, Azoxystrobin 11% +Tebuconazole 18.3% w/w SC, Epoxyconazole + Flyxapyroxald, Isopropanol azole, Mancozeb, Pyarclostrobin 13.3%+ Epoxyconazole 5% EC, and Tebuconazole 25.9% EC Fungicide tested to analyze their effect on growth of pathogen . Each fungicides was dissolved in water to obtain a final concentration 100 ppm, 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm, 3000 ppm, 3500 ppm, 4000 ppm, then inoculated into sterile PDA with three replicate.

These five dilutions for each chemical were made based on their recommended dose. Three plates poured with PDA medium served as control. Then 5 mm disc was cut from the edge of 7 days old culture of the fungus and disc was placed in the center of Petri dish and kept in BOD incubator for 8 days for the full growth of fungus was observed, in case of control where no chemicals were applied in medium. The inhibition in the radial growth of pathogen mycelium was recorded and percent inhibition in the mycelium of pathogen was recorded by comparing the mycelial growth of fungus in the treated plates with control. The effect of each chemical was evaluated as percentage of reduction (Sultana & Ghaffar 2010).

Figure 1: Wilted cumin sample

Figure 2: Growth of pathogen on PDA
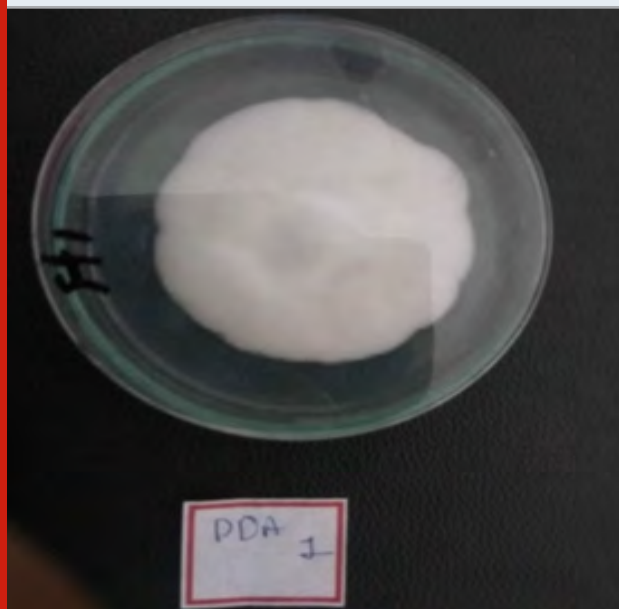
Figure 3: Microscopic observation of macro and micro conidia
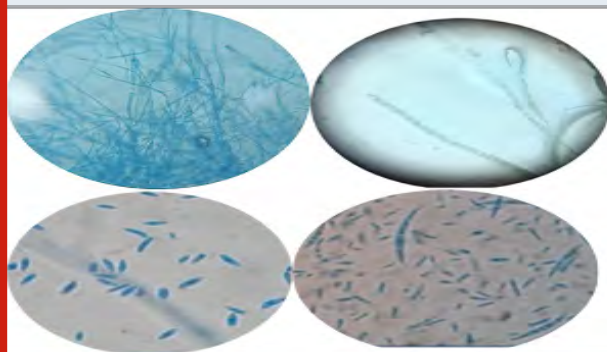
Figure 4
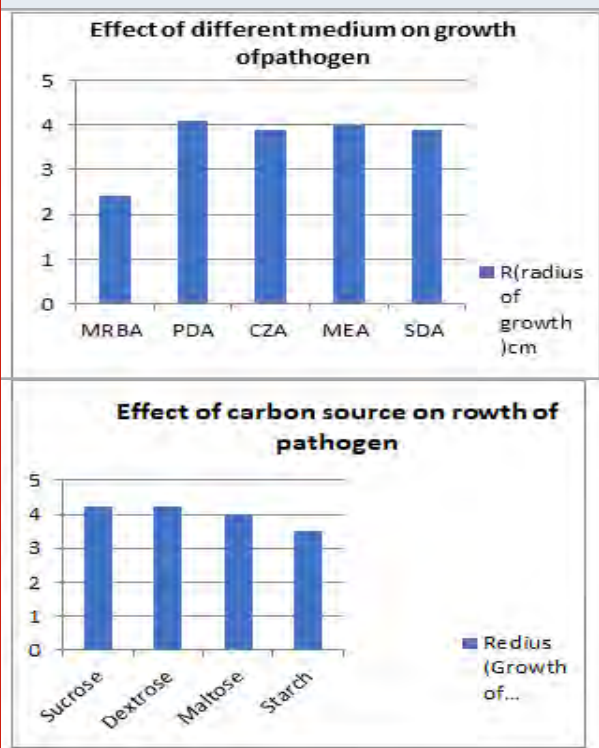
Where: R = dc – dt x 100/dc 25   Whereas:
R = Percentage of reduction
dc = diameter of control colony
dt = diameter of treatment colony

Figure 5

RESULTS AND DISCUSSION

In the present study, the experiments were carried out under laboratory conditions including all the available management practices and further their efficacy was tested in farm conditions. Standard tissue isolation technique was followed to get culture of causal organism from the wilted
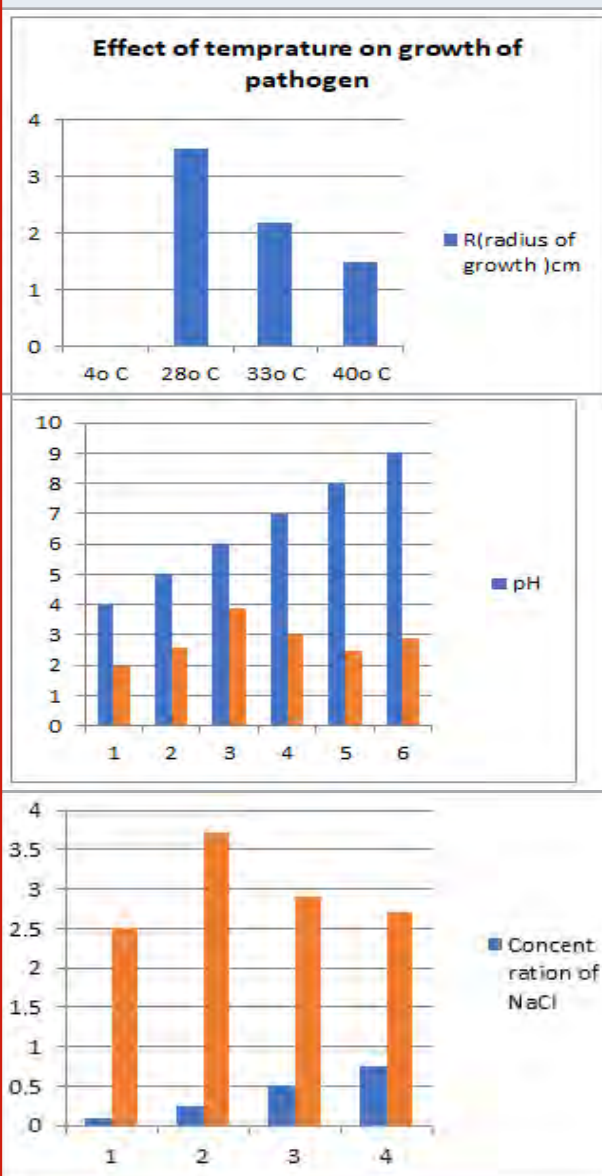
plants (figure 1), showing the symptoms. *Fusarium* was isolated consistently from wilted cumin sample. Repeated sub-culturing was done to obtain pure culture on basal medium PDA (figure 2).

Hyphal tip method was used to obtain pure culture as detailed in material and methods. The pathogen was identified as based on their morphological and cultural characters as *F.oxysporum* by referring to Booth (1971)

Cultural studies: Cultural characters were studied on five different solid media and four carbon sources were studied to analyze effect of carbon source on linear growth of pathogen, maximum growth was observe in Potato dextrose agar medium, dextrose and sucrose carbon source. For the majority of the isolates, the 27°C temperature, pH of 6, and salt content of 0.25 percent resulted in the fastest *in vitro* radial growth.

The symptoms of *Fusarium* wilt caused by *Fusarium oxysporum* appeared as leaf chlorosis, yellowing of foliage followed by wilting and dropping of leaves. The xylem became brown in colour of the stem and finally death of the above ground parts. Similar symptoms were also reported by Altinok (2005) and Mac Hardy and Beckman (1981). Isolated fungal pathogens were identified based on cultural and microscopic characteristic and using available literatures. Altinok (2005) and Mwaniki *et al.* (2011) isolated *F. oxysporum* from the eggplant vascular tissues in Tanzania. Sahar *et al.* (2013) also isolated *Fusarium oxysporum* from infected roots of egg plant in Pakistan. *F. oxysporum* was isolated from *Fusarium* wilt of tomato plant by Amini and Sidovich (2010). *F.oxysporum* was also isolated from roots and stem of wilt infected tomato plant (Nirmaladevi and Srinivas, 2012 and Joshi *et al.*, 2013).

Chemical agents used were found to be effective for the inhibition of mycelial growth of *Fusarium oxysporum* under *in vitro* conditions (Table 1). Khan *et al.* (1997) and Sharma (2006) found that Bavistin completely inhibited the mycelial growth of *F. oxysporum*. Hossain and Bashar (2011) also reported that complete inhibition of mycelial growth was observed in *Fusarium oxysporum* and *F. pallidoroseum* with Dithane M-45 and Cupravit at 50 and 800 ppm concentrations, respectively. The effectiveness of these fungicides against other fungi has been reported (Daradhiyar, 1980; Sommer, 1982; Kalra and Sokhi, 1985; Singh *et al.*, 1997; Patel *et al.*, 2005; Banyal *et al.*, 2008).

Table 1. Effect of chemical fungicides against *F. oxysporum* f. sp. *cumini*

No.	Trade name of fungicides	Name of active compound	Concentration of fungicides	Radial growth (mm)
1	Folicur	Tebuconazole	2000ppm	0.00
2	Amistar Top	Azoxystrobin	1500ppm	0.00
3	Opera	Pyarclostrobin + Epoxyconazole	4000 ppm	0.00
4	Adexar	Epoxyconazole + Flyxapyroxald	3500 ppm	0.00
5	Custodia	Azoxystrobin + Tebuconazole	1000 ppm	0.00
6	Curzate M8	Cynoacetamide	3500 ppm	0.00
7	Dithane M45	Mancozeb	5000 ppm	0.00
8	Captaf	WP Captan	3500 ppm	0.00
9	Contaf	Hexaconazole, Triazol	2800 ppm	0.00
10	Azaka	Azoxystrobin	1600 ppm	0.00

The pathogen was identified as based on their morphological and cultural characters as *F. oxysporum* by referring to Booth (1971). Generated Sequence from the Microbial Sample (CWP-1). DNA sequence was submitted to NCBI; accession number of DNA sequences is OR178141.1. Furthermore, *Fusarium oxysporum* f. sp. *cumini* was confirmed to be the causative agent of cumin wilt disease.

Among the fungicides tested in this study Folicur treatment reduced mycelium development at concentrations ranging from 500 ppm to 2000 ppm, similar to our founding Shcherbakova et al., 2020, reported Folicur at 400 ppm completely suppressed the germination of the pathogen.

Our findings of Custodia fungicide demonstrated that the fungicide was successful in stopping all mycelia development between 500 ppm and 1000 ppm, similarly, 500 ppm concentration of Custodia exhibits a fungistatic action, Poussio *et al.,* 2021. Opera was successful in inhibition of all mycelial development between 2500 ppm and 4000 ppm, 500 to 1500 parts per million concentrations of Amistar top fully inhibited growth of mycelia; similar to our study Amistar top 500 and 1000 ppm and opera 2500, 3000 ppm were concluded to be fungistatic and strongly suppressed the mycelial development of *F. oxysporum* f. sp. *lycopersici*, according to Chavan *et al.,* 2021. Curzate M8 successfully impeded mycelial growth from 1500

ppm to 3500 ppm. At doses between 1600 and 2800 ppm, Contaf fungicide proved to be effective. Similar to our investigation, Khamari and Patra's 2018 reported Curzate M8 - 1000 ppm has 99.60 % mycelial inhibitions and 1000 ppm of contaf was effective on *Fusarium spp*.

Mycelia had been significantly reduced by Dithen M45 between 3500 ppm. At doses between 1600 and 2800 ppm, Contaf fungicide proved to be effective. Similar to our investigation, Khamari and Patra's 2018 reported Curzate M8 - 1000 ppm has 99.60 % mycelial inhibitions and 1000 ppm of contaf was effective on *Fusarium spp*. Mycelia had been significantly reduced by Dithen M45 between 3500 and 5000 ppm. Similarly, Hossain and Bashar 2011 reported complete inhibition of mycelial growth with Dithane M-45.

Azaka concluded to be of an effective dose range of 400 ppm to 1600 ppm, similarly using 0.030% (300 ppm) Azaka (Azoxystrobin 18.2% + Difenoconazole 11.4 %), Undhad et al., 2022 achieved 98.80 % mycelial inhibition. Wieczyński, et al., 2016 found similar results Captaf, 2000 ppm and 3500 ppm was the efficacious dosage shown in our results, similar results were obtained by Garkoti *et al.,* 2013. Song *et al.* (2022) also reported that Tebuconazole 25.9% EC was found to be the most effective fungicide in inhibiting the mycelial growth of *F. oxysporum* in solidified poisoned PDA observed.

Similarly, among non-systemic fungicides, Mancozeb has the best performance and this result is supported by the findings of Singh *et al.,* 2000. They recorded Mancozeb to be best for growth inhibition of *F. solani* and *F. oxysporum*. Allen *et al.,* 2004 also recorded percent inhibition of all four *Fusarium* species by Mancozeb. The findings of the present study are in agreement with those of Pandey *et al.,* 2005). They reported that *Fusarium* is best controlled by the use of Mancozeb.

CONCLUSION

The observed wilt syndromes on cumin fields are the fungal disease symptoms caused by a soil borne pathogen known as *Fusarium oxysporum*. Salinity, pH, carbon source and temperature have a significant effect on fungal growth. These characteristics can aid in disease monitoring and raising farmer awareness.

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Availability of data and materials: All data and material used can be availed from corresponding author upon request.

Ethics approval and consent to participate: Not applicable.

Competing interests: Authors declare that they have no competing interest.

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Biotechnological Communication

Diversity of Avian Species in Upper Wardha Reservoir Morshi, Amravati, Maharashtra

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ABSTRACT

Birds have ecological value and constitute a vital element of the natural system, being important bio-indicators of the ecosystem. They also play an important role in seed dispersion, pest control and food chain. As there is a lack of data regarding avian diversity and its abundance in large reservoirs, the present study was carried out to determine the diversity and abundance of avian fauna at the Upper Wardha Reservoir of Maharashtra State, India. Five stations were selected and data were recorded from each station. Each site was visited four times in a month and point count method was used to collect the data. Total 151 bird species were recorded. Out of which, 84 species (belonging to 20 families), were of wetland birds and 67 species (belonging to 22 families) were wetland associated. The diversity indices, species evenness, relative density and abundance were studied. In this study, probably for the first time, we have recorded the significant sighting of Greater white-fronted goose. We also recorded occasional sighting of Grey lag goose, Barr headed goose, Oriental pratincole and Glossy ibis. It was also found that freshwater reservoir acts as a suitable habitat for significant avian diversity and species richness.

KEY WORDS: ABUNDANCE, AVIAN, AMRAVATI, DIVERSITY, WETLAND, UPPER WARDHA RESERVOIR,

INTRODUCTION

The Indian subcontinent is very rich in biodiversity. India hosts 1340 species (13 %), of birds out of the total 9000 birds species that are found in the world. Ali and Ripley (1987) considered 176 species endemic to the Indian subcontinent. Grasslands, Wetlands and wetland associated habitat provide appropriate dwelling places for these organisms. Out of 1340 species of the Indian subcontinent more than 577 species have been reported from Maharashtra State. In Vidarbha, a total of 417 species have been reported and overall Amravati district has 392 birds, species (Anon 2009, Kasambe 2016, Wadatkar et al., 2016, Praveen et al 2022).

Wagh and Tiwari, (2019) Wagh et al (2020) have studied the diversity and abundance of avian fauna in MIDC area of Amravati, similarly (Puri et al., 2020) surveyed water birds from Bodalkasa, Chorkhamara and Khairbandha Lakes of Gondia district, Maharashtra state (India). The diversity of birds in Simbhora Reservoir, Morshi, Amravati has been studied by Fule (2017). Simbhora reservoir inhabits several local and migratory bird. The biodiversity in the wetland is not studied in depth. Comparative studies of avian community composition in different habitats including wetlands, wetland associated habitat, forests, grasslands and even in urban and sub-urban areas can improve our knowledge of general patterns and processes that characterize bird species and communities.

Birds that depend on wetland and wetland associated vegetation have experienced a greater decline than any other habitats. Habitat loss and degradation of winter foraging and breeding ground were observed as leading causes of this decline. (Mankadan 2014, West, 2016, Johnson et al., 2019). As this Wardha reservoir is one of the largest one, supporting many bird species, the present study been made for the documentation of diversity, species richness, abundance and evenness wetland birds and wetland associated birds to know the present status of avian fauna.

MATERIAL AND METHODS

The present study was carried out from January 2022 to May 2022 in the Upper Wardha reservoir, Morshi. Five stations were selected in the study area and data were recorded from each station (Dam gate site, Fishing area near Nariman point, ITI Morshi backside, Durgwada village site

and MIDC Morshi site). Monthly four visits were made at each station (Table 1).

Upper Wardha reservoir is an open water body that holds tremendous potential for optimizing fish production in the country. Upper Wardha reservoir, also known as 'Nal Damayanti Sagar,' is constructed on the River Wardha, a tributary of the Godavari River, near the Simbhora village in Morshi taluka of Amravati district, Maharashtra. Although being one of the large reservoirs of Maharashtra (Total area at FRL 9748 ha; latitude-longitude at 21.2764° N and 78.0572° E) supporting the livelihood of thousands of villagers residing around the reservoir (noted during primary field data collection). It can be classified as a lacustrine wetland. It has periodically open mudflats and serves as the best foraging and breeding ground for residents as well as migratory wetland birds, (Fig.1).

Figure 1: Map showing the study area (Upper Wardha Reservoir) Amravati M.S.
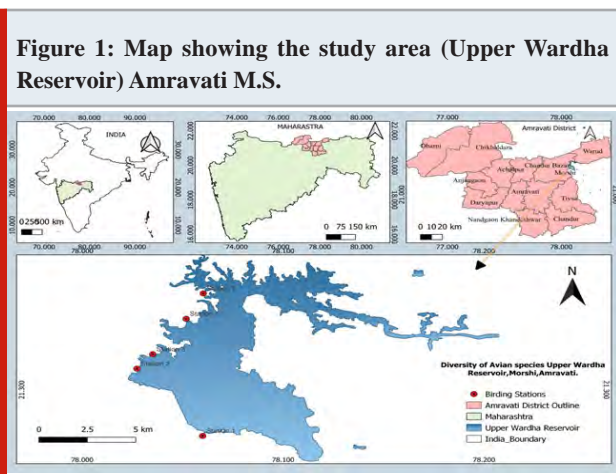
Table 1. The Co-ordinates of Study area.

Station Numbers	Birding Sites	Coordinates	
		Latitude (N)	Longitude (E)
Station 1	Dam gate site	21.272310°	78.059963°
Station 2	Fishing area near Nariman point	21.309220°	78.024225°
Station 3	ITI Morshi backside	21.316585°	78.028042°
Station 4	Durgwada village site	21.342680°	78.051739°
Station 5	MIDC site	21.357937°	78.060284°

Birds Survey and Identification: Point Count and Line Transects methods were used to study avian diversity in wetland and wetland associated habitat (periphery of the reservoir). This methodology also helps to determine their abundance in a given research area. Potential sites were selected. Observations were made during 6:30 am to 10:00 am and 5:00 pm to 6:30 pm. At each site birds were counted using a binocular. Birds were identified and listed with the help of available resources, books and checklist Ali and Ripley (1987), Grimmet et al., (2000). Rasmussen, and Anderton, (2012), (Sangha, 2021) and

book of Wetland and water birds of Amravati district (Wagh 2019). Observation were done using Nikon 8-16x40 mm Binocular. Photographic observations were taken by using Nikon D90, D5600 DSLR Camera with 70-300mm and 80-400mm Zoom lenses and 18-105mm normal lens. A Garmin TM 60 geographic positioning system (GSP) was used to mark each coordinates point of the study area.

Statistical Analysis: Biological diversity indices were calculated to compare studied sites. Various species diversity indices including Shannon–Wiener species diversity index (H), and Simpson index (D) were calculated (considering January to May for monitoring). The diversity indices were calculated and compared for both the habitats. Equally, the number of species, families, and abundance were calculated and compared through one-way ANOVA (Kruskal-Wallis Test) all analysis were performed using Software PAST version 4.03.

