

Bioscience Biotechnology Research Communications

Volume-15 Number (4) Oct-Nov-Dec 2022

Print ISSN: 0974-6455

Online ISSN: 2321-4007

CODEN: BBRCBA

Website: www.bbrc.in

An International Peer
Reviewed Open Access Journal

Published By:

Society for Science & Nature (SSN)

Bhopal India

website: www.ssnb.org.in

Online Content Available: Every 3 Months at

www.bbrc.in



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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.

Biosc Biotech Res Comm is an open-access international platform for publication of original research articles, exciting meta-reviews, case histories, novel perspectives and opinions in applied areas of biomedical sciences. It aims to promote global scientific research and development, via interactive and productive communications in these areas, helping scholars to present their cherished fruits of research grown on toiled and tilled trees of hard work in life sciences. Being the publication of a non-profit academic Society for Science and Nature, Bhopal India, since 2008, *Biosc Biotech Res Comm* strongly believes in maintaining high standards of ethical and quality publication.

Quality publication is one of the ways to keep science alive, and good journals have a leading role to play in shaping science for humanity! As teachers, we have great responsibilities, we have to advocate our students to accomplish and show them the path to test their mettle in hard times to excel, especially in the post COVID 19 era. Science and its advocates will rise more to the occasion and will soon provide succor to the already grief stricken humanity.

Sharique A. Ali, PhD
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Water Resources of Rivers and Erosion-Accumulation Processes

Fedor Lisetskii

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ABSTRACT

Basin territorial structures are defined as unified natural and anthropogenic systems, since the movement of matter, energy and information occurs naturally from the divide to the valley line of the river valley within them. Considering the global issues of soil degradation in watersheds and depletion of water resources, some regional experience in the design and implementation of soil and water protection of cultivated lands as part of the concept of the basin nature management and implementation of the program for environmental rehabilitation of rivers and water bodies is presented. The statement that it is necessary to develop a long-term strategy for the consistent arrangement of basin geosystems from a divide of catchments to valley lines of a fluvial network in the conditions of a crisis situation with the use of soil, land and water resources is justified.

KEY WORDS: RIVER BASIN, SOIL EROSION, WATERSHEDS, ENVIRONMENTAL FLOW, ENVIRONMENTAL REHABILITATION.

INTRODUCTION

European news resources are full of information about weather anomalies that have affected the shortage of water resources this year. On August 23, experts from the Joint Research Center (European Commission's science and knowledge service) believe that Europe is experiencing probably the worst drought in the last 500 years. The Danube River, which flows through the territory or is the border of ten states of Central and South-Eastern Europe, is the second (after the Volga) river in Europe in terms of its length and catchment area. The Danube basin (817 thousand km²) is the most international river basin in the world and it provides drinking water for 20 million people. As WWF notes, as of August 17, 2022, in recent weeks, the Danube River has been setting daily records for its lowest level since 1941. While back in early February, the Rhine river overflowed its banks due to melting snow and torrential rains, which resulted in many settlements being flooded, it was noted on 13 August 2022 that the dropped water level in the Rhine had become a threat to navigation. Countries such as France, Spain, Italy and the Netherlands are facing water shortages this year.

Water resources and their use have some features that require managing them as a whole. A river flow intended for use is a space and time category: it has intra-annual and interannual fluctuations and is unevenly distributed over the

entire territory. Natural features of the water flow dynamics change with the global climate changes. Another feature is that rivers that are used as a source of water supply can simultaneously be used as a wastewater receiver. However, it is important to emphasize that river flow is a key zonal factor for understanding territorial patterns of sediment yield formation (Yermolaev et al. 2021).

A conceptually new agriculture that is dynamically progressing economically resilience (adaptation), and removes greenhouse gases (mitigation), should not only be justified in terms of its feasibility, but also implemented in practice, which is especially important for such an agriculturally important country like India where over 600 million people are directly dependent on agriculture (Singh, 2013). And it is those densely populated countries with developed agriculture that soil erosion is most intense in. Annual global irreplaceable soil waste due to erosion reach 23 billion tons, of which the United States accounts for 6.5%, China - 14.3%, India - 20.4%. Since 1945, soil degradation has affected about 11% of the land free of ice from land mass of Earth (i.e. an area that, in total, exceeds the area of India and China). Innovative estimates for Earth (Borrelli et al. 2017) showed that on 9% of land and more, soil erosion is characterized as moderate and high intense, with the share of the latter class being 5.1%.