RESULT AND DISCUSSION

In the present study total 151 bird species were recorded from the study area. Out of which, 84 species (n = 795; belonging to 20 families) of wetland birds and 67 species (n = 915; belonging to 22 families) of wetland associated birds were recorded. The study area is richly diversified with patches of wetland associated habitat and wetland all over the Upper Wardha reservoir (Table 2&3). In the wetland habitat, maximum 16 Species from Anatidae family and in the wetland associated habitat, maximum 08 Species from Accipitridae family were recorded (Fig 2 and Fig. 3). Recorded birds species were also categorized as per the recent IUCN's list of threatened species (Bird life 2020) Table:5 and Fig.5.

Data Analysis: The diversity indices, species evenness, relative density, species abundance were studied. For the wetland birds diversity, Simpson Diversity Index was 0.9539, Shannon Diversity Index was 3.775, relative density was 10.56 and evenness was 0.519. Similarly in wetland associated area Simpson Diversity Index was 0.9654, Shannon Diversity Index was 3.761, relative density was 07.322 and evenness was 0.6416. Whereas abundance on the Upper Wardha reservoir was 1710 (Table 4). Kruskal-Walli's test for equal medians H (chi2): 11.16 Hc (tie corrected): 11.24 p (same):0.0008014. There was a significant difference in the avian diversity between wetland habitat and wetland associated habitat in the Upper Wardha Reservoir. (Fig.4).

R- Widespread Resident, W- Widespread Winter Visitor, PV- Passage visitors, RM- Resident Migrant, BM- Breeding Migrant, V- Vagrant or irregular visitors.

IUCN's list of Threatened species (2020), categorized as Least Concerned (LC), Near Threatened (NT) and Vulnerable (VU).

R- Widespread Resident, W- Widespread Winter Visitor, PV- Passage visitors, RM- Resident Migrant, BM- Breeding Migrant, V- Vagrant or irregular visitors.

IUCN's list of Threatened species (2020), categorized as Least Concerned (LC), Near Threatened (NT) and Vulnerable (VU).

In this study, probably for the first time, we have reported Greater white-fronted goose from the study area and it was a significant record for the Amravati district of Maharashtra state. The study site serves as suitable habitat for diversity and species richness of avian fauna (Plate1). As per the IUCN status, in the wetland habitat, out of the total 89 % birds species were least concerned, 8 % were near threatened and 03% were vulnerable species. While in wetland associated habitat, all the recorded avian species were least concerned. The recorded bird's species were also classified according to their migration status.

Table 2. Systematic checklist of Wetland avian fauna recorded from study area

Sr. No.	Common Name	Scientific Name	Family	ST	IUCN Status
1	Lesser Whistling Duck	Dendrocygna javanica	DENDROCYGNDIAE (1)	R	LC
2	Bar-Headed Goose	Anser indicus	ANATIDAE (16)	W	LC
3	Northern Pintail	Anas acuta		W	LC
4	Common Teal	Anas crecca		W	LC
5	Indian Spot -Billed Duck	Anas pocilorhyncha		R	LC
6	Gadwall	Anas strepera		W	LC
7	Garganey	Anas querquedula		W	LC
8	Northern Shoveller	Anas clypeata		W	LC
9	Eurasian Wigeon	Anas penelope		W	LC
10	Ruddy (Brahminy) duck	Tadorna ferruginea		W	LC
11	Comb Duck	Sarkidiornis melanotos		W	LC
12	Red-Crested Pochard	Rhedonessa rufina		W	LC
13	Common Pochard	Aythya ferina		W	VU
14	Ferruginous Pochard	Aythya nyroca		W	NT
15	Tufted Duck	Athya fuligula		W	LC
16	Cotton Pigmy goose	Nettapus coromandelianus		R	LC
17	Greater White-Fronted Goose	Anser albifront		V	LC
18	Common Kingfisher	Alcedo atthis	ALCEDINIDAE (1)	R	LC
19	White-Throated Kingfisher	Halcyon smyrnensis	HALCYONIDAE (1)	R	LC
20	Pied Kingfisher	Ceryle rudis	CERYLIDAE(1)	R	LC
21	Blue-Tailed Bee-eater	Merops philippinus	MEROPIDAE(1)	BM	LC
22	White- breasted Waterhen	Amanrornis phoenicurus	RALLIDAE (4)	R	LC
23	Purple Swamphen	Porphyrio porphyrio		R	LC
24	Common Moorhen	Gallinula chloropus		R	LC
25	Common Coot	Fulica atra		R	LC
26	Black-tailed Godwit	Limosa limosa	SCOLOPACIDAE (13)	W	NT
27	Pintail Snipe	Gallinago stenura		W	LC
28	Common Greenshank	Tringa nebularia		W	LC
29	Spotted Redshank	Tringa Erythropus		W	LC
30	Wood Sandpiper	Tringa glareola		W	LC
31	Green Sandpiper	Tringa Ochropus		W	LC
32	Common Sandpiper	Actitis hypoleucos		W	LC
33	Eurasian Curlew	Numenius orauata		PV	LC
34	Little Stint	Calidris minuta		W	LC
35	Ruff	Calidris pugnax		PW	LC
36	Curlew Sandpiper	Calidris ferruginea		PV	NT
37	Marsh Sandpiper	Tringa stagnatilis		W	LC
38	Dunlin	Calidris alpina		W	LC
39	Pheasant-Tailed Jacana	Hydrophasianus chirurgus	JACANIDAE (2)	R	LC
40	Bronze-winged Jacana	Metopidius indicus		R	LC
41	Indian Stone Curlew	Burhinus indicus	BURHINIDAE (2)	R	LC
42	Great Thick-knee	Esacus recurvirostris		R	LC
43	Black-winged Stilt	Himantopus himantopus	CHARADRIDAE (5)	RM	LC

Table 2 Continue

44	Little-Ringed Plover	*Charadrius dubius*		W	LC
45	Kentish Plover	*Charadrius aleandrinus*		BM	LC
46	Yellow-Watteled Lapwing	*Vanellus malabaricus*		R	LC
47	Red wattled Lapwing	*Vanellus indicus*		R	LC
48	Small Platincole	*Glareola lactea*	GLAREOLIDAE (2)	R	LC
49	Oriental Platincole	*Glareola maldivarum*		BM	LC
50	Brown Headed Gull	*Larus brunnicephalu*	LARIDAE (7)	W	LC
51	Black Headed Gull	*Larus ridibundus*		W	LC
52	Palas's Gull	*Larus ichthyaetus*		W	LC
53	Gull-billed Tern	*Gelochelidon nilotica*		PV	LC
54	River Tern	*Sterna aurantia*		RM	NT
55	Little Tern	*Sterna albifront*		BM	LC
56	Whiskered Tern	*Chlidonias hybridus*		W	LC
57	Little Grebe or (Dabchick)	*Tachybaptus ruficollis*	PODICIPEDIDAE (1)	R	LC
58	Darter	*Anhinga melanogaster*	ANHINGIDAE (1)	R	NT
59	Little Cormorant	*Phalacrocorax niger*	PHALACROCORACIDAE(3)	R	LC
60	Indian Cormorant	*Phalacrocorax fuscicollis*		R	LC
61	Great Cormorant	*Phalacrocorax carbo*		R	LC
62	Little Egret	*Egretta garzetta*	ARDEIDAE(11)	R	LC
63	Great Egret	*Casmerodius albus*		R	LC
64	Intermediate Egret	*Mesophoyx intermedia*		R	LC
65	Cattle Egret	*Bubulcus ibis*		R	LC
66	Grey Heron	*Ardea cinerea*		R	LC
67	Purple Heron	*Ardea purpurea*		R	LC
68	Striated Heron	*Butorides striatus*		R	LC
69	Indian Pond Heron	*Ardeola grayii*		R	LC
70	Cinnamon Bittern	*Ixobrychus cinnamomeus*		R	LC
71	Yellow Bittern	*Ixobrychus sinensis*		R	LC
72	Black Bittern	*Dupetor flavicollis*		R	LC
73	Glossy Ibis	*Plegadis falcinellus*	PHOENICOPTERIDAE(4)	W	LC
74	Black Headed Ibis	*Threskiornis melanocephalus*		R	NT
75	Black Ibis	*Pseudibis papillosa*		R	LC
76	Eurasian Spoonbill	*Platalea leucorodia*		RM	LC
77	Painted Stork	*Myeteria leucocephala*	CICONIDAE (3)	RM	NT
78	Asian Openbill	*Anastomus oscitans*		W	LC
79	Woolly-Necked Stork	*Ciconia episcopus*		R	VU
80	White Wagtail	*Motacilla alba*	PASSERIDAE (5)	W	LC
81	White-browed Wagtail	*Motacilla maderaspatensis*		R	LC
82	Citrine Wagtail	*Motacilla citreola*		W	LC
83	Yellow Wagtail	*Motacilla flava*		W	LC
84	Paddy-field pipit	*Anthus rufulus*		R	LC

R- Widespread Resident, W- Widespread Winter Visitor, PV- Passage visitors, RM- Resident Migrant, BM- Breeding Migrant, V- Vagrant or irregular visitors. IUCN's list of Threatened species (2020), categorized as Least Concerned (LC), Near Threatened (NT) and Vulnerable (VU).

In the wetland habitat, 38 wide spread resident, 34 winter visitors, 4 breeding migrants, 3 passage visitors and 01 vagrant or irregular visitor's species of birds were recorded. In wetland associated habitat, 56 resident species and 10 winter visitor species of birds were recorded (Table 6 & Fig.6). The Upper Wardha reservoir have extensive wetland associated region that supports the large number of birds species including wetland birds, which provides places for feeding and roosting and hence, it is a preferred habitat for various migratory and resident birds.

Birds like Green Bee-eater, Red-vented Bulbul, Indian Robin, Common Myna, House Sparrow, Black Drongo, House Crow, Grey Francolin, Bluethroat were amongst the most sighted species in the wide spread wetland associated area. This area also hosts a wide-ranging duck population. This wetland also hosts a variety of other migrating ducks which migrate from the colder regions like Siberia and Mongolia, Russia for various reasons. Migratory birds recite here for definite period and one of

such very rare fascinating migratory bird is Greater-White Fronted Goose (Anser *albifront*) which visits the Upper Wardha reservoir. Barr headed geese is winter visitor for upper Wardha reservoir.

Table 3. Systematic checklist of Wetland associated Avian Fauna recorded from study area.

Sr. No.	Common Name	Scientific Name	Family	ST	IUCN Status
1	Grey Francolin	Francolinus pondicerianus	PHASIANIDAE (5)	R	LC
2	Common Quil	Coturnix coturnix		W	LC
3	Rain Quail	Coturnix coromandelica		R	LC
4	Painted Bush Quil	Turnix suscitator		R	LC
5	Indian Peafowl	Pavo cristatus		R	LC
6	Yellow-Crowned Woodpecker	Dendrocopos mahrattensis	PICIDAE(3)	R	LC
7	Golden-rumped Flameback	Dinopium benghalense		R	LC
8	Common Flame Black	Dryocopus javensis		R	LC
9	Coppersmith Barbet	Megalaima haemacephala	MEGALAIMIDAE (1)	R	LC
10	Indian Grey Hornbill	Ocyceros birostris	BUCEROTIDAE (1)	R	LC
11	Green Bee-Eater	Merops orientalis	MEROPIDAE(1)	R	LC
12	Common Hawk Cuckoo	Hierococcy varius	CUCULIDAE (2)	R	LC
13	Asian Koel	Eudynamys scolopacea		R	LC
14	Greater Coucal	Centropus (senensis) parroti	CENTROPODIDAE (1)	R	LC
15	Barn Owl	Tyto alba	TYTONIDAE(1)	R	LC
16	Spotted Owlet	Athene brama	STRIGIDAE(2)	R	LC
17	Eurasian Collared Dove	Streptopelia decaocto	COLUMBIDAE (4)	R	LC
18	Red Collared –Dove	Streptopelia tranquebarica		R	LC
19	Spotted Dove	Streptopelia chinensis		R	LC
20	Laughing Dove	Streptopelia senegalensis		R	LC
21	Black-shouldered Kite	Elanus caeruleus	ACCIPITRIDAE (8)	R	LC
22	Black Kite	Milivus migrans		R	LC
23	Shikra	Accipiter badius		R	LC
24	Osprey	Pandion haliaetus		W	LC
25	Montagu's Harrier	Circus pygarguss		W	LC
26	Eurasian Marsh Harrier	Circus aeruginosus		W	LC
27	Pied Harrier	Circus melanoleucos		W	LC
28	Short toed Snake Eagle	Circaetus gallicus		R	LC
29	Common Kestrel	Falco tinnunculus	FALCONIDAE (1)	R	LC
30	Bay-backed Shrike	Lanius vittatus	LANIIDAE (3)	R	LC
31	Long-tailed Shrike	Lanius schach		R	LC
32	Brown Shrike	Lanius cristatus		W	LC
33	House Crow	Corvus splendens	CORVIDAE (5)	R	LC
34	Large Billed (Jungle) Crow	Crow Corvus macrorhynchos		R	LC
35	Common WoodShrike	Tephrodomis pondicerianus		R	LC
36	Small Minivet	Pericrocotus cinnamomeus		R	LC
37	Black Drongo	Dicrurus macrocercus		R	LC
38	Bluethroat	Luscinia svecica	MUSCICAPIDAE (5)	W	LC
39	Orientle Magpie Robin	Copsychus saularis		R	LC
40	Indian Robin	Saxicoloides fulicata		R	LC
41	Common Stonechat	Saxicola torquata		W	LC
42	Pied Bush Chat	Saxicola caprata		R	LC
43	Brahminy Starling	Sturnus pagodarum	STURNIDAE (4)	R	LC
44	Rosy Starling	Sturnus roseus		W	LC
45	Asian Pied Starling	Sturnus contra		R	LC
46	Common Myna	Acridotheres tristis		R	LC
47	Dusky Crag Martin	Hirundo concolor	HIRUNDINIDAE (4)	R	LC

48	Barn Swallow	Hirundo rustica		W	LC
49	Wire tailed Swallow	Hirundo smithii		R	LC
50	Red Rumped Swallow	Hirundo daurica		R	LC
51	Red vented Bulbul	Pycnonotus cafer	PYCNONOTIDAE (1)	R	LC
52	Zitting Cisticola	Cisticola juncidis	CISTICOLIDAE (4)	R	LC
53	Jungle Prinia	Prinia sylvatica		R	LC
54	Ashy Prinia	Prinia socialis		R	LC
55	Plain Prinia	Prinia inornata		R	LC
56	Indian Bush Lark	Mirafra erythroptera	ALAUDIDAE (5)	R	LC
57	Ashy- Crowned Sparrow Lark	Eremopeteri grisea		R	LC
58	Rufous-tailed Lark	Ammomanes phoenicurus		R	LC
59	Greater Short-toed Lark	Calandrella brachydactyla		W	LC
60	Sykes's Lark	Galerida deva		R	LC
61	Purple Sunbird	Nectarinia asiatica	NECTARINIDAE (1)	R	LC
62	House Sparrow	Passer domesticus	PASSERIDAE (6)	R	LC
63	Baya Weaver	Ploceus philippinus		R	LC
64	Red Avadavat	Amandava amandava		R	LC
65	Sliver-bill Munia	Lonchura striata		R	LC
66	Black headed Munia	Lonchura malacca		R	LC
67	Scaly-breasted Munia	Lonchura punctulata		R	LC

R- Widespread Resident, W- Widespread Winter Visitor, PV- Passage visitors, RM- Resident Migrant, BM- Breeding Migrant, V- Vagrant or irregular visitors. IUCN's list of Threatened species (2020), categorized as Least Concerned (LC), Near Threatened (NT) and Vulnerable (VU).

Figure 2: Graph showing family-wise birds species number recorded from wetland habitat.
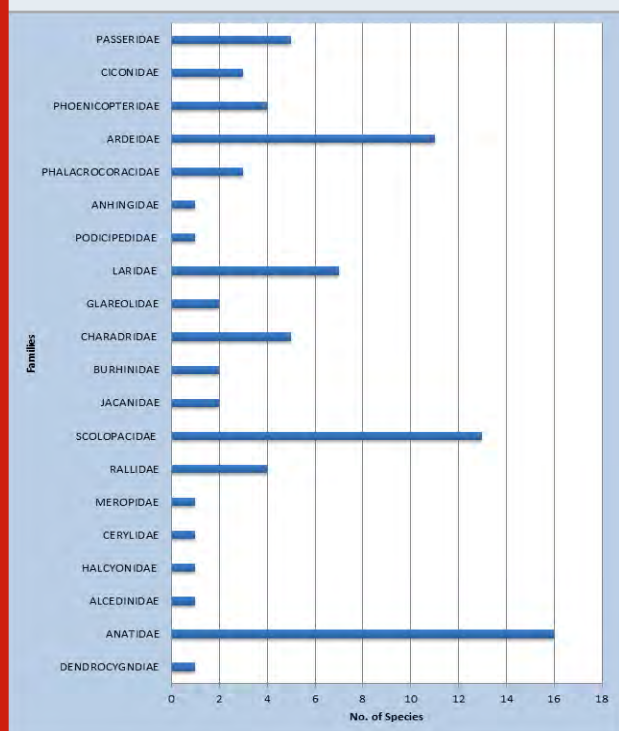
Figure 3: Graph showing family-wise birds species number recorded from wetland associated area.
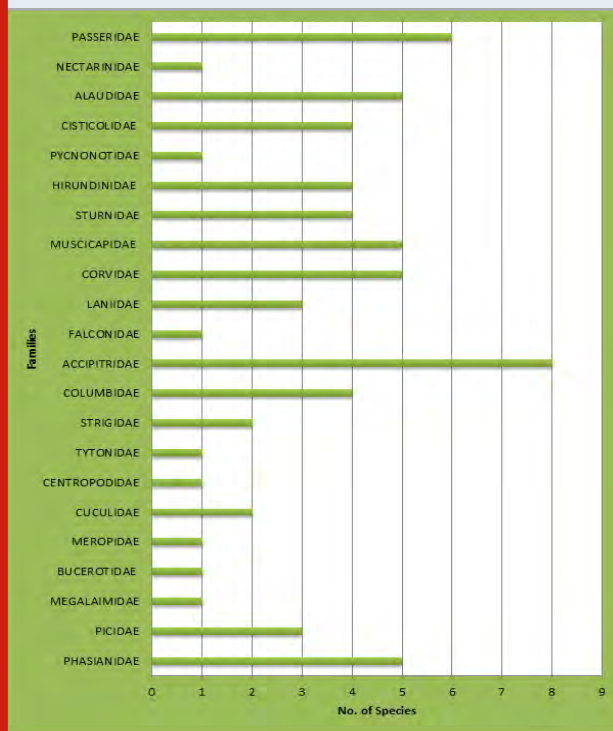
Table 4. Summary of Data Analysis.			
S.N.	Observations	Wetland Bird Species	Wetland Associated Bird Species
1.	Species numbers	84	67
2.	Total Individuals	795	915
3	Simpson Diversity Index [D]	0.9539	0.9654
4	Shannon Diversity Index [H]	3.775	3.761
5	Evenness	0.519	0.6416
6	Relative density	10.56	07.322
7	Fisher alpha	23.72	16.65

Figure 4: Diversity of Avian species in Upper Wardha Reservoir, Morshi, Amravati, Maharashtra.
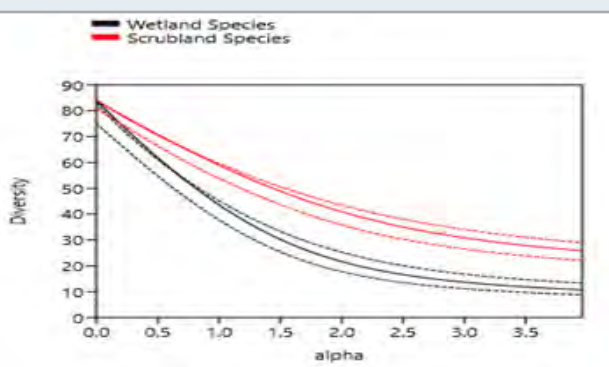
Figure 6: Diagram showing percentage of resident (R) and migratory (M) birds in Study Area.
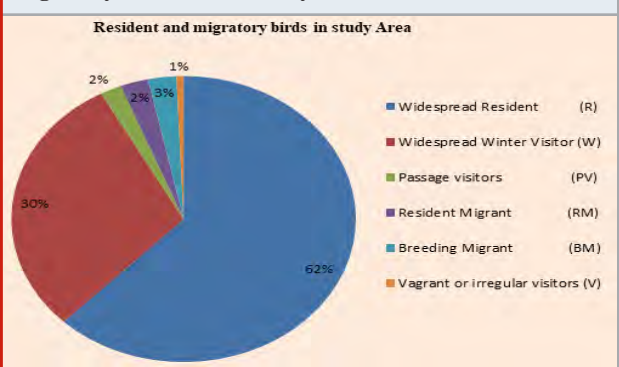
Figure 5: Diagram showing IUCN status of birds in Upper Wardha Reservoir.
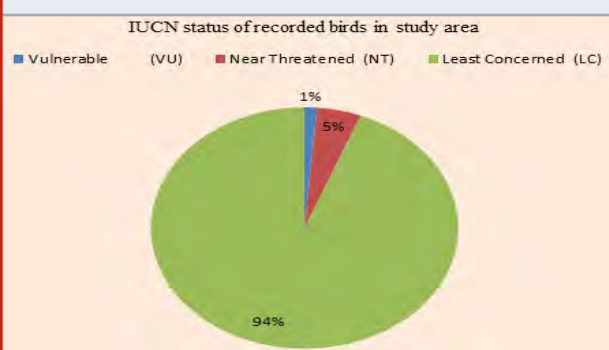
Figure 7: Diagram showing the percentage of in Wetland (Duck, Waders) and Wetland associated birds.
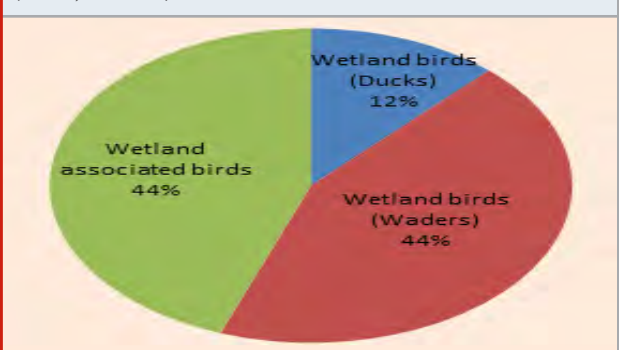
In the Wetland habitat; Storks, Sandpipers, Plovers, Ducks, Water Hens, Gulls, Terns and many other waders were recorded. The wetland patches also host the large number of migratory birds such as Bar-headed Geese which migrate in the study site in large numbers. Apart from these, little waders including Stints, Plovers, Pratincole and Sandpipers also sighted in large numbers (Fig.4).