Selective nature of effects of the water erosion leads to the fact that silt particles enriched more in organic carbon (by an average of 40%) are carried out of watersheds, which leads to decarbonisation of arable soils and siltation of water

Article Information:*Corresponding Author: fnliset@mail.ru

Received 15/10/2022 Accepted after revision 25/11/2022

Published: Dec 2022 Pp- 480-482

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Available at: <https://bbrc.in/> DOI: <http://dx.doi.org/10.21786/bbrc/15.4.1>

bodies. Sediments on slopes with variable steepness are redeposited with the formation of pollutant accumulation zones. The use of fertilizers and plant protection products in cultivated lands determines the need to develop a priority list of pollutants in the soil-ecological monitoring system, as is shown e.g. for the regional conditions of the black earth zone: Cu, Cd, Cs, Pb, Zn (Zelenskaya et al. 2019). Monitoring is necessary both to control the translocation of heavy metals in the soil-plant system and to predict the zones of their accumulation in subordinate landscape positions. Excessive use of agrochemicals and in particular pesticides on watersheds leads to accumulation of their residues in the water and fishes (Hasan et al. 2021).

Suppression of the self-purification process and a decrease in the self-purification ability of an aquatic environment occurs as a result of a rupture in individual links of the food chain and turnover disturbances due to the toxic effect of individual pollution components (heavy metals, pesticides, surfactants, biogenic elements in case of their excessive concentration). The FAO compiled Global Soil Partnership reports (GSP, 2017), noting that 75 billion tons of soil are eroded every year from arable lands worldwide, which equates to an estimated financial loss of US \$400 billion per year. Although there is disagreement among different authors that soil erosion costs for the US economy range from US\$30 billion to US\$44 billion annually, however, it can be assumed that the ratio of the total annual losses from erosion due to decreased productivity to the losses from water pollution as a result of erosion, but with dispersed sources (in the form of suspensions, soluble substances, nitrogen, phosphorus, bacteria), is 1:4.6 (Alt, Putman, 1987). Indirect and environmental consequences of water erosion are diverse (Borrelli et al. 2022).

In addition to damage to water supply sources from pollution with fertilizers, pesticides, and heavy metals, reduced fish stocks due to eutrophication of water bodies, changes in the composition of aquatic organisms, reduced recreational potential of coastal zones, it also includes siltation of navigable rivers and harbours, inflow of solid runoff into water intake facilities of the main canals, sediment deposition in roadside ditches, at spillways, etc.

Various criteria and indicators are used to assess the state of water resources in terms of quality and sufficiency of the volume for water use. The hydrological concept of the “minimum river flow” has at least two dimensions: a resource and an environmental one. The former is important for assessing the self-purification ability of the water flow to move sediments, which directly affects the water quality, as well as the balance of sediments in the aquatic environment and in case of accumulation in the bed. Turbidity of water of lowland and mountain rivers differs by 20–50 times, which is due to their river flow velocity differences. The environmental dimension of the minimum flow is determined by its potential for transporting ionic runoff and pollutants. Environmental runoff is an important tool for management of water resources.

Disruptions to environmental flows, which are widespread and occur in half of the sub-basins of the world, have

become twice as common as in the pre-industrial period (Virkki et al. 2022). From the point of view of the theory, the environmental flow and the hydroecological safety of water use increase in those situations when the flow characteristics of the summer and autumn low-water season increase, while the risks of hydroecological emergencies are reduced due to a decrease in the extremes of the flow volumes in the spring flood (Dmitrieva, Zhigulina, 2020).

The concept of basin nature management involves the development of a strategy for consistent development of basin geoecosystems from the divide of catchments to the valley lines of the fluvial network. Informational parameterisation of river basins is reasonable within the framework of a dedicated geoportal for large regions, as it is done for the East European Plain (Yermolaev et al. 2018), while the development and implementation of soil and water protection projects in watersheds within the framework of the basin nature management concept (Yermolaev et al. 2015).

At the national level, the Belgorod Oblast has become the territory of the pilot project of soil and water protection development of cultivated lands on the basis of the basin principles. The choice of this region is in no way accidental. Belgorod Oblast is a part of the Central Black Earth Economic Region (CBEER), which is part of the Eurasian Black Earth Zone, with a total area of 168,000 km², with its arable land occupying 60% is a large agricultural region, which provides the national agricultural industry with 40% of sugar beet, 25% of sunflower, and 10% of grain. Located on the south-western slopes of the Central Russian Upland, Belgorod Oblast has a large many slopes (72%, of which 34% of arable land exceed 3°), and is the region among the five regions of the Central Black Earth Region that is most prone to soil erosion (the share of eroded soils is about 50%). According to various estimates, the average annual rate of soil erosion in Belgorod Oblast ranges from 4 to 11 t ha⁻¹.

As shown in a large body of evidence (Yermolaev et al. 2022), changes in land use and climate (i.e. meltwater runoff and rainfall erosivity) can act as specific triggers in the transformation of the fluvial system on hillslopes. Small rivers and their hydrological regime, as shown in many studies (Lisetskii, 2021, Lykov, Melenchuk, 2022), are the most sensitive to anthropogenic transformations compared to watercourses of higher orders. For example, siltation of river beds on the territory of the Central Black Earth Region for 200–250 years of agrarian development of watersheds (on average it 1 mm yr⁻¹) has led to a change in the water regime on the floodplains and often leads to flooding of settlements. Compared to late 18th century, drainage density in the region has decreased 1.6-fold. Total length of small rivers (up to 100 km) has reduced by 40%. As a result of a decrease in water flow in small rivers and an increase in the amount of sediment, many tributaries have become non-perennial ones since they were separated from the main river bed.

Environmental rehabilitation of the surface water bodies now becomes a logical continuation of the basin nature

management projects for 52 river basins completed in 2015 and subsequent implementation of comprehensive soil protection measures at watersheds in each river basin (optimization of the structure of agricultural land, differentiated crop rotation depending on the erosion hazard, depression resting on arable land, creation of new forest strips and afforestation zones at the peaks ravines, etc.). Belgorod Oblast is one of the first constituent entities of the Russian Federation to launch a large-scale water rehabilitation program. To reduce the ingress of suspended sediment into a water body, it is required to arrange for protective forest belts or waterworks (water-retaining walls, drainage channels and flow diffusers) in the areas of the flow accumulation.

Water protection zones near a water body should include three zones: sanitary protection, moderate and partial restrictions. In addition to the damage caused by gullies to soils, land use and infrastructure (Yermolaev et al. 2022) active erosional forms that open as mouths on narrow floodplains into the river bed serve as powerful providers of sediments. Therefore, it is necessary to create silt filtrating plantations on the fans of gullies, on their bottom, as well as on the bottom of water supply troughs. The urgent need for comprehensive water management and environmental protection measures for small rivers has caused the commencement of development of the “Preservation of Unique Water Bodies project (Belgorod Oblast)”, including the water protection reconstruction of 72 water bodies back in 2022 (33 rivers and 39 ponds or reservoirs). It is planned to clear sections of river beds with a length of at least 260 km and lakes with an area of at least 730 ha by 2024.

Thus, the critical environmental situation in most rivers and water bodies requires a long-term rehabilitation strategy based on a deep understanding of hydrological, hydrodynamic, hydrochemical, and other processes in watersheds and in river beds. The results of said study will establish a scientific basis for implementation of comprehensive systems for environmental rehabilitation and protection of rivers and water bodies. The work is carried out in accordance with the Strategic Academic Leadership Program "Priority 2030" of the Kazan Federal University of the Government of the Russian Federation.