In the Wetlands habitat, maximum number of bird's species was recorded from the Scolopacidae and Anatidae family which include Snipes, Sandpipers, Shanks, and Stints. Their abundant number was due to the Food and foraging

preference they exhibit. Bar-headed Goose prefers small lush green grasses around the water bodies. Other regular visitors were Painted Stork, Asian Openbill stork and Woolly Necked Stork is a member of the Stork family, which was found in the wetlands, and prefers fishes as a major food item. Hence, their presence in the studied wetlands was justifiable.

While wading across the banks of the water body, these birds constantly search for small fishes, snails, plants and invertebrates. Regular surveys for diversity study and awareness among the people should be conducted for a

real assessment of environmental conditions prevailing in the area. The number of birds recorded during this study period was compared with earlier records (Wagh et.al .2015), and it was found in decreasing trend, particularly the abundance of waders.

Plate 1: Significant Bird Sighting At the Upper Wardha Reservoir, Morshi, Amravati.

Greater White-Fronted Goose (*Anser albifront*)

Bar-Headed Goose (*Anser indicus*)

Grey Lag Goose (*Anser anser*)

Black-Tailed Godwit (*Limosa limosa*)

Oriental Platincole (*Glareola maldivarum*)

Blue-Tailed Bee-eater (*Merops philippinus*)

Osprey (*Pandion haliaetus*)

Dunlin (*Calidris alpina*)

Ruff (*Calidris pugnax*)

Monthly variation was recorded in birds diversity and its was found that avifaunal diversity was more in January, February and March, as there was good water storage as well as open mudflat, availability of abundant food, increased vegetation and the arrival of migratory birds. During the present study, it was observed that this wetland was under serious threats due to widespread network of nylon fishing nets in the dam area, extensive fishing, cultivation and anthropogenic activities. These threats severely affecting the bird's population and their existence. Various factors like grazing by domestic animals, disturbance by stray dogs, and excessive fishing activities through wetland patches forces the birds species to choose another area.

To mitigate these issues, there is an urgent need of patrolling in the more sensitive areas, particularly during the breeding season. For the long-term management of this water body, there is a urgent need to spread awareness among the local peoples about these issues. So that they can also contribute for the conservation and management activities in collaboration with Government authorities, local administration and various NGOs. The present study provides the baseline data of Avian diversity at Upper Wardha Reservoir Morshi and this data will help for various conservation and management activities in near future.

CONCLUSION

In the present study, 151 bird's species (84 species from wetland and 67 species from wetland associated) were recorded. The observation shows that this water body supports a large number of birds species. This water body has been a main source of water for recharging the surrounding wells, bore wells and agriculture fields and also supply water to industries of MIDC, Amravati and also to the people of Amravati area. Large number of cattle graze and inhabit at the mudflats of the dam, due to this number of eggs and nests of the birds get destroyed. These threats along with some others, severely affected bird's population. Mitigation of various threats is urgently needed and for this combined efforts from the concerned departments of the government, local people, volunteers and NGOs are needed. The present study has documented the avian fauna of Upper Wardha reservoir which will help in further bird diversity research, management and conservation.

ACKNOWLEDGEMENTS

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Biotechnological Communication

Quantum Secure Communication: A Comprehensive Literary Analysis with In-Depth Insights

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ABSTRACT

This article helps us to understand the world with quantum secure communication. In this quantum landscape, the classical barriers of secure communication are transcended, and a brand-new era of impenetrable information safety emerges. The laws of quantum mechanics will be helpful in quantum secure communication for data transmission. It highlights the inherent security blessings conferred by using quantum properties, including the no-cloning theorem and the uncertainty precept, rendering eavesdropping impossible. In addition, this article gives us an understanding of how encryption can be done using quantum computing techniques compared to the classical ones. This article also outlines the challenges and ongoing research within quantum secure communication, addressing problems like sensible implementation, community scalability, and quantum key management. It underscores the collaborative efforts of researchers, industries, and governments in advancing quantum cryptography.

KEY WORDS: QUANTUM SECURE COMMUNICATION, QUANTUM KEY DISTRIBUTION, QUANTUM CRYPTOGRAPHY, INFORMATION SECURITY, QUANTUM COMPUTING, DATA PROTECTION, POST – QUANTUM CRYPTOGRAPHY.

INTRODUCTION

Imagine this 20 Years from now: all your bank account passwords, your messages, Photos, your browsing history, and finally, all the information that defines your societal status can be accessed by anyone with only a few seconds of computing. Scary right? Well, it is true, it is possible through Quantum Computing. If it is that dangerous, why should we even develop it? Here is an example of quantum computing. John is in a maze searching for an exit; he needs to find the route to the exit. He goes through a route and determines whether that route leads to the exit or not, and then he tries another route like that he goes on until he finds a route to the exit. In this time-consuming process, John thinks, what if someone like him searches another route while he is searching in his route? Well, the quantum computer does the same. Simply, it searches for the number of routes that lead to exit in different dimensions simultaneously and gets the shortest path to it.

This is possible because of the Qubits and their properties, such as superposition, entanglement, quantum sifting, and some dedicated search algorithms. After reading this, we get a question: well, a faster solution, so is it worth the risk of losing all kinds of encryption? Shouldn't we abort it like a failed experiment or potential dangers? We are already reaching speeds that are less than milliseconds. Here, speed is a by-product; its real value is how to solve and handle more complex problems like How complex? Experts expect solutions for, quote, "The birth of the universe and its mysteries," it is expected to solve other real-world problems that are more complex for classical computers. Coming to security, there is genuine concern about data privacy and communications, so why don't we use the same technology to solve this problem? Quantum Secure Communication Came into the thoughts and is actively being implemented globally (Alhayani et al., 2023).

Quantum Key Distribution (QKD): Quantum Key Distribution (QKD) is a technique for establishing secure communication channels. This can be done by exchanging keys, which are in encrypted format, between the sender and receiver. This method uses the principles of quantum physics, which can help us achieve security measures that can withstand the threats from quantum computing. Mechanics Behind QKD: QKD ensures secure transmissions by sending packets of quantum particles, commonly known as photons, through fiber optic cables (Cao et al., 2022). Each photon carries a random quantum state or qubit, representing a binary digit 0 or 1. These qubits are measured at the recipient's end, forming a line-up of bits or sequences. This series of bits then becomes the key for encrypting and deciphering messages. Any attempts made by external sources to meddle with or monitor the transmission can be detected due to intrinsic attributes governed by quantum mechanics.

Types of QKD Protocols: QKD protocols fall into two primary classifications: prepare-and-measure and entanglement-based. The former involves measuring the states of a particle. At the same time, the latter depends on an exclusive quantum entanglement phenomenon where measurement actions performed on one particle have consequential effects on another particle's behavior. These protocols ensure thwarting unauthorized access attempts, thereby securing communications effectively.

Challenges and How It is Accomplished: Quantum Key Distribution (QKD) confronts issues, including merging into present infrastructure, restricted transmission ranges for quantum photons (typically 100 kilometers), and the necessity for an initially protected communication path. Regardless, QKD is gaining importance as a safeguard against quantum computers, especially in view of the imminent threats they pose. Real-world QKD measures have discovered weaknesses such as phase remapping and photon number splitting attacks. To combat such risks, decoy state QKD protocols were developed to intensify security.

Evolution and Future of QKD: The roots of QKD go back to the 1970s when Stephen Wiesner started the idea of quantum cryptology. Charles H. Bennett later released the BB84 protocol, which became the cornerstone for quantum cryptology relying on non-parallel states. In 1990, Artur Ekert made a noteworthy contribution to QKD by investigating quantum entanglement as a bedrock for secure communication. The outlook for QKD presents a favorable future. Gradients such as the Quantum-Safe Security Working Group (QSSWG) endeavor to endorse the acceptance of QKD alongside other quantum-safe technologies to combat emerging threats ushered in by quantum computing. Researchers are engrossed in augmenting data velocities and extending distances for deploying QKD. Companies have also delved into the QKD space with commercially available systems on offer.

Quantum Repeaters
Illuminating Pathways towards Quantum Communication Network: The prospect of a paradigm-shifting world with exceptional security in communication, advanced AI systems, and state-of-the-art medical imaging lies within sight with the advent of the quantum internet. However, crafting a global reach depends heavily on transmitting quantum bits or qubits across substantial stretches, which brings us face-to-face with an issue requiring resolution – introducing Quantum repeaters into the mix. Distinguishing Quantum Repeaters from Classical Counterparts: In classical cyberspace, information is conveyed as binary codes via optical fibers, with repeaters performing the essential task of dealing with signal loss caused by weak intensity. The traditional repeaters receive incoming signals, amplify them, and transmit them at higher power levels (Wallnöfer et al., 2022).

The Challenge of Loss in Quantum Networks: Quantum networks encounter a similar dilemma regarding signal degradation due to feeble intensity levels. However, conventional repeater techniques cannot be employed in quantum transmissions because of an intrinsic aspect of quantum information: the no-cloning theorem. Unlike standard data bits, quantum details cannot be replicated or measured without modifying their state. This unique characteristic inherent within quantum data enhances its unmatched resistance against hackers' spying activities. However, it simultaneously generates problems compensating for weakened signals within quantum links. Unleashing the Power of Entanglement Swapping: The primary objective of quantum networks revolves around disseminating entangled states across their members. Distributing entanglement unlocks a host of applications, such as qubit teleportation. Entanglement swapping is a nifty technique that cleverly mitigates signal loss without violating the no-cloning principle.

Teleportation & A Vital Player: If repeaters possess qubits entangled with pairs at Alice and Bob's ends, they can conduct measurements and transmit. The essential data to establish the newly entangled connection. By constructing a chain of repeaters, the project of spanning lengthy distances is damaged down into practicable segments for photon transmission. Experiments and Milestones: Quantum teleportation among nodes has been experimentally established via various studies and organizations in various scenarios, consisting of free-area hyperlinks over vast distances and ground-to-satellite uplinks. Although there have been successful quantum teleportation experiments, building realistic quantum repeaters gives distinct demanding situations. Notably, quantum repeaters must be tailor-made to paintings inside the limitations of modern-day quantum gadgets.

The Evolution of Quantum Repeaters: 1st Generation Repeaters: These preliminary repeaters depend on quantum processors, which might be inherently blunders inclined. First-generation repeaters appoint entanglement distillation to catch up on errors, 1st generation repeaters appoint entanglement distillation, where remarkable entanglement is distilled from several low-excellent copies. While these repeaters permit groundbreaking applications, their communique charge is restricted through the distillation procedure.

2nd Generation Repeaters: As mistakes charge decrease, second era repeaters transition from entanglement distillation to quantum error correction, which handles operational mistakes through encoding records into blocks of qubits. This transition helps extensively faster facts switch and unlocks additional programs. 3rd Generation Repeaters: With similar upgrades in quantum gadgets, third-generation repeaters hire quantum blunder correction to manage loss and operational errors. Then, it ensures that the information in the nodes will secure its spot irrespective of verification of the established order of entanglement. Future Vision: Quantum networks are already in improvement, with projects like the Center for Quantum Networks at the University of Arizona pioneering the development of quantum networks equipped with completely error-corrected quantum connectivity, made viable through quantum repeaters. Comparable endeavors are underway globally at universities and country-wide laboratories.

Post-Quantum Cryptography: Post-quantum cryptography is one of the departments of cryptography for developing algorithms on encryption and protocols, which will even be applicable in quantum computing. So, developing these algorithms has become vital to maintaining protection and safety in digital communication (Joseph et al., 2022).

Several households of cryptographic algorithms are considered promising applicants for post-quantum cryptography. These consist of lattice-primarily based cryptography, hash-primarily based cryptography, code-primarily based cryptography, and other tactics.Lattice-Based Cryptography: Lattice-based cryptography is one of the leading contenders for post-quantum cryptography. It depends on lattices, which are grids of points in a multi-dimensional area. Lattice troubles are believed to be complicated even for quantum computers, making lattice-primarily based cryptography a sturdy desire. Some prominent lattice-based cryptographic schemes consist of: NTRU Encrypt and Ring-LWE (Ring Learning with Errors).

Hash-Based Cryptography: Hash-primarily based cryptography is another approach to post-quantum cryptography. It is predicated on the properties of cryptographic hash capabilities, which can be believed to be resistant to quantum assaults. Some hash-primarily based cryptographic schemes include: Merkle-Damgård Construction SPHINCS (SPHINCS-256).Code-Based Cryptography: Code-based totally cryptography is based totally on error-correcting codes and is taken into consideration as a possible option for publish-quantum cryptography. It depends on how complex the decoding of error correction code is. Some code-based cryptographic schemes consist of: McEliece Cryptosystem Niederreiter Cryptosystem.

Other Quantum-Resistant Algorithms: Apart from the households of algorithms, other approaches and cryptographic schemes are being explored for submit-quantum cryptography. These encompass: Multivariate Polynomial Cryptography, LWE-Based Cryptography, Isogeny-Based Cryptography, Code-Based Signature Schemes. The closing intention is to ensure that touchy records stay stable in the era of quantum computing.

Quantum-Secure Communication Networks
Design Principles of Quantum Secure Communication Networks: Quantum Key Distribution (QKD): Since a discussion was already covered on QKD, integrating its security with the fastest communication, like quantum communication, will give us an efficient and secure communication network. End-to-End Encryption: This Quantum Communication is built to ensure a network almost invulnerable to eavesdroppers. Quantum Repeaters and Relays: These devices work between the sender and the receiver, which handle encryption and decryption of the data based on the quantum keys of the sender and the receiver. Authentication and Authorization: The quantum network implements strong authentication and authorization mechanisms to ensure that only authorized people access the data. Quantum Secure Hardware and Protocols: Hardware and Protocols designed to withstand and overcome various quantum attacks are employed .Components including Quantum Nodes, Optical Fiber, and Trusted Repeaters:

Quantum Nodes: There are two significant nodes in Quantum Communication Networks. They are Transmitters and Receivers. Transmitters are responsible for generating and transmitting quantum states like qubits. This also deals with the data encryption using its own Encryption Key. The other node is the Receiver, which handles the receiving and Decryption of data and ensures the safety of the data by measuring the quantum state of the data transmitted by the transmitter. Optical Fibers: These are the communication mediums that are used to transmit quantum states between the quantum nodes. They should be placed and maintained carefully so there will be no signal loss and reduce the effects of the transmission impairment on the data. Trusted Repeaters: Trusted Repeaters can sometimes be called Quantum Nodes. They act as intermediate devices within the quantum communication networks. Purpose Their primary purpose is to enable long-distance quantum-key distribution and quantum state transmission, which was a limitation of the Optical Fibers. Quantum Memory These devices are included with memory devices that can store the quantum states temporarily. This Favors the long-distance QKD.

Quantum Operations These devices can perform operations like entanglement swapping and quantum error correction to maintain the security and integrity of the quantum keys.

Security Since repeaters are intermediate devices, they are built to provide the maximum security possible for quantum states or quantum key distributions. Integration with classical Encryption for added security: Classical Encryption Layer: This layer uses traditional cryptographic algorithms, such as Advanced Encryption Standards (AES) or Rivest, Shamir, and Adleman (RSA). This layer is placed on the top of quantum-secure keys.Quantum Key as One-Tiem Pad: Any quantum key like KAB, KBA, etc., serves as a one-time pad. It means these keys are used only once to encrypt and decrypt a message by the transmitter and the receiver, respectively. This uses a matching one-time pad and key. Data Encryption and Decryption: Consider a

transmitter, Alice, and a receiver, Bob. Alice has a crucial KAB to encrypt and transmit the message. When Bob receives the encrypted message, the same KAB key is used to decrypt the message.

Double-Layer Security: Since we have placed Classical Layer on Quantum encryption, this gives us a two-layered data encryption in which even if a layer is compromised, there will still be another layer that should be compromised. This will enhance the safety of the data. Quantum Key Rotation: The quantum key generated through QKD is used only once, giving them a shorter lifetime duration. Then, another key is rotated between the quantum nodes. Even if a key is compromised, it will no longer be in use (Muralidharan et al., 2016; Bernstein & Lange, 2017; Villoresi, 2010; Nakahara & Sasaki, 2012).

Security Considerations And Threats: Overview of Potential Threats: Quantum Eavesdroppers: The Eavesdroppers are the ones who keep observing the data line silently, and they will have the potential to copy the encrypted data on the data line. They also can decrypt and break classical encryption algorithms like RSA, ECC, etc. Side-Channel Attacks: Whenever there is a vulnerability or a weakness in implementing the quantum system, the attackers will use it as an advantage to exploit the system, such as measuring quantum states and extracting information through unintended channels.Man-in-the-Middle Attacks: Sometimes, even the quantum-secure network is vulnerable to this kind of attack where the attacker tries to impersonate themselves as a quantum node to another quantum node and tries to get data directly from it. He could also intercept or modify quantum states and keys (Bennett & Brassard, 2014).

Discussion on counter measures and mitigation strategies against quantum attacks: Quantum Key Distribution (QKD): One of the best countermeasures for eavesdropping attacks that can be implemented in a quantum-secure communication network is QKD. Where the quantum states are encrypted, when an attacker tries to intercept, then the states are disrupted, which can be easily identified. Post-Quantum Cryptography: Implementing Post-Quantum Cryptographic Algorithms along with QKD ensures resistance against quantum attacks. The classical encryption layer remains secure if the quantum computer becomes a threat.

Security Key Management: Some of them are One-Time Pads and Key Rotation, which provide strict access control on the message and prevent tampering and side-channel attacks. Quantum Repeaters: These devices are used for long-range quantum communications that ensure long-distance secure key distribution and security. Comparison with Classical Security Measures: Security Posture: Quantum Security provides Security against quantum Security, whereas classical Security is vulnerable to quantum attacks. Key Distribution: Classical Security relies on third parties or pre-shared keys for secure key distribution. However, quantum Security does key distribution by itself without relying on other party keys.Practical Implementation: Quantum secure Communication is still evolving, and it might face some

practical challenges in the future, like limited range and specialized hardware. Classical Communication is already well-established and widely implemented.Key Length: Critical lengths of the classical encryption take longer than the quantum encryption. The keys of quantum encryption are shorter in length and more secure due to their unique properties (Scarani et al., 2009).

Quantum-Secure Hardware
Quantum Resistant Hardware components: Quantum-Safe Cryptographic Accelerators: These are the components that help to accelerate the post-quantum cryptographic algorithms. They can efficiently perform complex computations required by quantum-resistant encryption algorithms. Physical Security Measures: The hardware components are specifically built for tamper prevention and protection against side-channel attacks. Random Number Generators: These are used to generate random cryptographic keys. The Quantum-Resistant Random Numbers that are generated are resilient to quantum attacks. Secure Hardware Modules: Hardware Security Modules (HSM) ensure the security of the keys even after the post-quantum threat landscape. They are also known as Secure Enclaves.

Quantum-Secure Communication Devices: Devices like quantum nodes and quantum repeaters are known as quantum-secure communication devices that ensure safe long-distance data transmission in quantum communication networks.