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A Review of Tag-aware Recommender Systems for Future Applications in Research and Development

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ABSTRACT

Due to the recent growth in online data about customers and the growing social web content due to the ever-increasing popularity of social media services, tag-aware recommendation systems are attracting more attention. Tag-aware recommendation systems (TRS) effectively reveal user preferences and extract latent semantic information of items through social tag information. Therefore, a review of the present status of the literature on tag-aware recommendation systems is necessary to identify future research possibilities and directions. This article reviews the research direction in terms of approaches used, application domains, challenges and problems related to developing a system of recommendations, and evaluation metrics used to evaluate performance. It also, presents the insights gained and potential directions for further research. We evaluated 33 scientific papers thorough quantitative evaluation. Although TRS is a flexible approach to managing information, we found that the number of publications are few over the years. Also, scientific publications are limited to specific datasets and types of publications and focus on a specific field more than others. 73% of the papers were published as a journal, and 29% of papers used collaborative filtering approach. The most covered domin was the music domain with 26%, and the most used dataset was Last.FM with 20%.

KEY WORDS: TAG-AWARE, RECOMMENDER SYSTEMS, SOCIAL TAGGING SYSTEM.

INTRODUCTION

The information and content in our time are increase in the amount . And that becuase of extensively used by the users. Thus, access to appropriate and effective content from the vast amount of information has become a problem (Konstan and Riedl, 2012), (Isinkaye, Folajimi and Ojokoh, 2015), (Liang et al., 2018), (Zhao et al., 2021). And for this, recommendation systems (RS) have appeared which is a filtering tool that filtering the vital information part from a large amount of information which generated dynamically according to user preferences or interests or its observed behavior around the element in a highly personalized way (Isinkaye, Folajimi and Ojokoh, 2015), (Zhao et al., 2021).

These systems not only display preferences similar to the user's preferences, but also those that are unknown and of interest to the user. Techniques for creating personalized recommendations have been developed and suggested, such as Tag-aware Recommendation Systems (TRS). TRS helps find items that are important and reflect the user's personal preferences by using random words or phrases, which are

freely sets by the user (Liang et al., 2018). Through their labeling behavior, these systems provide complementary information to the recommender systems (Zhang, Zhou and Zhang, 2012). This type of recommendation system showed effective work, as were made recommendation systems through the fusion of collaborative filtering algorithms as in paper (Tso, Marinho and Schmidt-Thieme, 2008), and recommendation systems based on deep learning - Intelligent computing systems as in the paper (Liang et al., 2018). Also, based on deep reinforcement learning as in the paper (Zhao et al., 2021).

From our view, the field of recommender systems suffers from a lack of research papers in it. There may be some scientific papers on recommendation systems, but not especially on tag-aware recommender systems. From this direction, this paper contributes to publishing a new value to scientific papers and is a starting point for publishing specialized scientific papers in tag-aware recommender systems. From this, this paper aims to present a survey of the tag-aware recommender systems. This review article differs from previous ones as it provides more recent information on the tag-aware recommender systems.

The remainder of this paper is structured as follows. Section 2 presents the background information about recommender

Article Information:*Corresponding Author: rabduljabbar@ksu.edu.sa
Received 22/10/2022 Accepted after revision 30/12/2022
Published: Dec 2022 Pp- 483-493
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Available at: <http://bbrc.in/> DOI: <http://dx.doi.org/10.21786/bbrc/15.4.1a>

systems generations and tag-aware recommender systems. In section 3 we briefly review the related work of recommender systems and tag-aware recommender systems. Section 4 is about tag-aware recommender systems. We present a quantitative assessment of the comprehensive literature in section 5. Insights and discussions in section 6. Finally, a conclusion is given in section 7.