Secure Key Storage: The components of hardware that store the cryptographic keys should be highly secured against quantum attacks. Importance of Secure Hardware in Quantum Communication Networks: Protection Against Quantum Attacks: Now, classical security has become more vulnerable to quantum attacks. So, quantum communications came into play, with more algorithms implemented to ensure secure communication. Tamper Resistant: The Quantum-Communication Hardware is purposefully built to prevent quantum attacks and physical access to sensitive data like cryptographic keys. Preventing Side-Channel Attacks: Communication Hardware uses Double-Layered Encryption Algorithms that help prevent quantum and side-channel attacks. Long-Term Security: The hardware of quantum-communication networks has a longer life span than any other security hardware. They ensure network security even after the advancement of quantum computing technology (Ramakrishnan et al., 2022).

Uses And Implementations
A topic like secure communication in quantum technology has uses and cost efficacies of nearly millions or even billions, but is the cost worth it? Here are a few case studies and practical implementations: OTPs through QKD: using quantum key distribution, we can send the OTPs and verify persons, and their identities will be much more accurate. In the increasing cases of cloning mobile numbers/emails, etc., very important OTPs can be shared using Quantum technology, specifically the more significant transactions or authorization to Accounts that have a heavier influence. Voting through QKD: if this QKD becomes a household

norm, voting from anywhere could be possible since a Quantum secure connection is established once we can verify if there is any tampering or not so the voting process can happen in the home itself, which reduces the burden of going to a voting booth, problems like rigging, lousy influence on voters will be reduced. With a connection made through the QKD path, the voter can generally vote according to their will from the comfort of their home. Since any middleman tries to intervene in the connection, it can be spotted as a disturbance, and the voter can abort the connection and retry again till a safe connection is established.

Energy Sector: A never-ending threat to the energy sector from wrongdoers via MITM attacks or DDOS Attacks, etc these generally happen through a loophole in the communication system like weak passwords of office accounts or human error, accessing unknown links, etc., when the QKD comes into play ultra-sensitive data will be passed in this method hence reducing a significant chance for an attack. Cloud Computing: since there is a proposal of quantum computing through the cloud, if it happens, quantum computers are easily accessible, so most kinds of encryption can be broken.

To prevent this, QKD is beneficial, and by improving human verification methods, authorities can act fast since a trail in the QKD system is unerasable. Supply Chains: with even more security, the ordered goods and payments will be safer and end-to-end encrypted. This QKD in the supply chain can be quoted as "a double-edged sword. "Since the wrongdoers can have secure communication and access to the blockchain, AI, etc., their supply chain will be hard to track, but QKD is only used on a connection basis and not for anonymity purposes authorities with the right level of social engineering tactics can still have the upper hand (due to the wrongdoers are human so they are vulnerable to social engineering).

Satellite Communication: satellites currently use radio waves for long-distance communications, but if a Qubit's entanglement feature is enhanced, it can deliver information more instantaneously and, of course, more security. Defense: The need for a secure route for communication is mainly due to conflicts between nations or high military tensions. We human beings tend to think emotionally, but Defense is a sector where rational thinking is needed, and the highest level of problem-solving is utilized because there is no single solution for everything. Hence, this quantum technology aims to solve real-world problems the human mind cannot solve. Hence, the first step in protecting the nation's peace is to have a secure line of communication in the Defense sector.

IOT (Internet of Things): In the Day and age of IOT devices, quality of life is improved in the same way threats such as accessing the Devices and causing some inconvenience or even accidents. Quantum technology will be beneficial in determining if there is any risk for a device or even some unnoticed logins and attempts to manipulate specific IOT devices. Remote Devices: Remote accessing our Devices from one place to another is an excellent option, but it sometimes comes at the cost of leaking credentials etc. QKD will ensure the remote access is secure, and no tampering occurs during connection time.

There are many implementations such as QKD in Health Care, Data Centres, copyright protection, Networks, Legal Contracts, Auctions, identity verification, and much more; the thing is, with the rapid development of quantum computing, experts are preparing for potential crimes and trying to invent new cryptography methods to encrypt our data there are new types of cryptography to encrypt the end-to-end keys (Sheng et al., 2022; Hasan et al., 2023; Gill et al., 2022).

Beyond Moore's Law And The Potential Developments: According to Moore's Law, computational power doubles every two years, i.e., in specific terms, the number of transistors in an integrated circuit (IC) doubles about every two years. However, as we progress on silicon chips, we are reaching its limit on computational power as per the law of thermodynamics and laws of quantum mechanics; this is going to happen soon as naturally, we tend to look for other alternatives in terms of producing computational power and lo and behold, we have quantum computing, with the power of Qubits there are some drawbacks to it:

Decoherence: is a phenomenon where the qubit loses its superposition due to noise or other factors such as temperature change. AI and Quantum Technology: The deadly combo of Artificial Intelligence and Quantum computing is very dangerous and yields capricious results.

Cryptography: As quantum technology offers speedy results and more computational power, the current encryption could be easily decrypted, governments are trying new cryptographies now, but the major problem that is happening is SNDL (store now, decrypt later) actions, which means the encrypted data that is available today can be stored now, and when the quantum technology is available for the public this stored information with old cryptographies can be decrypted using a quantum computer in future. We predict that quantum technology will be the new normal in businesses and governments. Household quantum computers are a long shot since laypeople do not need that much computational power for their daily tasks. Developments: With this technology, Developments would be secure, i.e., all sorts of possibilities can be calculated faster and made. Since problem-solving happens in complex scenarios, we may find surprising cases of faults and errors and potential new solutions for them—the creation of the universe and predictions such as predicting accurate weather (Gill et al., 2022).

Risks In Quantum Technology: A Big Obstacle for Blockchain Technology: Some of us will have an idea about blockchain technology, which will depend on several algorithms. Blockchain uses algorithms like RSA and EC, which are asymmetric as quantum computing is compelling enough to crack those algorithms, which will result in the difficulties of blockchain. This will become a problem for many companies investing in blockchain technology.

Unknown Security issues: Quantum computing is the upcoming technology that has the power to change the entire digital system. Although it has certain advantages, many issues that cannot be foreseen today will arise. Everything goes differently than planned in this world. So, we need to be prepared for a counterattack. Usage Difficulties: This is one of the most critical risks in quantum computing. We must find where and when we must use it. There will be some areas where there is no necessity for the usage. Analyzing the situation, one can understand which aspect of quantum computing can be fitted. To implement this, companies must use high-end computers in addition to the software. DNN's Complexity: DNN (deep neural network), if a quantum computer is utilized in developing Deep neural networks, it is possible that the Neural Network might exceed Human Comprehension, and supervising it will become tough.

A Queen less Nation's Sorrow: Wars are a never-ending cycle; when a quantum computer is involved in Armies, a nation without a quantum computer is equal to playing chess without a queen. Digital Divide: Like in the real world, the divide between The Rich and The Poor here in the digital era Quantum computer holds the power to flip the tables completely; as usual, the wealthiest will uphold this power, and the poor will be left in the dust (Faruk et al., 2022).

Transparency Issues And Scalability Factor: Transparency issues in Quantum Secure Communication refer to how weak the accessibility and security of the network are. These issues might impact the reliability and trust of the network by the users and stakeholders. Some of the issues are: Complexity of Quantum Technology: Quantum Communication works on complex principles and technologies that are difficult for beginners and non-experts to understand. The users may need help understanding how it works, which might compromise transparency.

Limited Public Awareness: Since quantum communication is a developing concept, it might have limited implementations, leading to a need for more public awareness. Since only a few will be able to know about that, it might lead to skepticism among the public and the users. Opaque Quantum Key Distribution Protocols: Some aspects of QKD protocols are very complex to explain the technical concepts. This might hinder explaining the concept transparently, leading the user to lose trust in the network's security.The scalability factor in quantum communication speaks about the ability of the network to expand its capability to facilitate the increasing number of users, devices, and requests (like data traffic). Not only that, but it should also maintain its level of performance and security even after changing its capacity. Some of the scalability factors are:

Network Size and Range: One day, enormous users might be worldwide. This means the network should be able to cover a larger geographic area that requires scalable Quantum Key Distribution (QKD) technology. Quantum Repeaters: These are the quantum nodes that are used as the intermediate devices for long-distance communication. For this, there have to be multiple quantum repeaters that are capable of providing secure communication over large networks and distances to be deployed. Quantum Resources: Number of users are proportional to the number of resources required. Resources should be maintained in such a way that it serves the users even if there is any unexpected rise in the number of users. The Entangled Photons can be one of the critical resources. Integration with Existing Infrastructure: Quantum Communication is now a widely used network nowadays. So, implementing and integrating them with existing classical infrastructure is very important. Moreover, it has to be done without disturbing the network's security and the capability to serve the larger areas (Ben-Sasson et al., 2018; Lo et al., 2012; Gisin et al., 2002; Qi et al., 2019; Kumar & Garhwal, 2021; Diamanti et al., 2016; Long, 2017; Zhou et al., 2018).

CONCLUSION

It is the year 2023, a long way from the year 1998 when Quantum computing began. Quantum computing is being developed rapidly, and the development of computational power and newer algorithms will continue. We are looking for more and more resources and more experimentation processes to find new possibilities. There might be another way to get more computing power. Who knows, but in this race against computing power, we risk losing current security measures and our data privacy. This is a well-known fact; we experience what happens if we do not act fast and develop countermeasures. Experts are already inventing newer cryptography methods and communications using quantum technology.

It is like the quantum computer was never invented but, at the same time, getting benefits from the computational power of a full-fledged quantum computer. The Key takeaway from this paper is to provide most of the information available on this topic (Quantum Secure Communication) and understand the wonder of science that brought the technology we are using today. However, the cycle of wars, crimes, inventions, beliefs, the good and the bad, Rights, suffering, hunger, food, and Money will relentlessly continue even with all these technologies.

So, why should we develop our technology if there is no change? Quantum technology can solve more and more complex problems like food shortages, wars, crimes, and any tensions that include religion, Money, power, etc. if we humans cannot solve the problems like these. It is up to nature to provide us with the resources and knowledge to solve this. Quantum communication is a step closer to protecting the journey of unraveling the mysteries of humanity and, of course, the Universe.

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Machine Learning in Healthcare Technology: Insights, Challenges, Roles and Applications

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ABSTRACT

In today's ever-changing world, Machine Learning (ML) has become significantly important. It is reshaping what we thought was not going to be possible. Data plays a crucial role in this era. Thus, data is essential in various advanced technologies, like IoT devices, web services, deep learning models, and the expansion of Artificial Intelligence. This paper aims to explore Machine Learning by starting with an overview and then getting into its nature and overall mission. It discusses various factors such as transparency issues, scalability issues, and security issues that come with Machine Learning, also suggesting various techniques to overcome these concerns effectively. Furthermore, this article also highlights real-world examples which describe the impact of Machine Learning across various domains. It also describes the various kinds of learning in ML and the importance of data in ML—the Impact of Machine Learning in Industries: Enhancing Decision-Making Techniques. Along these, this includes applications, advantages, and disadvantages of ML. In conclusion, this article aims to deliver a detailed overview of Machine Learning in today's context and its developments.

KEY WORDS: MACHINE LEARNING, ARTIFICIAL INTELLIGENCE, NLP, COMPUTER VISION, ALGORITHMS, DEEP LEARNING, NEURAL NETWORKS, SCALABILITY, TRANSPARENCY, SECURITY.

INTRODUCTION

Machine learning (ML) is a branch of artificial intelligence that focuses on developing the various algorithms that help computer systems improve their performance. ML is capable of making the machines make use of information and then recognize the patterns. Further, it will be able to take the decisions by itself. ML also includes developing a different number of algorithms through the learning process. The history of machine learning started in the middle of the 20th century when the concept of "artificial intelligence" started. Alan Turing and John von Neumann have established the foundation for decision-making.

Between the 1950s and 1960s, researchers developed the first ML algorithm, including the perceptron, which also helped the study of neural networks. However, the process was stopped in the 1970s due to less access to data. This period has seen the comeback of interest in the 1990s with more advanced algorithms, and researchers have access to large datasets. Since then, machine learning has kept growing, advancing in deep learning, reinforcement learning, and other technologies (Baştanlar & Özuysal, 2014; Badillo et al., 2020).

Machine Learning algorithms are in different forms; they can be in prediction tasks and deep neural networks, which can control extensive data, including images and text. As ML is a quickly developing technology, it will come up with many of the latest improvements in the field of AI and other technologies; it will reshape the way we interact with

machines and overcome many limitations. It is capable of detecting information and making predictions on its own (Kubat & Kubat, 2017; Senders et al., 2018).

Machine Learning is a field that is evolving very rapidly. Future developments in machine learning certainly include more automation and integration with IOT, the creation of NLP models for text and speech analysis, etc. This is a brief overview of Machine Learning.

The objectives of this work are – To explain the mission and vision of machine learning as a discipline.To provide insights into the complexities and difficulties within the field. To explore professionals' diverse roles and challenges in machine learning.To examine issues related to transparency, scalability, and security in machine learning and offer solutions for addressing these concerns. To showcase the wide-ranging applications of machine learning across different industries and domains. To discuss potential future developments and advancements in machine learning.

Components of Machine Learning

- **a. Subset of Artificial Intelligence:** Machine learning is one of the fields of intelligence that creates models that can perform tasks independently without any human instructions. AI has many subfields, and ML is one such field.
- **b. Algorithms and Models:** ML involves creating and using various algorithms and models. These algorithms are designed to learn from data and then make predictions based on that learning. The ML algorithm is the "brain" behind all the processes/tasks.
- **c. Learning from data:** The idea of ML is that the system can learn from data. Here, we do not give any of the user's instructions; ML systems use data to make patterns and relationships. This learning process allows them to think and make predictions about new data.
- **d. Performance Improvement:** Here, the systems are designed in such a way as to improve their performance. This improvement helps itself in different ways, like making more accurate predictions and better recognition of processes.
- **e.Without Clear programming:** In Machine Learning, unlike general software, where developers write the code to perform the task, ML systems do not depend on particular rules or instructions. Instead, they improve themselves based on the data and feedback they receive (Dreossi et al., 2019).

Importance of Data in ML
The importance of data in Machine learning (ML) is very crucial. Data is essential for ML as it plays a crucial role in all aspects of the Machine Learning pipeline. Here, we can clearly understand the importance of data in ML (Jain et al., 2020).

- **a. Training the Models:** ML models highly depend on data to learn patterns and then make predictions based on the data. During the training process, the data is given as input, and their results are taken to retrain them repeatedly to make the model efficient. The model will learn based on the input data. The quality of the model can be improved when trained by giving larger datasets as input. So, the model will be able to produce the accurate results.
- **b. Generalization:** ML models are designed to generalize by the number of times they are trained. So, training the model as often as possible will help provide more accurate predictions. The model also learns from various historical data to make the right decisions about the future. If we provide sufficient data during training, the model can provide generalized data, which is appreciated.
- **c. Generating various Features:** The data is crucial in ML mode to generate various features. So, selecting the correct data and performing training more often will produce quality results. Data scientists are often involved in selecting the most relevant data, carefully selecting and modifying it for better results. Thus, this process is very crucial in making the model highly reliable.
- **d. Evaluation of Model:** To verify the quality of the model, it is essential to have the test and training data. This data is used to measure model performance, which includes factors like accuracy, F1 score, etc. So, with reliable test data, it is easier to determine how well the model performs and whether it is ready for real-world deployment.
- **e. Bias & Fairness:** Data quality is essential to find out the issues of abuse and justice in Machine Learning. More data will produce false predictions, which will have a positive impact. So, ensuring that the training data is bias-free is mandatory, which is an essential consideration in ML.
- **f. Continuous Learning:** In most situations, models have to quickly learn and adapt to the latest data, which improves their performance and quality in generating better results.Thus, the Data is a fundamental part of Machine Learning as Machine learning would only be successful now with providing better input/training data.

Advanced Technologies in ML:
- **a. Deep Learning:** Deep learning is a subbranch of ML that will focus on the topics of Deep Neural Networks. These are made to learn from the data and then represent the data automatically. These use various ML algorithms to train the neural networks. Deep neural networks help us in getting various features from the data directly. The best situation where Deep learning is widely used is in image and speech recognition.
- **b. Natural Language Processing:** NLP is also an Artificial Intelligence and ML subbranch. The main focus of NLP is to create interaction between computer and human language. This also includes understanding the input text and regenerating text and speech in human language. NLP depends upon ML algorithms; these primarily work on supervised techniques and Deep learning. ML models, including Recurrent Neural Networks (RNN) and Convolutional Neural Networks (CNN), are very much used to process and understand the language. These are trained on a large amount of input data to improve their performance.
- **c. Computer Vision:** Computer Vision is one of the

other sub-fields of AI and ML that mainly focus on making the machine describe and understand visual information from a more extensive set of input data of images and videos. Computer vision also uses ML algorithms, like deep learning techniques such as Convolutional Neural Networks (CNN), as these models are trained on labeled images, which helps the model to learn various image features and patterns. So, the learned data can be further utilized for various tasks such as image and object detection.

Thus, the latest Machine learning technologies have several applications, including image processing, text-to-speech, and automating tasks. The main link between Deep learning, NLP, and computer vision with ML is that they all use ML algorithms to perform various tasks on Data. Thus, all together, they help each other in creating the world into a more advanced place (Hassanien et al., 2021).

Learning's in Machine Learning: In Machine Learning, the models are designed with the ability to adapt to the data. So, it is essential to learn from data in various ways and choose the best learning method for each situation. Thus, it further helps build complex algorithms, generating accurate results. The learnings in ML are classified into three main categories. They are Supervised Learning, Unsupervised Learning, and Reinforcement learning. We will now clearly understand them and their sub-classifications (Naeini & Prindle, 2018).

Types of Learnings-
- a. Supervised Learning: Supervised Learning is one of the most widely used ML learning methods. The algorithm learns from input data, which is linked to output, so the model will have simultaneous access to both input and output, thus making it simple to learn for the machine. This Learning uses several algorithms, including linear and logistic regression decision trees. Supervised Learning is classified into two types based on their tasks. They are Classification and Regression.
1. **Classification:** The main aim is to classify the data into predefined classes or labels. It is suitable for image recognition, disease diagnosis, and many more.
2. **Regression:** Regression predicts continuous values, such as stocks, gold prices, currency exchange rates, etc. This is achieved by learning the relation between the input data and the numeric values.

Thus, both classification and regression models of supervised Learning are built based on the input data. These have a significant role in achieving discrete and continuous-based results.
- **b. Unsupervised Learning:** Unsupervised Learning differs from supervised Learning because we will train the model using unlabeled data here. The main aim is to find patterns and relationships within the input data. This Learning includes various algorithms like clustering and anomaly Detection. This is widely used in data analysis, feature engineering, and understanding data structure.
- **c. Reinforcement Learning:** Reinforcement Learning

is the other most important Learning in ML. Here, the agent is trained in such a way as to make sequential decisions by interacting with the environment. An agent is like a decision-taker who decides based on the environment. Reinforcement learning algorithms include Q-learning and DQN. This learning technique is used in applications like robotics, automated vehicles, and online games. This Learning is essential in AI, too.

Thus, there are various types of learning in ML. These learnings are done based on the type of model that is being developed. These learnings are attached to various algorithms Machine learning uses, like Supervised Learning, Unsupervised Learning, NLP, anomaly detection, and deep learning algorithms.

Now, we will look at a few of the most important algorithms used in machine learning.

Types of Machine learning Algorithms
- **a. Supervised Learning Algorithms:** In this algorithm, they learn from labeled data. The best algorithm under supervised Learning is Linear regression. These are developed by taking the human brain as a referential model.
- **b. Unsupervised Learning Algorithms:** These algorithms learn from unlabeled data. This always aims to find hidden/old patterns and the latest requirements. In unsupervised learning, Principal Component Analysis (PCA) helps us to reduce.
- **c. Reinforcement Learning Algorithms:** Reinforcement Learning Algorithms focus on training the agents. These algorithms are mainly used in AI agents, which is crucial during decision-making. These works are based on the principle of trial and error.
- **d. Anomaly Detection Algorithms:** These algorithms find very rare or uncommon information. The Isolation Forest algorithm freezes the anomalies by separating data into smaller subsets.
- **e. Time Series Forecasting Algorithms:** These algorithms are designed specially; these are capable of estimating future values based on the past. Autoregressive Integrated Moving Average (ARIMA) is effective for forecasting trends.
- **f. Dimensionality Reduction Algorithms:** These algorithms reduce the number of features in a dataset, making it more manageable. Singular Value Decomposition (SVD) is helpful for matrix factorization, and Isomap will enhance geodesic distances to uncover underlying structures in data.
- **g. Ensemble Learning Algorithms:** Ensemble learning combines various numbers of models to improve the predictive performance. Boosting manages the weights of data points, which is unnecessary; it also focuses on complex examples when the stacking combines the diverse models to create a more robust predictor.
- **h. Deep Learning Architectures:** Deep learning controls the deep neural networks for complex tasks. Convolutional Neural Networks (CNNs) help us analyze the image and can also be recognized using convolution layers. Whereas Recurrent Neural Networks (RNNs)

can catch sequential information, making them most suitable for tasks like text generation.

Thus, these are various algorithms used by Machine Learning. Models select the required algorithm according to task requirements. Selecting the suitable algorithm ensures us with the most efficient result. The whole ML concept runs with these algorithms (Sarker, 2021).

Machine Learning – Frameworks and Libraries:
Machine Learning has a large number of tools as well as libraries that help us in developing the models. These built-in tools and libraries will reduce task time and difficulty. Here are a few of the most widely used tools and libraries of ML (Nguyen et al., 2019).

a. TensorFlow:
- TensorFlow is one of the most popular frameworks of ML, which Google developed. The main task is to produce high-level and low-level APIs.
- This is considered very flexible for deep neural networks and to deploy the created models in various environments.

b. PyTorch:
- This open-source framework was developed by Facebook's AI Research Lab (FAIR). Researchers widely use this for their research works.
- It is known for its flexibility and ease of use ways. This framework truly helps us in reducing the complexity of tasks.

c. Keras:
- Keras is another open-source API. It shows high-end functionalities.
- It is specially designed to connect with TensorFlow and also with other backends.
- It is simple to use and most suitable for beginners.

d. Caffe:
- The Berkeley Vision and Learning Center (BVLC) developed this deep learning framework. It is known for its efficiency in training deep neural networks very quickly.
- However, the only limitation is that it is less flexible than other frameworks, but it is a potent tool.

e. Chainer:
- Chainer is also a deep learning-based framework. This specialty is that it uses dynamic computation, which is similar to PyTorch.
- It is more widely used in countries like Japan, and the best part of this comes with its flexibility and helps users to perform the necessary tasks very fast.

f. NumPy:
- It is one of the basic libraries of ML; NumPy is a shorthand representation of Numerical Python.
- It is used to perform many mathematical operations, like arrays, matrices, etc., in a much easier and faster manner.
- This library is used in various fields like universities, research centers, etc.

g. Pandas:
- Pandas are other ML libraries mainly used for data wrangling and analysis.
- With the help of pandas, it becomes easier to transform the data and prepare quality datasets.

h. Scikit-Learn (sklearn):
- It is a famous Python-based library for ML. It provides various algorithms for various tasks, including classification and data evaluation.
- It is a lot easier to use and straightforward to work on for the users, making it the most suitable library for data classification.

i. Matplotlib and Seaborn:
- These are the Python-based libraries that are useful for data visualization. Using these tools, we can create various kinds of charts and plots.
- The task becomes more accessible and straightforward when we use Matplotlib and Seaborn. Because they are trained well, they produce very accurate results.

Thus, these are some of the most widely used frameworks and libraries in machine learning. So, using these libraries and tools reduces the difficulty in doing tasks and completes the tasks much faster and easier. The rest of the paper is organized as follows: Section 2 contains the mission and vision of machine learning. Section 3 contains challenges within machine learning. Section 4 deals with various roles within the field of machine learning. Section 5 describes various transparency, scalability, and security issues in machine learning and the suggested methods for tackling those issues. Section 6 consists of applications of machine learning across various fields. Section 7 consists of merits and demerits of machine learning. Section 8 concludes with possible future advancements in machine learning.

Mission And Vision Of Machine Learning: Mission:
Machine Learning (ML) aims to create algorithm models that allow computers to learn from data, and then ML makes predictions or decisions without the need for explicit programming. Its applications include improving data analysis, automation, and decision-making in various tasks. This whole iteration process involves continuous training called fine-tuning. Thus, it reduces most of the errors and will train the ML systems to adapt to the environment and make more accurate predictions. Vision: Machine learning is a technology that is making rapid changes. Also, on the other hand, it aims to develop AI, various algorithms, and models that allow the computer to learn and make decisions independently. The vision of machine learning is to create intelligent systems that can perform tasks other than what is being performed at present. Its vision includes:

- **a. Automating Tasks:** Machine learning can automatically perform tasks using algorithms. However, making them more efficient and dependable is essential to use them in the long run. Let us consider the automatic vehicles; though they are proposed, they still need to be allowed to be used in a complete run as this requires much more training and

testing.

- **b. Adapt and Improving:** ML has to adapt to the latest information because generating older results might make the user feel misguided and lose trust in the model. So, the model has to be trained and improved according to the latest data to be dependable and trusted by users.

- **c. Enhancing the Decision-Making:** It can make predictions and decisions. Improving the model to the latest trends will also make it more robust. Thus, we can use it more accurately for advanced tasks like Disease diagnosis.

- **d. Natural Language Processing:** ML is intensely used in NLP. It can understand human text and speech, an essential requirement for most chatbots, voice assistants, etc. So, enhancing it to newer levels, like making it more interactive and easier to use, will make it more suitable for all age groups.

- **e. Innovation and Research:** Machine learning can analyze more extensive datasets. So, it reduces the burden of scientists and helps them focus on the original innovation instead of analyzing data.

- **f. Environmental and Sustainability Applications:** It is also capable of fetching out the environmental challenges and helping in finding better solutions. It can analyze a large number of problems that are contributing to environmental damage. So, we can fix those problems to protect the environment and sustainability.

- **g. Ethical Thinking:** ML has emerged as an essential technology. We can now make use of it make use of as a trustable model. A model must be unbiased and transparent to say it is better. So, later, we can combine ML with AI to get better results.

- **h. Personalization:** Machine Learning can learn from the data and interactions, making it more powerful. So, using this, it will sense the user interactions on various interests and make a list of preferences, and from then on, it will recommend the user based on his interests. However, the model should also focus on the things that should not stop the user from accessing all the content.Thus, these are the Missions and visions of Machine Learning. These must be carried out to create a world with advanced user experiences, accurate decision-making, and solve complex problems in different fields (Moye, 2019).

Challenges Of Machine Learning

Though ML has rapidly developed, this field has a few challenges (Holzinger et al., 2018). These challenges must be overcome to make ML stand out as a better technology. Here are some of the critical challenges in the ML:

- **a. Data Quality and Quantity:** High-quality data is crucial for better results. In most advanced topics, we might need more accurate and high-quality data, which results in less accurate results. Data preprocessing is vital because it reduces disturbance, noise, and inaccurate data. Thus, ensuring more quality outcomes.

- **b. Overfitting and Underfitting:** This problem is faced when we get excess data or less data than we require for the model. Overfitting is when the model is trained well with

the data but fails to produce new or prediction-based data. Underfitting occurs when the model is straightforward but still shows poor/less performance.

- **c. Model Selection:** Even after having many models, it is essential to select the right and most suitable model according to our task requirements. When we choose a suitable model, it might lead to positive results.

- **d. Bias and Fairness:** Training the model with unbiased datasets is essential to ensure the model is safe. Otherwise, giving ambiguous data might result in unfairness or discrimination, resulting in privacy and unsafe issues for the user.

- **e. Continuous Learning:** Another essential challenge occurs while training the model because the data continuously develops and changes happen. So, ensuring the model is trained continuously to get better results is essential.

- **f. Scalability:** As said above, the machine learning models have to quickly adapt to extensive set data and real-time applications, which is difficult as it needs continuous learning.

- **g. Evaluation Metrics:** Selecting the most suitable evaluation metrics is essential. When we choose the right-suited metrics for the task, it leads to interpretation.

- **h. Security:** Providing security to the users is crucial for any application or model. If the model is not designed using solid algorithms, it might be vulnerable to attacks. So, maintaining security is a crucial challenge.

- **i. Hyperparameter Tuning:** ML always requires hyperparameters, which must be set correctly. However, finding the correct hyperparameters takes time and effort. This turns out to be a big challenge.

- **j. Concept Drift:** It is a phenomenon where the statistical properties of the target variables in machine learning keep changing. So, to keep all of them up to date is a challenging thing. However, the main problem occurs when we do detection, adaptability, mitigation, and labeling.

- **k. Cost and Resource Management:** Managing the cost is complicated in ML projects because it requires lots of cloud storage for data storage. So, managing the cost and available resources is very important to reduce the cost.

- **l. Collaborative Learning:** Collaborative learning in ML refers to protecting the privacy of the developed model. It lets the multiple parties to share the data without letting others know this. It ensures the model's efficiency and also makes the model lot secure.

- **m. Cultural and Organizational Adoption:** This refers to ML and AI's various challenges and processes. It requires lots of support from leaders to get it done. Thus, Cultural and Organizational adoption is mandatory for the smooth going of the tasks.

- **n. Reproducibility and Documentation:** This is another essential aspect of ML for ensuring the transparency and accountability of the model. So, maintaining the kind of data used in the language is vital for further usage. Thus, documentation is essential.Thus, these are the various challenges that occur in Machine Learning. So, managing all the above requirements is very important to overcome these challenges.

Roles Of Machine Learning

Machine Learning (ML) is a subbranch of artificial intelligence that has gained much importance recently. Its ability to enable systems to learn and adapt to various environments without any explicit programming has made it develop a wide range of applications. Since it does not require explicit programming, it can provide many features and capabilities, making it very popular. Now, we will look at the various roles that Machine Learning plays in our modern world (Waqas et al., 2022).

- **a. Data Analysis and Insights:** Machine Learning plays a crucial role in data analysis. It is capable of processing larger datasets and finding patterns and valuable information that might not be possible to do using traditional data analysis methods. ML algorithms can identify correlations, anomalies, etc., essential for decision-making in various fields like finance, healthcare, and marketing.

- **b. Predictive Modelling:** One of the primary goals of Machine Learning is to make correct predictions. ML algorithms are capable of making the right predictions based on historical data. For example, in the financial sector, these models can predict stocks. ML can predict diseases in healthcare, and in e-commerce, it can predict customer needs based on reviews.

- **c. Natural Language Processing (NLP):** Machine Learning algorithms are key for Natural Language Processing (NLP), a field that focuses on making computers understand and generate human languages. NLP is incorporated into chatbots, virtual assistants, and language translation services to make the interaction between humans and computers much more accessible.

- **d. Image and Speech Recognition:** Machine Learning has reshaped image and speech recognition technology. ML models can now describe objects from the given input data, allowing applications like facial recognition and medical image analysis. In speech recognition, ML enables voice-activated assistants and various transcription services.

- **e. Recommendation Systems:** Machine Learning boosts recommendation systems, which have become extremely common in online platforms. These systems can analyze user behavior and preferences to create personalized recommendations, improving user experiences in e-commerce, online streaming services, and social media platforms.

- **f. Healthcare:** In healthcare, Machine Learning helps in disease diagnosis, drug discoveries, and treatment. ML models are also used to analyze medical images to detect disease in early stages, identify suitable drugs for patients, and help doctors make more accurate decisions.

- **g. Autonomous Systems:** Machine Learning is an integral part of developing autonomous systems. Self-driving cars, drones, and robots use ML algorithms, which further help in navigation and make real-time decisions based on training and sensor data. This technology has also got the potential in transportation and logistics.

- **h. Anomaly Detection:** Machine Learning is widely used for anomaly detection. ML models can identify unusual patterns in domains, including network security and cyber threats. Manufacturing (predicting equipment failures), and finance (to detect fraud transactions).

- **i. Environment Conservation:** Machine Learning helps in the conservation of the environment. It can grab data from sensors and satellites, which further helps track and protect endangered species, observe climate changes, and predict natural disasters, contributing to our planet's conservation.

- **j. Personalization:** Machine Learning plays a crucial role in personalization. Online platforms use ML algorithms to deliver content, products, and services to each user based on preferences, which enhances user satisfaction.

- **k. Quality Control in Manufacturing:** Machine Learning plays an essential role in quality control during manufacturing processes. It can identify various problems in real-time by analyzing the sensor data. Thus, it produces the lines, ensuring that only high-quality products are released.

- **l. Dynamic Pricing in E-commerce:** Machine Learning is capable of dynamic pricing strategies in e-commerce. It continuously analyses market conditions, competitor prices, and customer behavior to adjust prices in real time, increasing profit.

- **m. Sentiment Analysis in social media:** Machine Learning is also designed for sentiment analysis on social media and other platforms. It can automatically analyze and categorize public opinions and emotions expressed in posts and comments, providing valuable data for businesses.

Machine learning (ML) has become a part of our world, which impacts various fields and industries. Its knowledge in data analysis, predictive analytics, natural language processing (NLP), image and speech recognition, recommendation systems, healthcare, autonomous systems, anomaly detection, environmental protection, personalization, manufacturing quality control, impact analysis, and social media and many more have changed the way we work in different areas. Machine learning can make valuable information from large data sets, make accurate predictions, and enable human-machine interactions, making it a trustworthy and dependable technology.

Issues In Machine Learning: Machine learning is a powerful tool, but it does face some issues. These issues are classified into three types. They are Transparency issues, Security issues, and scalability issues. Overcoming these issues will further help us to resolve issues and make ML lot dependable.

Transparency Issues in Machine Learning: Transparency Issues are one of the issues in ML (Pynadath et al., 2018). These deal with the awareness of the people who use Machine learning. It refers to how much Machine Learning is open to people for use or practical implementation. Some of the transparency issues are given below.

- **a. Black Box Models:** The name is Black Box, which means that the box or the room we are working in is dark because of its black color. So, we will be unable to see what is inside and how it works. Similarly, ML is being improved by

researchers and scientists to reduce ambiguity and increase understandability.

- **b. Interpretability and Explainability:** Interpretability means the ability to understand, and Explainability means the ability to explain a concept. In Machine Learning, it may be very difficult to explain and understand some algorithms which are very complex. Models like Deep Neural Networks are a few of those cases. Their decision-making is complex to demonstrate.
- **c. Fair and Bias:** Machine Learning makes decisions based on the data it receives and the training data set. The outcomes are biased, based on the data set and data we provide, which might affect the Fairness towards some aspects of situations which is not preferred for a model.
- **d. Data Compliance Regulations:** General Data Protection Regulation (GDPR) issued some rules to avoid and decrease the discriminatory and unfair results of a model that is acquired from a data set or another data model. Its main objective is to increase Fairness and the outcomes of a data model.
- **e. Privacy and Data Protection:** Any user or developer, including the data models, works on the data that has to be protected. Machine Learning must prioritize protecting data from unwanted data access and unwanted attacks on databases.

Security Issues in Machine Learning: Any real-world application must be provided with basic security (Xue et al., 2020). Security is the central aspect of working in the world. Security means being safe and well protected from any unpleasant and unwanted access or theft of the data or resources. Implementation of security and privacy is crucial and also a tricky job. Some of the security issues are given below.
- **a. Adversarial Attacks:** It is a process in which the data that is inputted into the data training model is modified in such a way that the outputs may raise conflicts or they may also increase vulnerabilities of the model.
- **b. Data Poisoning:** It is the same as an Adversarial Attack. However, instead of inputting misleading data, the data model is compromised by poisoning the data set during or before training.
- **c. Model Stealing:** In this process, the crucial information of the data training model is attacked by its essential components due to its weaknesses and vulnerabilities. It is achieved either by attacking the weak points or by reverse engineering.
- **d. Reverse Engineering:** Reverse Engineering is a process in which the essence of a full-fledged model is obtained by analyzing from its outer components but not from its inner logic. The information is obtained with knowledge of its infrastructure.
- **e. Robust Models:** The built Machine Learning Models must be robust and unexposed to stand firm against attacks like Adversarial Attacks, Data Poisoning, etc.
- **f. Privacy-Preserved Machine Learning:** Machine Learning Models must also respect the privacy of the users and developers. There are emerging ideas and implementations like multi-party computations and collaborative working

environments. Here, the model should only expose raw data and crucial information with precautionary measures.

Scalability Issues in Machine Learning: Scalability is the ability of the model to increase and decrease its capability of serving multiple requests at a time (Mohammed et al., 2018). Whenever there is an increase in demand, the model should be able to scale itself by increasing its capacity. And it should be able to decrease the capacity when there is no/less demand. Increasing the capacity when needed will help the model serve multiple requests in high demand. It improves the trust and reliability of users on the model. Moreover, decreasing the capacity when there is no demand helps the model to maintain the resources efficiently and decrease the unnecessary load on the system. Machine Learning depends on training with larger data sets and producing predictions using complex situations and algorithms.

- **a. Magnitude of Data:** Since the data being used for Machine Learning is very large, it keeps growing daily. So, it would be difficult when there is an unexpected rise in the data used to train the model.
- **b. Resources for Computation:** When dealing with larger and more complex models, we come across concepts like Deep Learning that require physical hardware resources such as Graphical Processing Unit (GPU) and Tensor Processing Unit (TPU). So, when there is an increase in the amount of data being used, the hardware must be capable of handling such extensive data and prove necessary resources.
- **c. Real-Time Processing:** Machine Learning models are explicitly made to train on data sets and then produce predictions by performing complex calculations and analysis. So, in case of any increase in the size of the data set, then the calculations should be repeated to gain accurate results. If the size of the data set is increased at a high rate, then it might take more time to produce results.
- **d. Model Deployment:** When multiple devices and users require the data model to get deployed, there is much work and care to do. Increasing the number of use cases will require the deployment of multiple models. If the model is too large and complex to handle, it might become more accessible to finish the job accurately.
- **e. Efficiency of Algorithm:** When there are multiple places to deploy the model, the algorithm implemented in the model should be solid and efficient to handle large requests, users, and data.

Thus, these are vital issues of Machine Learning. Overcoming these problems will make the model much more efficient than now.

Applications Of Machine Learning: Machine Learning is a subject of artificial intelligence (AI) that allows computers to look at data and improve their performance on duties without being explicitly programmed. Over the years, the demand for ML has increased in various industries. This article gives us ML applications in healthcare, finance, advertising and marketing, autonomous vehicles, and natural language processing as shown in figure 1 (Sarker, 2021; Dhall et al., 2020).

Figure 1: Applications of ML

Figure 1: Applications of ML
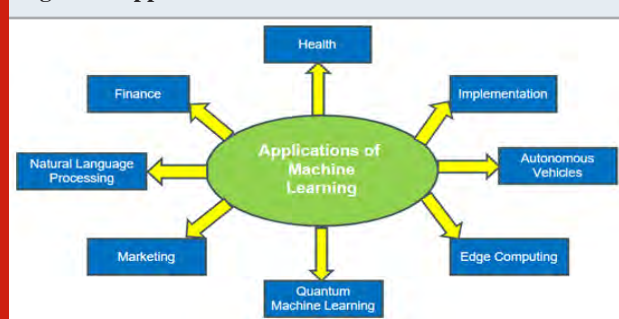
a. Healthcare: Machine Learning has rebuilt the whole healthcare system with the help of enhancing evaluation, remedy, and affected individual care. Some essential packages include:

- **Diagnosis:** ML algorithms can look at scientific records, images, and genetic data to assist in the early analysis of illnesses, which includes most cancers, diabetes, and coronary heart situations.
- **Medicine Discovery:** ML has fastened drug discovery by predicting the requirements of drug candidates, improving molecular interactions, and figuring out novel drug goals.
- **Predictive Analytics:** Healthcare companies use ML to expect affected characteristic outcomes, optimize sanatorium operations, and decrease readmission charges.
- Personalized Care: ML enhances plans to character sufferers, considering their genetics, medical records, and their lifestyle.

b. Finance: In the financial industry, device studying is included for diverse duties, including:

- **Trading:** ML models analyze market statistics to predict buying and selling stocks; it is being trained with more data to expand performance and profits.
- **Management:** Banks and economic establishments use ML for credit score rating hazard assessment, fraud detection, and portfolio manipulation.
- **Service:** Chatbots and digital assistants are a few of the most widely used services of ML. It enhances customer service by addressing inquiries and facilitating transactions.
- **Analysis:** ML is used to make news and social media analysis. Thus, it accesses sentiment, emotions, and many other positive results.
- **Marketing:** ML has also made its mark in advertising strategies. Thus, it also supports various businesses.
- **Customer Segmentation:** ML fashions organization clients primarily based on behavior and alternatives, permitting targeted advertising campaigns.
- **Recommendation Systems:** E-trade systems use ML to suggest products to customers, boosting income.
- **Ad Targeting:** ML algorithms optimize ad placements, growing clicks on on-via charges, and ad effectiveness.
- **Churn Prediction:** Companies count on purchasers to churn the usage of ML, allowing them to take proactive retention

measures.
- **Autonomous Vehicles:** Machine learning plays a crucial role in automatic vehicles for their functioning.
- **Detection:** ML agents understand the people, vehicles, and road surroundings for safe navigation.
- **Planning a route:** Algorithms in ML are meant to be for planning correct routes based on real-time online visiting websites.
- **Maintenance:** ML is useful for early prediction of car renovation needs, reducing problems, and improving safety.

c. Natural Language Processing (NLP): NLP, a subset of ML, has made intense progress in understanding and producing human language. NLP is a subfield of ML that makes a specialist in the interplay between computers and human language. It has several programs beyond what becomes noted in advance. Its applications are:

- **Language Generation:** NLP fashions like GPT-three can generate human-like textual content; this is applied in content advent, chatbots, and even innovative writing.
- **Language Translation:** Services like Google Translate use NLP to offer actual-time translations among languages.
- **Chatbots and Virtual Assistants:** NLP-powered chatbots and digital assistants understand and respond to natural language queries.
- **Sentiment Analysis:** Companies analyze social media and patron opinions to gauge public sentiment.
- **Speech Recognition:** NLP permits voice assistants like Siri and Alexa to understand and respond to spoken language, enhancing character stories in numerous devices and programs.
- **Text Summarization:** ML fashions can summarize lengthy texts or articles, making it more straightforward for customers to digest statistics rapidly, which is treasured in records aggregation and studies.

d. Challenges and Ethical Considerations: Despite its considerable ability, system studying additionally gives worrying conditions and moral troubles. These encompass bias in algorithms, records privacy worries, and the capacity for assignment displacement.

e. Future Trends and Opportunities: The destiny of gadget studying holds promise, with possibilities for growth in areas like reinforcement learning, quantum computing, and explainable AI. Continued research and development are crucial to help with complex conditions and maximize the benefits of ML.