Background

Recommendation systems appeared in the 1990s and have evolved more over the days for the algorithms used and for deploying applications that use these systems (Felfernig et al., 2013). Recommender systems are in their development stages and have been developed from the first generation to the third generation (Singh, Chuchra and Rani, 2017). First-generation recommendation systems deal with e-commerce (Singh, Chuchra and Rani, 2017). Objects and users are the two basic blocks of this generation, and they have a binary relationship (Singh, Chuchra and Rani, 2017). Based on their preferences, users rate the items. The rating could be binary or on a scale from 1 to 5. Researchers have classified this generation into 11 approaches according to (Singh, Chuchra and Rani, 2017). In the second generation, recommendation systems are used in the social network and social contextual information (Singh, Chuchra and Rani, 2017).

Social tagging sites have grown, and thus tag recommendation has become a topic of interest in this generation of recommending systems. Social tagging systems rely on three building blocks: Users, Items, and Tags to create recommendations, and these blocks have relationships with each other. According to (Singh, Chuchra and Rani, 2017), there are nine approaches for this generation. The third generation appeared after the increase in the use of mobile devices, as this generation uses location-based information or the Internet of Things to create recommendations. Location-based recommendation systems and RFID tags are examples that used in this generation. There are two approaches to this generation according to (Singh, Chuchra and Rani, 2017), where it was used Collaborative recommender with space and time similarity in (Organero et al., 2010), and Location-aware recommender system (LARS) which was used in (Levandoski et al., 2012).

Recommendation systems were developed from the first generation to the third generation through the second generation (Singh, Chuchra and Rani, 2017). As the available options increased and with the increase in its applications, topics related to recommendation systems appeared, including social tagging systems (STS) (Tang, Hu and Liu, 2013), (Malmström, 2019). Where items can be social entities such as people or a group of people (Singh, Chuchra and Rani, 2017). Tags are generally a way to make it easier to display content by topic, and this content is grouped by category (Ricci et al., 2011). The interested content of the user can be found by used this approach. (Ricci et al., 2011). Social recommendations include tag recommendations, people recommendations, and content recommendations (Singh, Chuchra and Rani, 2017).

The tag recommendation system is a system that

recommends tags to the user, and these tags are defined as words that the user freely adds to an object (Malmström, 2019). The tag recommendation system uses a database that contains the objects, which in turn contains the tags that organize and describe them, and thus it is easy to search in this database for objects (Malmström, 2019). Through this database, the user can create tags on objects or add tags to new objects (Malmström, 2019). And because the Internet of things technologies are used in social networks such as NFC and RFID, which are used in (Organero et al., 2010) and others, tag recommendation systems fall under the third generation. It is one of the most successful approaches of increasing the level of relevant content as more content is available on the Internet.

Literature Review

Recommendation Systems (RS) have improved many different services in various fields. A systematic literature review (Alyari and Jafari Navimipour, 2018) discussed RS from 2005 and compared the different algorithms and limitations. They concluded the classic recommendations approaches play a dominant role in almost all types of applications. Still, hybrid RS is more popular than a recommendation based on a single-recommendation technique to avoid the drawbacks of the single-recommendation approach. The results are consistent with the survey in (Malik, Rana and Bansal, 2020).

Although the classic approaches of RS have been successful, they still suffer from many problems. Based on this, authors in (Da'u and Salim, 2020) presented a systematic literature review of deep learning-based learning resources that can better guide researchers and practitioners to understand trends and new challenges in this field. The results indicate that the most widely exploited deep learning architectures for RS are autoencoder (AE) models, followed by Convolutional Neural Networks (CNNs) and Recurrent Neural Networks (RNNs) models. As for the datasets most used to evaluate RS based on deep learning, the two datasets are Movie Lenses, followed by Amazon. In (Batmaz et al., 2019), they presented a survey study of deep learning-based recommendation system approaches, categorized into four main aspects. Furthermore, they provide a quantitative assessment of the literature and a discussion of the insights gained. As a result's review, the promising and encouraging results can be seen from the deep learning recommender system. In addition, scalability and accuracy consedred as challenges for this review for improvement and future work.