These are a few of the many applications of machine learning. In all the above fields, ML influences tasks directly or indirectly because the users depend on these agents as they complete the work very fast and efficiently.

Advantages & Disadvantages of Machine Learning: Advantages:Machine Learning is an actual trump card when it

comes to Automating tasks with the help of AI; here are a few more advantages of machine learning (Khanzode & Sarode, 2020).

Advantages based on Machine Learning:

- **a. Real-Time Processing:** Let us consider a data set and you want to do some peculiar tasks from that data set. With machine learning, you can do that in real time; it works like magic, giving rapid responses and improvised suggestions in real-time, which will help boost productivity.
- **b. Personalization:** Imagine you have access to an image-based dataset for machine training, a text-based dataset, and a mathematical dataset. The good news is you can tailor your model to your liking. However, the choice of dataset for training and personalization should align with the user's specific interests and needs.
- **c. Scalability:** Suppose you have a large data set; it is possible to train it in machine learning. It is one of the most significant advantages of machine learning. Training a machine with a large data set will benefit because it can perform in any environment.
- **d. Data-based Decision-making:** Consider a machine that is well-trained with data regarding English grammar. When a situation is provided, it can make the right decision or changes based on the data it is trained on; if the data is of good quality, the chances of producing a good result are higher.
- **e. Uphill Improvement:** A machine learning model with more data will generally improve continuous improvement; it will only improve with more training of good data.
- **f. Predictions:** When an ML is based on one bunch of predictable data sets and mathematics, then that machine has attained the capability to predict. For example, if a machine is trained on a bunch of weather data, we can predict the future weather (mostly). Hence, this is one of the most essential advantages of machine learning.
- **g. Recognizing Patterns:** Let us consider a chess game as an example; the first step you take when the match starts are either the movement of the pawn or a knight. There are over 3000 openings, like Sicilian defense, French defense, Scandinavian defense, Italian game, Queen's gambit, King's gambit, etc. These are patterns that commonly occur with the starting move from both opponents in chess, and this trained machine can recognize what pattern it is, and based upon that, it can predict results or make moves if this machine itself is playing.
- **h. Cost Reduction:** A model that is a well-trained machine is significantly better in the manufacturing process than a human; a human can work a fixed amount of hours and need more money to maintain if a machine is trained to do human tasks such as packing boxes, the machine would be more cost-efficient and work 24/7 compared to a human.
- **i. Risk Analysis:** Analyzing the risk once or if we get into the problem is only possible. Let us consider that we want to analyze the risk of investing in a specific company; if an ML model is trained on the data of investments, it can analyze all sorts of risks involved. In this way, we can apply it to the medical scene, transport scene, etc.
- **j. Language translation:** We can train a machine when we

have enough data; we can train a machine that responds in real-time and translates our language to another language; it is also capable of generating text-to-speech results, which helps hundreds of users to do their tasks.

Advantages of ML Based on particular domains:

- **a. Natural Language Processing:** An NLP is a domain where a model is required to understand the language of the person communicating with the machine. For example, a well-developed ML model in NLP can rearrange all the mail from the customer as good and bad reviews so that the organization can efficiently work on the bad ones and provide good feedback and solutions to the customer.
- **b. Autonomous Vehicles:** A machine would make mistakes like humans do. We can observe over 1000 accidents a day; most of these accidents happen due to human error. What if there is a model that is trained on all the traffic rules and patterns and predictions and the dos and don'ts? Thus, the term autonomous vehicles came into focus; we have already seen cars like Tesla, BMW, and Mercedes, which have inbuilt assisted autonomous steering where, on highways, the car can drive itself under the guidance of a human driver.
- **c. Health care:** ML is also trained with medical facts that are proven with evidence. It is beneficial to help the patients. If any personal bias is involved, it can lead to totally biased treatment instead of evidence-based treatments. This might become a severe backlash if the patient's health is affected negatively. So, there are several benefits to having an ML model in health care, like giving a free analysis of a patient's condition, and if the condition is terrible, referring to a doctor visit would be beneficial. If it is a common cold, the machine can suggest treatment options, etc.
- **d. Quality analysis:** When a large number of products are given to a machine with a trained data set, it could figure out the defective pieces; this will be especially useful in pharma manufacturing, where even the slightest mistake could have horrible consequences.
- **e. Agriculture:** This is a domain where weather prediction, soil analysis, and plant condition have to be measured to give accurate instructions to the farmer to take the necessary steps to get a good yield. Even this task can be done using ML algorithms.

Disadvantages: Disadvantages based on Machine Learning:

- **a. Dependent on the dataset:** When a machine is trained on a good-quality data set, it naturally tends to be on the good side, and the same thing is true in the opposite scenario; if a machine is trained on bad-quality data or biased data, it will not be helpful. In terms of analogy, training a machine can be a double-edged sword (Khanzode & Sarode, 2020).
- **b. Storage Safety:** We all know that if a company trains a large-scale ML agent, an enormous data set lies somewhere in a data center. One day, if those data centers are attacked and bad actors gain access to the data set, it will lead to severe consequences. Personal data might be used for the wrong purposes, which causes a loss of user privacy.
- **c. Expensive:** Some ML models with large data sets require

a large amount of computational power, which can only be provided by a few organizations, hence reducing the power of machine learning too little.

- **d. Overfitting:** This is a case where an ML model is trained and tested in its environment. It is fast and responsive, but if a new condition or scenario is given out of its data set as a question, we can see some struggle in its answering capabilities.

- **e. Ethical Concerns:** If the ML model is trained with only hateful content or made for biased purposes and is implemented in robots or hardware, it turns out to be very dangerous. Giving our video captures from recordings or footage to a machine learning model is ethical. So, it can learn ethical constraints in developing a machine learning model.

- **f. Complexity of a Model:** When working with large amounts of data, keeping track of what kind of task is being implemented is essential. It will be complicated if new employees take on the journey of developing an already existing model since keeping track of almost all functions is very difficult, increasing the model's complexity.

Disadvantage Of ML Based on particular domains:

- **a. Legal Domain:** Let us consider a document regarding the case given to the model to verify and generate unbiased judgment; if the model is not trained with enough data required for accurate results, it will surely fail. So, providing and training the model in the best possible way is essential.

- **b. Drug Manufacturing:** The daily routine is going on, and the machines work as usual. Suddenly, a sensor fails or faults, allowing the faulty drug to go by the quality check. If a patient consumes this faulty drug, their health is at risk; these mistakes are hazardous in drug manufacturing.

- **c. Health care:** when a model is trained under only a particular demographic, then that model might produce a wrong judgment, leading to a potential misdiagnosis, which can confuse healthcare workers and even risk the patient's health.

- **d. Autonomous Transport:** we know autonomous transportation is much safer than a human being, but with all the good things, there are some potential harmful elements. If the model is not well updated with the routes and geography, it will probably make a mistake that might risk the lives of passengers.

- **e. Content Moderation:** we are well aware of the recommendations Tab where we will get our daily recommendations; what if an ML model is only trained with single users' data based on a short period of content consumption? It will lead to a content loop where the consumer will tend to get the same repetitive content. Hence, the consumer will not be able to see the available diverse content.

Thus, these are the various advantages and disadvantages of Machine learning. As ML is a fast-growing language, most cons would be identified and corrected. However, with ML's advantages, users trust this advanced technology.

Future Scope Of Machine Learning:

The future of Machine learning will be much more advanced than that of now because it has a smaller number of limitations and is also very effective on most of the tasks (Ghosh & Dasgupta, 2022).

- **a. Safe Healthcare:** Machine learning can profoundly contribute to healthcare areas where manual tasks cannot be performed. Then we will be using these ML and AI-based agents which are going to replace the tasks of humans (doctors in this case)

- **b. Fraud Prevention:** All financial institutions are heading towards ML to let their tasks be done with enhanced security. It develops the trust of users in those organizations. It restricts malpractices from being happening.

- **c. Natural Language Processing (NLP):** This a subfield of artificial intelligence where a machine can generate human-based outputs. To achieve this, models must be trained on large amounts of data. Machine learning will play a significant role in enabling applications such as sentiment analysis models, chatbots, language translation models, etc.

- **d. Job Empowerment:** ML has rapidly grown in various parts of the globe. Thus, it has also produced various job opportunities for the people. So, the ML can produce jobs and complete tasks efficiently. In the future, it is going to get much better when it is enhanced with AI.

- **e. Recognizing Patterns:** Recognizing the patterns is very important for the model. Let us consider a chess game. The first step you take when the match starts is either the movement of the pawn or a knight; there are several different openings in itself. These patterns commonly occur with the starting move from both opponents in chess. These trained machines can recognize what pattern it is and, based on that, can predict results or do moves if this machine is playing.

- **f. Automobile field:** ML is reshaping the concept of driving. As it is being trained in such a way that the car is becoming automated. However, this technology is being implemented now; these must be improved and tested in various environments.

Thus, these are some of the best examples that showcase the importance of ML in the present day and also what field it will focus on in the future.

CONCLUSION

Machine learning has slowly grown more extensive and more significant. Its ability to analyze massive datasets and patterns has reshaped many fields like healthcare, transportation, and various industries. ML has found many new things during this process, like various challenges to adapting to new environments. The most significant success behind ML is its quality and relevance in data analysis. With reliable data, it is easier to produce accurate results predictions. So, in machine learning, the data has a crucial role. Along with the data, its algorithms are designed so that they are secure and robust. The results are always unbiased because they have trained models with real-time data, bringing us better

results. The model is also trained so that it continuously evolves according to the latest conditions and updates.

This machine will play a key role everywhere in the future due to its high performance and very few limitations. Thus, making it more trained and powerful will make it an outstanding technology to depend on for better output generation. Finally, machine learning is not just a technology but a transformative force. Its ability to make decisions according to the environment without bias makes it more dependable for users for various tasks. Joining the ML with advanced technologies like AI will make it more robust and secure.

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Biotechnological Communication

Evaluation of Antioxidant and Antidiabetic Potential of *Acalypha indica* leaf extract: Identification of Bioactive Factors

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ABSTRACT

The present investigation is intended to prepare *Acalypha indica* leaf extract and assess the antioxidant and glucose-lowering effects utilizing in vitro models. The plant methanolic extract of *Acalypha indica* leaves was exposed to phytochemical examination to identify the bioactive factors by GC-MS. We assessed the antioxidant properties of the extract by using the DPPH scavenging method, ABTS assay, TBARS assay and NO assay. Likewise, the anti-diabetic activity was assessed by α-amylase and α-glucosidase enzyme inhibition and glucose diffusion inhibitory techniques. We found that methanolic extract of *Acalypha indica* leaves contains a high measure of phenolics, flavonoids and tannins. The GC-MS assay identified the bioactive components. We also found that methanolic extract of *Acalypha indica* leaves had significant higher antioxidant and glucose-lowering effects. In conclusion, it could be reasoned that due to the nearness of antioxidant components, the methanolic leaf extract of *Acalypha indica* has good potential in the control of hyperglycemia, diabetes and the related state of oxidative stress.

KEY WORDS: ANTIOXIDANTS, DPPH, GC-MS, DIABETES MELLITUS AND FLAVONOIDS.

INTRODUCTION

Diabetes mellitus (DM) is a glucose or insulin associated metabolic disorder that affects the digestion of carbohydrates, fats and proteins. It is a combinatorial disorder accompanied by high blood glucose level (hyperglycemia) and glucose intolerance due to the relative inadequate hypoinsulinemia or growing insulin tolerance in peripheral tissues. This is accompanied by several complications like hyperlipidemia (abnormal state of lipid in the blood), oxidative stress, and advanced enzymatic glycation of proteins. Approximately 450 million adults (20-79 years) are living with DM; by the year 2045 (Galicia-Garcia et al., 2020). this estimate will ascend to 629 million. 79% of adults with DM are living in low and center salary nations, 1 of every 2 (212 million) individuals with DM are undetected, and 352 million individuals are in danger of acquiring type 2 DM. DM is prevalent in most developing nations like India. According to ICMR-INDIAB reports, India has the highest number of diabetic patients in the world and has been notoriously named as the "diabetic capital of the world" (Anjana et al., 2011, Parim 2019, Banday & Nissar 2020 & Sun 2021).

There is substantial evidence that the biochemical and molecular pathways enacted by hyperglycemia are related to the increase in reactive oxygen species (ROS), resulting in increased oxidative stress. Excessive ROS production prompts the initiation of stress sensitive intracellular signaling pathways that, in turn, promote cellular damage and contribute to several complications and disease progression. In recent times, several plant species have been widely characterized for their antioxidant activity. Antioxidants from fragrant, stimulating, therapeutic, and other plants are concentrated to create natural antioxidant formulations for nourishment, anti-diabetic properties, and other applications (Seebaluck 2014, Zahidin et al., 2017, Parim, 2019, Sun 2021).

On the other hand, the number and classes of oral glucose lowering agents in use for the treatment of DM have increased in last couple of years. At present, viable and safe medications are not adequately available, and they have significant adverse side effects. A large number of these drugs have even been pulled back from the market due to undesirable impacts. As a result of these adverse reactions and limitations, there is rising popularity of plant-based formulations. Dietary considerations and nature-based cures can be used as a supplement to local systems of prescription for managing DM more efficiently (Uddandrao et al., 2020).

Acalypha indica Linn is a herb belonging to family Euphorbiaceae. The twigs and leaves of *A. indica* posses flavonoids, catachols, alkaloids, saponins, volatile oil, fatty acids, phenolic compounds and steroids (Ravi et al., 2017, Ninave & Patil 2022). It plays an important role

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in diverse folk medicines including the Ayurveda system and is supposed to boost defense against various diseases. Hence, in this study we made an endeavor to evaluate the ameliorative potentials of *A.indica* and assess their antioxidant and glucose lowering potential (Brahma et al., 2015).

MATERIAL AND METHODS

Sample Collection: Dried *Acalypha indica* Linn leaves were obtained from the botanical garden, Osmania University, Hyderabad in the month of June 2018, their identity was authenticated by a taxonomist Dr L Rasingam, Scientist-E, Botanical Survey of India, Hyderabab, India, voucher number BSI/DRC/22-23 Identification/552 dated 16-10-2022, and a specimen has been preserved at the departmental herbarium. Leaves were shade dried and made into course powder. The powder (2.5kg) was soaked sequentially with hexane, ethylacetate, methanol, and double distilled water each 6 d at room temperature in a 10 L aspirator jar to collect the extracts. These extracts were evaporated by using a rotavapor to obtain respective dry extracts to be used for further studies. The extracts were dissolved in Tween 80 for pharmacological studies (Erum Iqbal, 2015) (Erum et al., 2015).

Preliminary Phytochemical Screening: The methanolic extract of *Acalypha indica* Linn leaves was screened for the presence of various phytoconstituents by the methods described by Mopuri et al (Shafodino et al., 2023). Quantitative Estimation of Phytochemicals: In light of the fundamental phytochemical examination results, further, we exploited the *Acalypha indica* Linn leaves extracts to quantitative estimation of secondary metabolites, for example, total phenolic content (Ninave & Patil 2022), total flavonoids [13] and total tannins (Erum et al., 2015) were measured according to the respective protocols.

GC-MS (Gas Chromatography-Mass Spectrophotometry) Analysis: The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 mL/min. The injector temperature was set at 260°C during the chromatographic run. The 1 μL of extract sample injected into the instrument the oven temperature was as follows: 60°C (2 min); followed by 300°C at the rate of 10°C min−1; and 300°C, where it was held for 6 min. The mass detector conditions were: transfer line tem- perature 240°C; ion source temperature 240°C; and ionization mode electron impact at 70 eV, scan time 0.2 sec and scan interval of 0.1 sec, the fragments from 40 to 600 Da. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) and the compounds were identified (Mopuri et al., 2017).

Evaluation of Antioxidant Activity: The antioxidant ability of *Acalypha indica* leaves extract was measured through 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, ABTS assay, TBARS assay and NO assay activity as per phosphomolybdate assay proposed by

(Mopuri et al., 2017) & (Oyebode et al., 2021).

Assessment of in vitro Glucose-lowering Effects: Inhibition of α-amylase Enzyme Assay: A total of 500 μL of test samples and standard drug (100- 1000μg/mL) were added to 500 μL of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/mL) solution and were incubated at 25°C for 10 min. After these, 500 μL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 mL of 3, 5 dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 mL distilled water and absorbance was measured at 540 nm. Control represented 100% enzyme activity and was conducted in a similar way by replacing extract with vehicle [19]. All assays were carried out in triplicate and values were presented in percentage of inhibition. The activity of α-amylase was determined as pursues (Chekuri et al., 2023):

$$\% \text{ Inhibition} = \frac{\text{The absorbance of control} - \text{Absorbance of the sample treated with the extract}}{\text{Absorbance of control}} \times 100$$

Inhibition of α-glucosidase Enzyme Assay: The inhibitory activity on α-glucosidase was determined by incubating a solution of the starch substrate (2% w/v maltose or sucrose) with 1mL 0.2 M Tris buffer (pH 8.0) containing concentrations of *Acalypha indica* leaves extracts for 5 min at 37°C. The reaction was initiated by adding 1mL of the α-glucosidase enzyme to it, followed by incubation at 37°C for 10 min. Then, the reaction mixture was heated for 2 min in a boiling water bath to stop the reaction. The amount of liberated glucose is measured by the glucose oxidase peroxidase method (Pallapothu et al., 2021). All assays were carried out in triplicate and values were presented in percentage of inhibition.

Glucose Diffusion Inhibitory Study: Glucose diffusion inhibitory study was carried out by using in vitro model consisted of a dialysis tube (6 cm X 15 mm) (Spectra/ Por®, MWCO:2000) into which 6 mL of *Acalypha indica* leaves extracts and 2 mL of 0.15 M NaCl containing 1.65 mM D-glucose were added (Penumala et al., 2017). The dialysis tube was sealed at each end and placed in a centrifuge tube containing 45 mL 0.15 M NaCl. The tubes were shaken occasionally and incubated at 37°C for 3 h. The concentration of glucose within the dialysis tube was measured and control tests were conducted in the absence of test samples. Glucose concentrations were analysed by the DNS method. All tests were carried out in triplicate and the results were presented as means ± SD (Penumala et al., 2017).

Statistical analysis: The experimental results were expressed as mean ± standard deviation (SD) of three replicates. EC50 values (concentration at which 50%

inhibition was achieved) were obtained from the regression plots. Where applicable, the results were treated to a one-way analysis of variance (ANOVA) and the significant difference (p<0.05) between means was determined by the least significant difference (LSD) using Statistical Package for Social Sciences (SPSS) version 15.0 for Windows.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening: Phytochemical screening was performed on all the studied fractions, and the results are given in Table 1. The methanol extract of *Acalypha indica* leaves contained almost all classes of secondary metabolites such as alkaloids, flavonoids, steroids, phenols, carbohydrates, amino acids, proteins, terpenoids, diterpenes, triterpenes, polyphenols, and lipids.

However, saponins were absent in methanol extract of *Acalypha indica* leaves [Table-1].

Quantitative Estimation of Phytochemicals: Table-2 depicts the quantitation of secondary metabolites such as phenolics, flavonoids, and tannins present in the methanol extract of *Acalypha indica* leaves. Compared to other extracts, methanolic extract contains the highest amount of phenolics, flavonoids and tannins. These estimations revealed that the amounts of phenolics and flavonoids were higher than tannins. The other extracts had a considerably good amount of these secondary metabolites.

GC-MS Analysis: GC-MS analysis of the methanol extract of *Acalypha indica* leaves, identified several compounds as shown in Figs. 1, 2, 3 & 4.