The study (Mu, 2018) provides a reference for developing and reviewing the limitations of deep learning-based recommendation systems. Furthermore, exploration of the advantages of deep learning over traditional recommendation systems, they learn latent features of the user and item automatically by integrating different types of heterogeneous data from multiple sources, modeling hierarchical patterns of user behavior, and more effectively reflecting different user preferences, and improving the accuracy of recommendations. Furthermore in (J. Y. Liu, 2018), they presented a recommender system survey focusing on deep learning approaches and application

systems. Whereas the deep learning neural network is customized to the recommendation system to extract the users and items features or latent and explicit features. The results showed that the Deep Belief Network (DBN) is usually used to create a user profile, the CNN is usually used to extract the image or visual features, and the autoencoder model is usually used to find latent or implicit features.

Due to the recent growth in online customer data, tag-aware RS is attracting more attention. Based on this, A systematic review are conducted (Shoja and Tabrizi, 2019) provides the challenges and problems related to developing the recommendations system, the application areas, the proposed methodologies, the evaluation criteria used to evaluate performance, limitations, and defects that require investigation and improvement. The results indicate that CNN significantly outperforms traditional approaches of tag capture. In conclusion, the tag-aware RS is witnessing the interest of researchers in recent years and given the lack of literature reviews that have been conducted in this field, from our point of view, the field requires more studies to summarize the progress made and application domains. In addition, to the advantages, problems, and evaluation metrics of the TRS. Thus, quantitatively assessing and discussing the findings and inferences that we reached to contribute to TRS development and provide new research directions in the future.

Tag-Aware Recommender System

Tags allow information to be retrieved and shared in the future to determine user preferences. In this section, we will review suggested approaches for establishing a tag-aware recommendation system, application areas, evaluation metrics used to evaluate the performance of the proposed model, advantages and problems related to the development of the recommendations system.

A. Tag-aware Recommender System Approaches: The approaches analyze user data based on tags to help users find the items they want by producing a predicted likelihood score or a list of top-N recommended items (Bogers, 2018). In this part, the techniques used will be reviewed and categorized into traditional approaches and deep learning approaches.

1) Traditional Approaches: Traditional approaches have played a key role in helping users to make decisions, such as collaborative filtering, content-based models, and hybrid filtering approaches.

a) Collaborative filtering: To Taking users' preferences advantage, Collaborative filtering (CF) approach is used. Which is the most widely used by assume the same interest of users. . CF is categorized into memory-based and model-based methods. User-based and item-based methods are Memory-based methods. based on similar ratings of users the user-based methods are depend on the target , while item-based methods depend on ratings of similar items given by the user. (Shoja and Tabrizi, 2019).

b) Content-based filtering: Content-based recommendation systems use information about the items stored in tags. The

similarity between items consumed by the user and other available items are measured by the system to find item similar to the item liked by the user .(H. Liu, 2018).

c). Hybrid Approaches: Different recommendation algorithms are collected to create a recommendation algorithm that can take advantage of the algorithms' strengths and mitigate their weaknesses, as clustering-based methods deal with redundancy in tags and ease ambiguity when there is a vague word (Shepitsen et al., 2008).

2). Artificial Intelligence Approaches: Machine learning and deep learning plays a significant role in extracting hidden patterns from data for building effective and dynamic behavior modeling in RSs. Convolutional neural network (CNN), recurrent neural network (RNN), and attention models are an examples of neural networks. Which have been used recently to deal with tag-aware recommendation systems problems. In addition to address the traditional approaches limitations (Shoja and Tabrizi, 2019).

B. Applications of Tag-aware Recommender System: There are many areas of application of the tag recommendation system to provide improvements that help users in making decisions, which we will review in this section.

1) E-learning: A tag-based recommendation system assists e-learning that helps in providing suggestions to users, such as finding relevant educational materials that match the time and content based on the availability of information (Tang and McCalla, 2005). In (Tang and McCalla, 2005), a web-based learning system model based on collaborative filtering and data clustering are developed, to provide intelligent and adaptive recommendations based on system feedback and adaptive recommendations based on system feedback of learners' activities throughout their learning period and the cumulative assessments made by learners.