Table 1. Phytochemical analysis of *Acalypha india* extracts

S. No	Phytoconstituents	Hexane	Ethyl acetate	Methanol	Ethanol
1	Alkaloids	+	+	+++	+
2	Carbohydrate	+	+	++	+
3	Glycosides	+	+	++	+
4	Phenols	+	+	+++	+
5	Flavanoids	+	+	+++	-
6	Proteins&amino acids	+	+	++	+
7	Steroids	+	+	++	+
8	Tannins	+	+	+++	-
9	Saponins	+	–	+++	-
10	Terpenoids	+	+	+++	+
11	Diterpenes	+	+	++	+
12	Triterpenes	+	+	++	+
13	Polysterols	+	+	++	+
14	Lipids	+	+	+	+

(+ : Present, – : Absent of the particular compound).

Table 2.*Acalypha indica* leaf methanolic extract quantitative phytochemical analysis

S. No	Phytochemical	Quantitative analysis
1	Alkaloids	7.20±0.05
2	Flavonoids	2.18±0.03
3	Glycosides	0.05±0.00
4	Saponins	4.30±0.02
5	Steroids	0.50±0.00
6	Phenols	0.080±0.00
7	Terpenoids	0.40±0.01
8	Anthraquinones	1.40±0.03
9	Taninns	4.80±0.03

Figure 1: GC-MS analysis of the methanol extract of *Acalypha indica* leaves
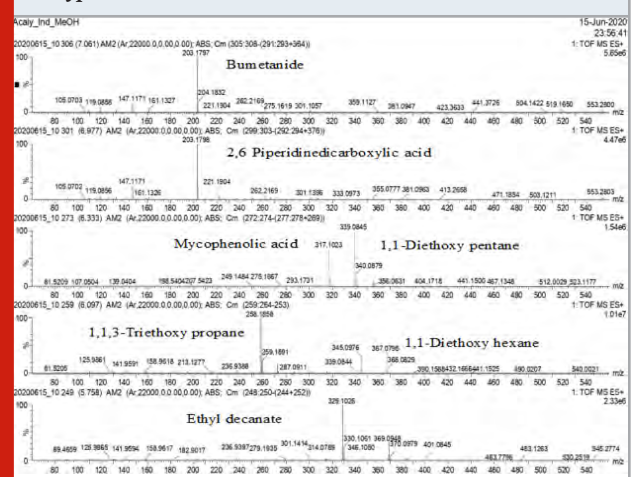
Figure 2: GC-MS analysis of the methanol extract of *Acalypha indica* leaves
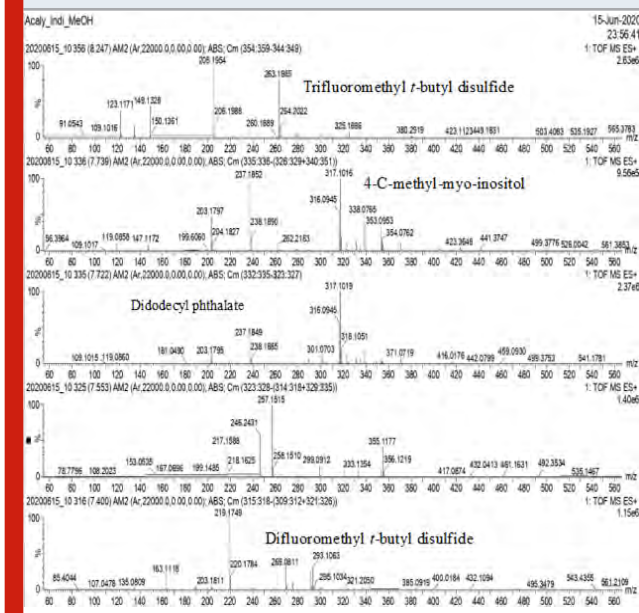

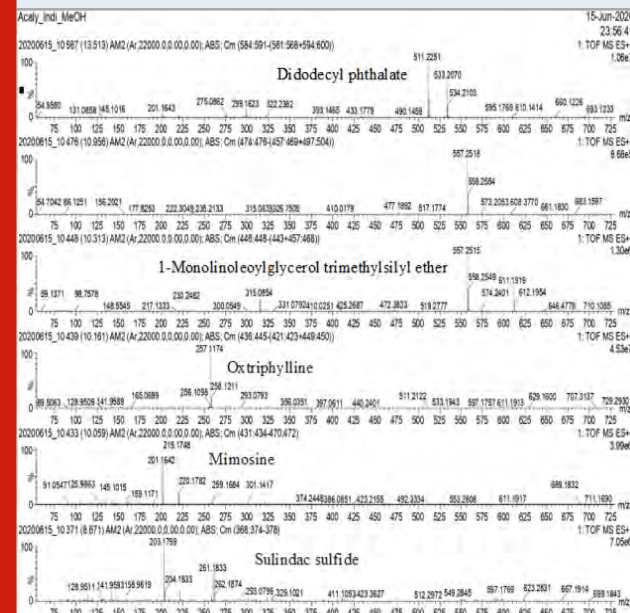**Figure-3: GC-MS analysis of the methanol extract of *Acalypha indica* leaves**

Figure 4: GC-MS analysis of the methanol extract of *Acalypha indica* leaves

concentrations. methanol extract of *Acalypha indica* leaves showed DPPH decomposition activity in a dose dependent manner, measured highest at 25μg/mL. Moderate DPPH scavenging activities were noted with other extracts of *Acalypha indica* leaves but less compared to methanol extract of *Acalypha indica* leaves or ascorbic acid (Erum et al., 2017).
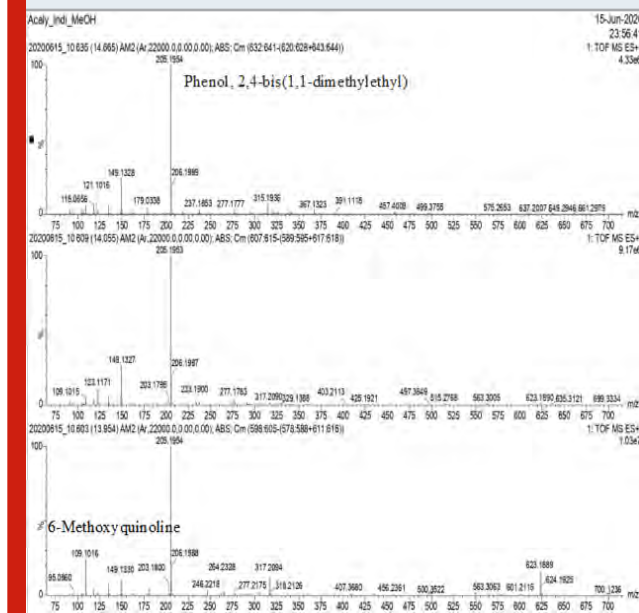
Antioxidant Activity: The DPPH radical scavenging activities of extracts of *Acalypha indica* leaves at different concentrations are shown in Table-3 and Figure-5, and were compared to ascorbic acid as reference. methanol extract of *Acalypha indica* leaves demonstrated the highest DPPH radical scavenging activity at concentrations ranging from 5μg/mL to 25μg/mL, when compared to the other extracts, which showed only moderate activity.

Figure-5 demonstrates the DPPH scavenging activities of extracts of *Acalypha indica* leaves at different

Table 3. DPPH activity MEAL

Concentration (μg/ml)	% Inhibition of DPPH free radical	
	BHA	MEAL
5	72.1± 1.7	66.0 ± 2.1
10	79.6 ± 2.4	73.7 ± 3.5
15	93.3 ± 2.3	78.9 ± 2.6
20	95.6 ± 1.7	84.1 ± 2.3
25	96.9 ± 1.5	89.8 ± 2.5

Figure 5: DPPH activity of MEAL

Figure-6, table-4 and Figure-7 & table-5 depicts the ABTS and NO percentage inhibition using the extracts of *Acalypha indica* leaves at different concentrations. These results clearly indicated that the methanol extract of *Acalypha indica* leaves can potentially inhibit ABTS and NO in a dose-dependent manner.

other hand, the other extracts of *Acalypha indica* leaves did not show significant α-amylase inhibitory activities when compared to methanol extract of *Acalypha indica* leaves and acarbose. A comparison of α-amylase inhibitory activity between the standard drug acarbose and *Acalypha indica* extracts is depicted in Fig. (8).

Table 4. % Inhibition of ABTS radical scavenging activity		
Concentration (µg/ml)	% Inhibition of ABTS radical scavenging activity	
	BHA	MEAL compound
5	70.2 ± 2.9	62.0 ± 232
10	81.3 ± 2.4	68.3 ± 3.5
15	92.5 ± 2.4	72.2 ± 3.7
20	94.7 ± 1.8	77.1 ± 1.3
25	96.1 ± 1.5	82.5± 1.5
25	96.9 ± 1.5	89.8 ± 2.5

Figure 6: % Inhibition of ABTS radical scavenging activity

Figure 7: % Inhibition of Nitric oxide scavenging activity
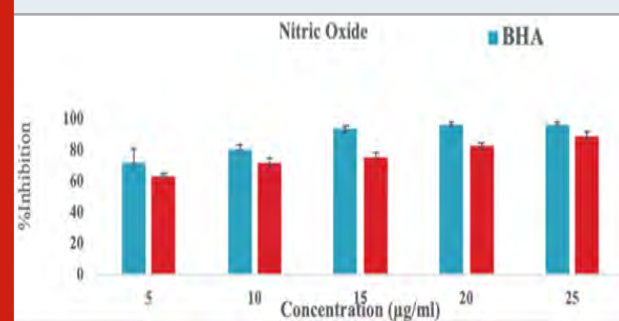
Figure 8: In vitro Alpha-amylase Inhibitory Activity of MEAL

Table 5. % Inhibition of Nitric oxide scavenging activity		
Concentration (µg/ml)	% Inhibition of Nitric oxide scavenging activity	
	BHA	MEAL
5	70.9 ± 9.5	65.0 ± 2.1
10	70.5 ± 2.6	72.2 ± 3.6
15	93.1 ± 2.2	75.9 ± 2.7
20	95.9 ± 1.8	82.1 ± 2.2
25	96.3 ± 1.2	88.4 ± 2.8
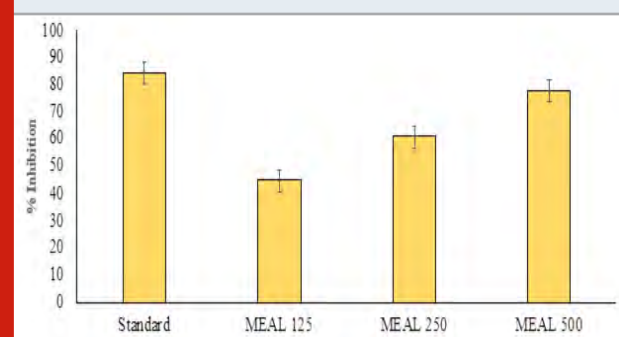
Figure 9: In vitro α-glucosidase Inhibitory Potential Activity of MEAL

In vitro Alpha-amylase Inhibitory Activity: The results of the current study demonstrated a dose dependent increase in percentage inhibitory activity against the α-amylase enzyme. The methanol extract of *Acalypha indica* leaves had significant inhibitory activity ($p < 0.05$) of the α-amylase enzyme that was the highest at a dose of 500µg/mL, in contrast with the standard acarbose. On the
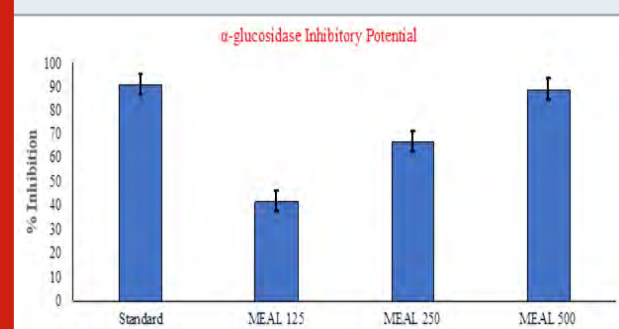
In vitro α-glucosidase Inhibitory Potential: The results of the antidiabetic study using α-glucosidase inhibitory assay of the extracts of *Acalypha indica* leaves are shown in Fig. (9). Among al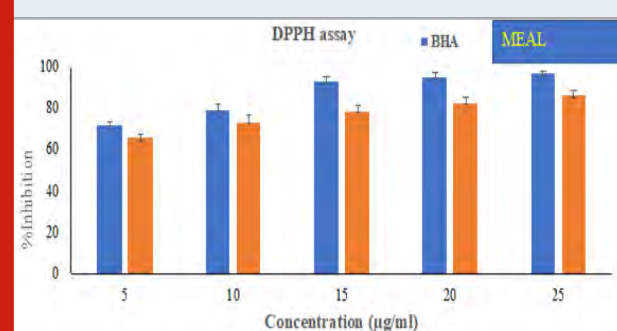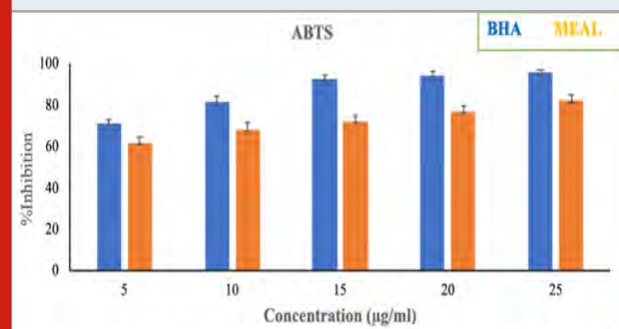l the extracts, methanol extract of *Acalypha indica* leaves established a significant ($p<0.05$) inhibitory action of α-glucosidase enzyme in a dose-dependent manner when compared to other extracts.

Figure 10: Glucose Absorption Inhibition Activity of MEAL.
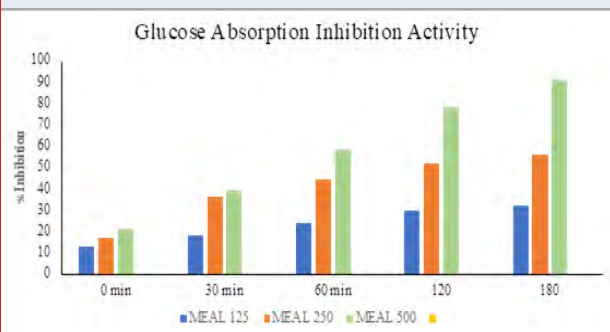
Glucose Absorption Inhibition Activity: A simple model was used to investigate glucose absorption inhibition by extract of *Acalypha indica* leaves shown in Fig. (10). methanol extract of *Acalypha indica* leaves exhibited the highest potential in inhibiting the movement of glucose molecules across the dialysis membrane when compared to the control. Other extracts were less effective in preventing the diffusion of glucose molecules when compared to methanol extract of *Acalypha indica* leaves.

Diabetes mellitus (DM) is a metabolic disorder, with an increasing trend in occurrence all over the world. Conventional medication offers great clinical chances and demonstrates a splendid future in the treatment and management of DM and its complications (Parim et al., 2019). In spite of the fact that various types of oral antihyperglycemic drugs along with insulin are used for the treatment of DM, there is a renewed interest in patients to use natural products with antidiabetic action (Montero-Muñoz et al., 2020). A considerable amount of conventional or indigenous drugs are also compelling the cutting edge arrangement of prescription. In the present investigation, we made an endeavor to prepare different solvent extracts of *Acalypha indica* leaves and assessed their antioxidant and antidiabetic activities (Brahma et al., 2014).

Since DM is a life style and health threat to our population; researchers around the globe are trying to identify natural active agents by screening phytochemicals (Ninave et al., 2022). In a previous study, a phytochemical screening of different plant extracts of the demonstrated the presence of exacerbates, which accounted for its activities (Brahma et al., 2015). We found that our methanolic extracts of *Acalypha indica* leaves contained very high amounts of total phenolics, total flavonoids, and tannin content compared to individual plant extracts. Phenolics, flavonoids and tannins are one of the most widespread groups of natural constituents found in the plants, which can neutralize the free radicals and showed antioxidant activity through scavenging or chelating, and also reported having antidiabetic activities (Erum et al., 2015) (Shafodino et al., 2023).

Free radicals like reactive oxygen species (ROS) are associated with DM, metabolic disorders, obesity, inflammation, cardiovascular disarranges, atherosclerosis, aging and neoplastic ailments (Shafodino et al., 2023) (Mopuri et al., 2017). The balance between the rate of free radical generation and elimination is essential. Abundance in cell radical generation can be hurtful; in any case, if there is a critical increment in a radical generation or abatement in radical elimination from the cell, oxidative cellular stress follows (Penumala et al., 2017). Experimental evidence and clinical proof suggest that oxidative stress is associated with DM initiation and progression (Oyebode et al., 2021).

In the present investigation, we assessed the antioxidant activities of different solvent extracts of *Acalypha indica* leaves by using DPPH radical and H_2O_2 scavenging action, TBARS test and measuring total antioxidant activity. DPPH is a steady, nitrogen focused free radical, which, after receiving hydrogen from the antioxidants present in the polyphenolic extract, is converted to diphenylpicryl hydrazine (Mouli et al., 2012). The noted decrease of DPPH by the methanolic extracts of *Acalypha indica* leaves was either because of the exchange of a hydrogen atom or the exchange of an electron.

Phenolic compounds and flavonoids are additionally thriving hydrogen benefactors, which make them great antioxidants (Priya et al., 2016). H_2O_2 is a weak oxidizing agent and can directly inactivate a few enzymes, usually by the oxidation of essential thiol groups. H_2O_2 can cross cell membranes rapidly, and once inside the cell, H_2O_2 likely reacts with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals, which are detrimental for the cells. The secondary metabolic compounds may go about as free radical scavengers on account of their hydrogen giving capacity and scavenging capacity (Agrawal et al., 2023).

Our investigation showed that the total antioxidant capacity of methanolic extracts of *Acalypha indica* leaves was much higher compared to other solvent extracts. A few reports have indicated direct correlation between total phenolic contents and antioxidative activity. The chemical composition and chemical structures of active components of the extracts are significant elements contributing to the adequacy of natural antioxidants (Chakraborty et al., 2023) (Thepwiwatjit et al., 2022). In this study, we found high amounts of total phenolic content, flavonoids, tannins, and bioactive factors by GC-MS in methanolic extracts of *Acalypha indica* leaves. These components may account for the potent antioxidant activity shown by methanolic extracts of *Acalypha indica* leaves. However, a further detailed study is necessary to confirm the action of individual compounds present in the methanolic extracts of *Acalypha indica* leaves.

Carbohydrate hydrolyzing enzymes, principally α-amylase and α-glucosidase, contribute to postprandial hyperglycemia. α-amylase initiates the procedure of

carbohydrate digestion by hydrolysis of 1, 4-glycosidic linkages of starch and glycogen (polysaccharides) to disaccharides, and α- glucosidase mediates the further hydrolysis of disaccharides to monosaccharides, which leads to postprandial hyperglycemia (Penumala et al., 2017) (Parim et al., 2015. Therefore, inhibition of α-amylase and α- glucosidase are useful in the management of hyperglycemia as they delay carbohydrate digestion, which subsequently diminishes the postprandial plasma glucose level. Ongoing advances in understanding the action of intestinal enzymes (α-amylase and α-glucosidase both are significant in starch assimilation and glucose retention) have led to the improvement of pharmacological agents. High postprandial blood glucose levels are related to micro and macrovascular complications in diabetes and its associated cardiovascular diseases. an α- glucosidase enzyme secreted by the intestinal lumen plays an important role in the digestion of carbohydrates by breaking down starch and oligosaccharides to monosaccharides before they can be absorbed.

It is recommended that concealment of the movement of such digestive enzymes would defer the debasement of starch and oligosaccharides, which would reduce glucose absorption and subsequent decrease in postprandial blood glucose levels (Hanuma et al., 2021). α-glucosidase inhibitors like acarbose and miglitol impede the processing of carbo- hydrates and hinder the assimilation (Meriga et al., 2012). Consequently, one of the restorative methodologies for decreasing postprandial blood glucose levels in patients with hyperglycemia is to counteract the assimilation of carbohydrates after meals. Restraining these enzymes (α-amylase and α-glucosidases) diminished the high postprandial blood glucose peaks in diabetes (Sahukari et al., 2021). Acarbose and miglitol also inhibit α-glucosidases and reduce the assimilation of starch and disaccharides (Sivasankari et al., 2014), (Perez et al., 2006) & (Penumala et al., 2017).

The α-amylase inhibitors are used as aide supplements that deter the assimilation and ingestion of carbohydrates. Acarbose, a perplexing oligosaccharide, restrains the activity of pancreatic amylase in breaking down starch. At the same time, synthetic inhibitors cause several side effects, such as abdominal pain, diarrhea, and soft feces in the colon (Oyebode et al., 2017). On the other hand, plant-based compounds and products derived from natural sources have been documented for their effective inhibitory activities of the enzymes, but have no or fewer side effects (Penumala et al., 2017). Our finding uncovers that different solvent extracts of *Acalypha indica* leaves productively holds back α-amylase enzyme activity in vitro, although the mechanism of inhibition requires detailed investigation. However, a previous report suggests that the plant secondary metabolites may cause conformational changes in structure (Montero-Muñoz et al., 2020).

Methanolic extracts of *Acalypha indica* leaves also showed noteworthy inhibition of glucose movement into the external solutions across the dialysis membrane. These outcomes suggest that the glucose lowering effects of methanolic extracts of *Acalypha indica* leaves might be due to reduced glucose absorption. The main mechanism behind the role of dietary fiber in bringing down postprandial serum glucose is the viscosity of various dietary strands, which hamper diffusion of glucose, thus, interfering with absorption and digestion of carbohydrates (Brahma et al., 2014). Gallagher et al. examined the capacity of various plants to repress glucose dispersion.

The various phytochemical compounds such as polyphenolic compounds, triterpenoids and other bioactive compounds present in the plant may contribute to the observed glucose lowering impacts of the extracts (Mopuri et al., 2017). Tannins, flavonoids, saponins, alkaloids and other bioactive mixes present in the plant are theorized to contribute to the hypoglycemic impact (Oyebode et al., 2018) (Penumala et al., 2017). Hence, the observed α-amylase and α-glucosidase inhibitory effects might be due to the presence of these phytoconstituents. In the current study, we found that the glucose lowering effect of methanolic extracts of *Acalypha indica* leaves is higher than that of any of the different solvent extracts, which might be due to the less number of phytochemicals.

CONCLUSION

In conclusion, our results reveal that the methanolic extracts of *Acalypha indica* leaves contains a high amount of secondary metabolites and bioactive components, which have therapeutic potential. We found that the methanolic extracts of *Acalypha indica* leaves has free radical scavenging activity and inhibitory action against α-amylase and α-glucosidase. These properties could be explored for the management of postprandial glycemia in individuals with type 2 DM. Although the effects have been set up in vitro, these results suggest that methanolic extracts of *Acalypha indica* leaves may be a potential dietary health supplement. Further detailed examinations are in progress to elucidate whether the methanolic extracts of *Acalypha indica* leaves has antidiabetic potential by in vivo for corroborating the traditional claim of *Acalypha indica*.

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Biotechnological Communication

Isolation of Alkaline Protease from Fruit Waste and its Application as Detergent

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ABSTRACT

The modernization of the society has made our environment face a series of changes. One among them is the enzymatic method produced by microorganisms from any waste. Considering the fruit waste globally several industrial uses have been fulfilled by the fruit waste. The most abundant enzyme now a days is protease from papaya waste isolated from *Bacillus spp*. Alkaline protease has been choosen for the detergent industries widely since it has pH range over 7.5. It performs more effectively in decomposing proteins. Since, it is stable over wide temperature and pH range.

The aim of the study was to isolate alkaline protease enzyme from fruit waste and its applications as detergent against three different stains such as blood stain, banana stain and tea stain. Papaya fruit waste was used to extract protease enzyme to create a useful detergent for stain removal. The enzyme's activity was tested at different pH and temperature, and the best results were obtained from three samples. The study suggests that alkaline protease enzyme from papaya waste is effective in removing protein stains and could help manage solid waste while being produced at lower temperature.

KEY WORDS: ALKALINE PROTEASE,BACILLUS SPECIES,

INTRODUCTION

Fruit and vegetable waste is the ever growing global question. Several techniques have been developed to facilitate the waste into possible sources of energy and helps in reducing the environmental pollution. Globally, the overall production of fruits and vegetables is about 675 million metric tons annually and out of the which 1.3 billion ton wastage is produced. India alone produces 86.602 million metric tons of fruits and vegetables and constitute about 5.6 million ton of waste annually. Manyof the garbage are removed in ecologically unsustainable manner or the minimum quantity of waste was enzymatically decomposed with microorganisms due to their ability to secrete enzymes (Elvira C et al. 1998 Chao et al. 2017).Papaya is a popular fruit consumed worldwide and its well known for food and nutritional values. It is used in food industries, detergent industries, chewing gum industries etc. As a result, the industries generate huge amounts of papaya peel (PP) and seeds as by-products, which are typically considered a waste, and thus discarded, but that can be converted into many value-added products (Pathak et al. 2019).

Now a days enzymes play a key role in industrial areas. The two major enzymes from papaya include lipase and protease. Commercial proteases account for nearly 60% of the total industrial enzyme market (Katsuhisa et al., 2007). *Bacillus* sp is the most important group of bacteria that are involved in the the enzyme industry and this bacterium is also known to produce proteolytic enzymes quite effectively (Patel 1985). Although many other classes of enzymes are currently in industrial use, the focus of this paper is on alkaline proteases from bacterial species. This is because the organisms producing enzymes capable of catalysing the reactions at the extremes of pH above neutrality (Arunachalamand & Saritha 2009). The genus Bacillus is vital for commercially important alkaline protease which is active at alkaline pH ranging between 9 and 11 .The detergent industry consumes alkaline proteases most abundantly (Moon, & Parulekar 1991). The largest application of protease is in laundry detergents and leather industry, where they remove protein based stains from clothes and dehairing purpose, respectively. As the modern world focuses on ecofriendly products and product output, subsequently more chemical processes are being replaced by enzymatic methods (Manavalan et al., 2020 Bektas et al 2023).

Protease is one of the important enzymes used in textile industry. The presently available proteases are not sufficient to meet industrial demands. Hence; there is continuous search

for new proteases with novel characteristics for industrial application from diverse bacteria isolates. Microbes from varied habitats have been examined by many researchers to obtain the industrially suitable proteases. This present study was to discuss about the isolation of alkaline protease from the fruit waste papaya peel and discuss the stain removal (detergent activity) against the different stains as made.

MATERIAL AND METHODS

Isolation and characterization of microorganism: Protease producing isolates were analysed from the papaya peel using a skim milk agar plates after incubation. Among the isolates, *Bacillus* species exhibited a prominent clear zone. Pure culture was obtained for the best three isolates of clear zone after the incubation period of 24-48 hrs at 37⁰C. This was selected for the study of protease production. This was maintained at 4⁰C in skim milk agar plates[10]. The morphological and physiological properties of the isolate were investigated according to Bergey's manual of determinative bacteriology (Towatana 1999 & Becerra et al., 2016).

The three bacterial isolates were inoculated separately to three conical flasks containing 100 ml nutrient broth and incubated over 2-3 days period at 37⁰ C in a rotary shaker. Nutrient broth without bacterial inoculation kept as control. The contents of the flasks were collected in a centrifuge tube and it was centrifuged at 10000 rpm for 10 minutes at 4⁰ C in order to get the cell free supernatant containing crude enzyme.

Quantitative analysis of protease enzyme activity: Protease activity in the culture supernatant was determined a by using casein as a substrate. A mixture of 500 μl of 1% (w/v) of casein in 50 mM phosphate buffer, pH 7 and 200 μl crude enzyme extract were incubated in a water bath at 40°C for 20 minutes. After 20 minutes, the enzyme reaction was terminated by the addition of 1 ml of 10% (w/v) trichloroacetic acid (TCA) and was kept at room temperature for 15 minutes. Then, the reaction mixture was centrifuged to separate the unreacted casein at 10,000 rpm for 5 minutes. The supernatant mixed with 2.5 ml of 0.44M Na2CO3. 1 ml of 3-fold diluted FollinCiocalteus phenol reagent was added (Lu et al., 2018).

The resulting solution was incubated at room temperature in the dark for 30 minutes and absorbance of the blue colour developed was measured at 660 nm against a reagent blank using a tyrosine standard (Sony & Potty 2016). This was modified as Lowry's method (Lowry et al., 1951). One unit of protease is defined as the amount of enzyme that releases 1 μg of tyrosine per ml per minute under the standard conditions of supernatant solution.
Enzyme activity was calculated by the formula

$$\text{Units/mL enzyme}: \frac{\text{Umol tyrosine equivalents released} \times \text{total volume of assay}}{\text{Vol.of enz used} \times \text{length of assay} \times \text{vol.used in calorimetric determination.}}$$

Effect of pH on enzyme activity: The culture was incubated at 370C for 48hrs and the pH was adjusted using different buffers ranging from 3-9. The OD values of enzyme activities were taken in spectrophotometer at 600nm (Badhe et al., 2016).

Table 1. Activity performed with different dilutions against different stains

Stains	Tap water (C)	Broth 10^{-5}	Broth 10^{-7}	Broth 10^{-9}	Detergent + Broth	Detergent
Blood stain	20ml	20ml	20ml	20ml	0.2g in 20ml of tap water, $10^{-5}, 10^{-7}, 10^{-9}$	0.2g in 20ml tap water
Banana stain	20ml	20ml	20ml	20ml	0.2g in 20ml of tap water, $10^{-5}, 10^{-7}, 10^{-9}$	0.2g in 20ml tap water
Tea stain	20ml	20ml	20ml	20ml	0.2g in 20ml of tap water, $10^{-5}, 10^{-7}, 10^{-9}$	0.2g in 20ml tap water

Effect of Temperature on enzyme activity: To study the optimum temperature where an enzyme shows its maximum activity were exposed to different temperature ranging from 4⁰C – 100⁰C. After incubation the OD values were taken in spectrophotometer at 600nm (Olajuyigbe et al., 1980).

Stain removal activity with crude enzyme: The three sample broths were taken for the washing activity.

Application of protease enzyme by isolated organism as a detergent additive was studied (Kalapana et al., 2008). The washing test is performed with the three prepared broth samples for three different stains.

Partial Purification Method: Based on this washing test the best stain removal test sample medium 10^{-5} was identified and done partial purification for the sample by

saturated Ammounium sulphate method (Mark et al., 2002).

Characterization of Proteins Using SDS-PAGE analysis: Electrophoresis is the process of migration of charged molecules in response to an electric field.Proteins have a net charge at any pH other than their isoelectric point (pI), thus when placed in an electric field, proteins will migrate towards the electrode of the opposite charge. This principle is used to separate molecules of differing charges (Sanbrook et al., 1989).

Table 2. Activity performed with effective broth dilution against different stains

Stains	Tap water	Broth 10^{-5}	Detergent + Broth	Detergent
Blood stains	20ml	20ml	0.2g in 10ml tap water and 10ml broth	0.2g in 20ml tap water
Banana stain	20ml	20ml	0.2g in 10ml tap water and 10ml broth	0.2g in 20ml tap water
Tea stain	20ml	20ml	0.2g in 10ml tap water and 10ml broth	0.2g in 20ml tap water

Stain removal capability after partial purification: The washing test activity is done again after the partial purification method to identify the capacity of the enzyme in stain removal ability. The three different stains were tested against the sample 10-5 medium. The stain removal of the enzyme was studied along with the detergent powder. The stain removal was studied under the following ways in table 2

RESULTS AND DISCUSSION

Isolation and characterization of microorganism: Out of 86 isolates screened the best hydrolysis zone were obtained for three isolates after 48hrs of incubation at 370C . The zone of isolation of three three isolates were named as S1,S2,S3. The zone of hydrolysis on skim milk agar is shown in Fig.1 Their morphological and physiological study for casein hydrolyse study as as shown in table 2 and 3.

Estimation of Protease activity: The maximum enzyme activity was given by the sample test 1 which showed its higher activity at 0.45nm. The enzyme acidity gradually increased as the substrate concentration also increased and also there was a gradual decrease in the test 2 and 3 with its substrate concentration. Table 4 shows the test samples enzyme activity.

Optimization of growth using Temperature and pH: The enzyme showed the best activity between temperature range 250C and 450C with maximum activity at 370C. As the graph shown in (Fig 2) the enzyme was constant and stable at 370C. At 450C and above it started to decrease its activity and get completely inactivated by 120 min at the further temperatures. And in the same way the enzyme showed the reasonable activity between the pH 8 and 11 with maximum activity at 10 as shown in (Fig 3) which shows enzyme was stable at 10. At above pH 11 the enzyme started to reduce its activity after few minutes and get inactivated by 90min at further pH.

Fig 1(a): Growth of microbes in 10-5dilution
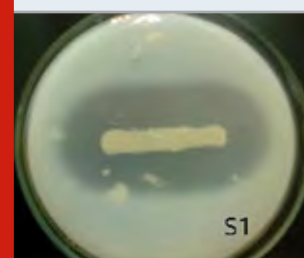
Fig 1(b): Growth of microbes in 10-7dilution

Fig 1(c): Growth of microbes in 10-9dilution

Table 3. Shows the results of Morphological study

SI.No	Gram staining	Negative staining	Spore staining
S1	Positive	Short chains	Endo spores
S2	Positive	Short chains	Endo spores
S3	Positive	Short chains	Endo spores

Stain removal activity with crude enzyme: A good detergent additive protease enzyme must have a cabaple to remove the protein stains by cleaving the protein bond breakage. Therefore, the effects of various oxidizing agents

and surfactants on the protease enzyme were studied. stability of enzyme towards these stains were also analysed with the incubation period of 60min and 120mins (Kumar; Takagi 1999 & Joo 2005). protein stability has commercially wide applications in detergent industry has been reported in many studies (Kumar & Bhalla 2004) Oxidizing agents are major ingredientsof modern bleach based detergents. Alkaline protease from bacillus shows extreme stability towards oxidizing agents (Nadeem et al., 2013).

Table 4. Shows the results of Physiological study : Casein hydrolyse test:

Sample	Growth	Zone of inhibition(mm)
S1	Yes	15mm
S2	Yes	11mm
S3	Yes	9mm

By considering these facts the washing test were perfomed for the blood stain, banana stain,tea stain with crude enzyme of three isolates were washed after the incubation period

of 2hrs .The best results were obtained from the sample 1 isolate as shown in (Fig 2,3 and 4).

Partial purification and molecular weight determination: To obtain proteins from cell free culture filtrate the sample 1 isolate were partially purified with ammonium sulphate at 75%(w/v)saturation by adding powdered Ammonium sulphate slowly with continuous stirring . The yield of protein was 2.30mg/mL, with a total activity of 415.26U/mL per min. This yield of partially purified protein was undergone for molecular weight determination by SDS -PAGE analysis and the result was shown in the(Fig 5)

Washing test with partially purified enzyme: The enzyme with extreme stability towards oxidizing agent is commercially significant as the peroxidise are common ingredients of bleach based detergents. The enzymes prepared are mostly stored under low temperatures which will prevent its denaturation if the enzymes can withstand room temperature it is very useful in lifetime of a detergent product. So by consisdering these issues the partially purified enzyme sample 1 isolate has withstand its room temperature and also removed the blood stain, banana stain, tea stain. Thus this reveals its use in detergent industry. The washing test performed is shown in (Fig 6,7, and 8.)

Table 5. Estimation of protein by Lowry's method

Test tube	Tyrosine Vol. Conc.		Alkaline reagent	Cond.	Dis. water	Folins phenol	Cond.	OD at 660nm
B	-	-	2	Incubation	2.00	0.2	Keep	0.00
S1	0.05	5	2	in	1.95	0.2	in	0.25
S2	0.10	10	2	dark	1.90	0.2	dark	0.33
S3	0.15	15	2	room	1.85	0.2	room	0.40
S4	0.20	20	2	for	1.80	0.2	for	0.53
S5	0.25	25	2	10 mins	1.75	0.2	30mins	0.60
T1	1.00		2		1.00	0.2		0.45
T2	1.00		2		1.00	0.2		0.37
T3	1.00		2		1.00.	0.2		0.33

Graph 1: Shows the growth rate in pH values
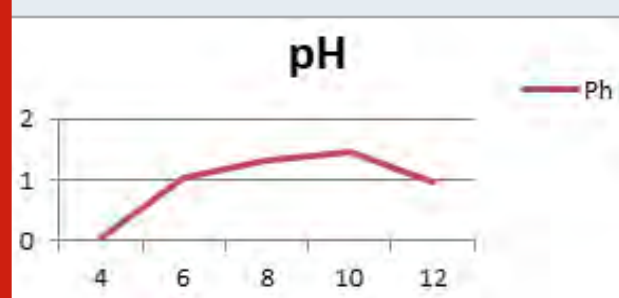
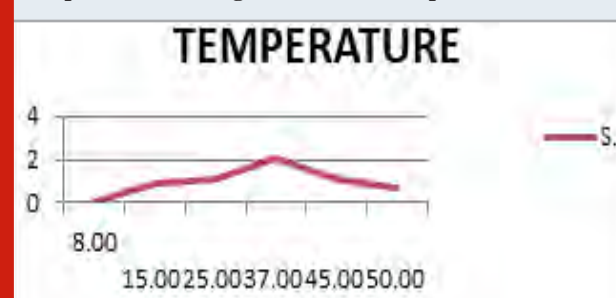**Graph 2: Shows the growth rate in Temperature values**

Figure 2(a) : Stained blood clothes in S1 sample.

Figure 2(b) : After 2 hrs the washing activity shows best
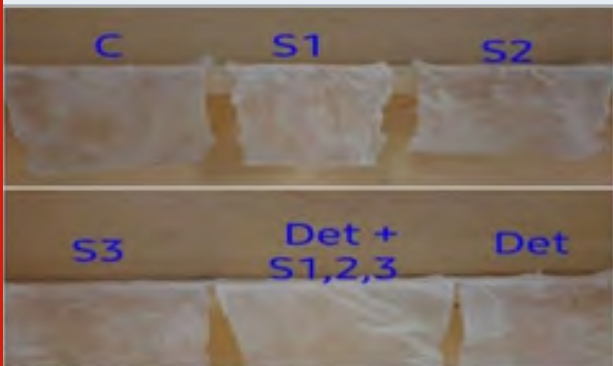
Figure 3(a): Stained banana clothes in S1 sample.

Figure 3(b) : After 2 hrs the washing activity shows best
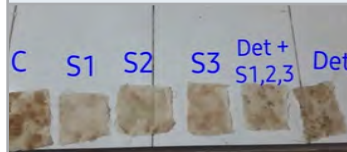
Isolation and characterization:

Growth of Bacillus sp., in different dilutions

Figure 4(a) : Stained tea clothes

Fig 4(b) : After 2 hrs the washing activity shows best in S1 sample
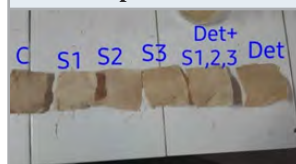
Partial purification and molecular weight determination:

Figure (5) : SDS-PAGE analysis of isolated protease from papaya peel waste.
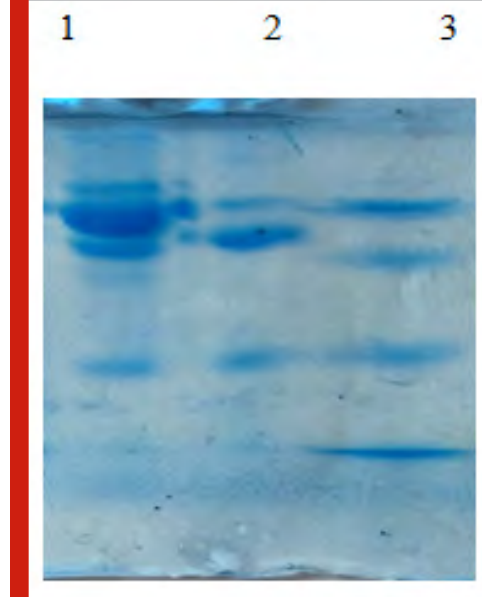
Lane -1 : Marker, Lane -2 : Crude lane, Lane -3 : Partial Purified lane   Marker : 10kda , 50kda, 75kda, 100kda

Lane -1 : Marker, Lane -2 : Crude lane, Lane -3 : Partial Purified lane   Marker : 10kda , 50kda, 75kda, 100kda

Molecular weight of crude proteins:

1st band – 82kda, 2nd band- 68kda, 3rd band- 12kda
Molecular weight of partially purified proteins:

1stband -81kda, 2nd band- 47kda, 3rd band – 10kda

Washing test with partially purified enzyme:

Banana stain:

Figure (6a) Blood stained clothes purified protein

Figure 6(b) : Washing activity after 2hrs proved best partially results in 10^{-5}

Fig (7a) Banana stain clothes in partially purified protein

Fig: (7b) Washing activity after 2hrs proved best results in 10^{-5}

Fig (8a):Banana stain clothes in partially purified protein

Fig (8b): Washing activity after 2hrs proved best results in 10-5

Tea stain:

CONCLUSION

Papaya fruit waste was used to extract protease enzyme to create a useful detergent for stain removal. The enzyme's activity was tested at different pH and temperature, and the best results were obtained from three samples. Washing activity was performed with three different stains, and the best sample was identifies as 10 - 5 broth, showing the best stain removal activity after 2 hours.The sample was partially purifies and tested again, confirming the presence of protein by SDS - PAGE analysis with a specific molecular weight The study suggests that alkaline protease enzyme from papaya waste is effective in removing protein stains and could help manage solid waste while being produced at lower temperature.

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