Social Media: The popularity of social content published online is significantly influenced by tags. Tag suggestion systems assist users in tagging their submitted photographs, increasing the likelihood that they will become popular (Zhang et al., 2017). Many studies have been conducted to improve the accuracy of social media recommendations based on tags. A framework based on collaborative filtering has been proposed (H. Liu, 2018), and several machine learning models have been developed (Zhang et al., 2017) and (Xu et al., 2018). Furthermore, studies have been conducted to develop models based on deep learning and neural network (Li, Huang and Zhong, 2018), (X. Chen et al., 2020). To improve the performance of systems, machine learning models have been proposed (Pan et al., 2021) and (Xu et al., 2018), also the deep learning (Huang et al., 2020). On the other hand, studies have been conducted to recommend images using collaborative filtering in (Mauro and Ardissono, 2019), and recommend images and videos based on tag-aware deep learning in (Malmström, 2019). In addition, to recommending restaurants and food, the cooperative liquidation model in (Cagliero, Fiori and Grimaudo, 2014).

3). Movies: The tags are used to develop recommendation systems to help movie and series providers to make

recommendations appropriate to users' interests (Kim and Kim, 2014). Tag-aware movies recommendations are an active research domain, as both traditional and deep learning approaches have been used. A new model has been proposed for the collaborative filtering approach, which is one of the famous traditional methods (Bang and Lee, 2016), (Kim et al., 2011). While in (Kim and Kim, 2014) a hybrid framework has been proposed. The tag-aware based on deep learning enhances the movies recommendation system to overcome the problems of traditional approaches, as many studies have been conducted to present proposals to achieve this goal (Liang et al., 2018), (Huang et al., 2020) and (B. Chen et al., 2020). Furthermore, a Tag-aware recommender system based on a deep reinforcement learning model is proposed in (Zhao et al., 2021).

4) Music: Social tagging is one of the most important sources of essential information for developing recommendation systems in music. Moreover, they are considered the cornerstone of the algorithms of recommendation systems based on the similarity of tags, taking into account several considerations such as time periods, the name of the band or singer, etc. (van den Oord, Dieleman and Schrauwen, 2013). Collaborative filtering is the most widely used approach based on tag-aware music recommendation systems (Tso, Marinho and Schmidt-Thieme, 2008), (Chen et al., 2021)–(Li et al., 2019) and (Jäschke et al., 2007), and a hybrid approach has been proposed in (Zheng et al., 2018). Moreover, the machine learning approach has been applied in (Pan et al., 2021) and the deep learning approach in (B. Chen et al., 2020) and (Huang et al., 2020).

5). Tourism: Photographs displaying motion and paths shared by photographers can be utilized to make route recommendations based on geo-tagging, as they contain sequential spatial-temporal information and implicitly contain spatial semantics (Cai, Lee and Lee, 2018).

C. Advantages of Tag-aware Recommender System:

Tag recommendation Systems help users with the manual commenting effort of tagging by recommending tags to them. Tags are helpful because they give RS useful supplemental information as a flexible and effective method of managing information by summarizing item characteristics and reflecting user desire. Tags act as a bridge to create an implicit relationship between users and items by assigning several personal tags (Huang et al., 2020).

D. The Problems of Tag-aware Recommender System:

There are two main sub-problems with tag recommendations. There are the object-centric problem and the personal problem (Malmström, 2019). The object-centric approach in recommender systems aims to suggest relevant tags to an object and then recommend the same tags to another object regardless of the user (Malmström, 2019). This problem revolves around parsing a specific object. As for the other problem, the system will also consider the user. This means that different users will get different recommendations for the same object depending on the history of interactions with the recommendation system (Malmström, 2019).

Another problem related to the tag recommendation system that must be solved separately is the cold start problem (Malmström, 2019). It is a common problem in this type of system and is also called an out-of-matrix recommendation problem. Indicates that the element does not have tags already added (Singh, Chuchra and Rani, 2017). This is a problem in associative tag recommendation systems that rely on pre-added tags. A cold start problem can also refer to a person who hasn't rated anything yet, or to a new item that no one has rated yet (Singh, Chuchra and Rani, 2017), (Ricci et al., 2011).

E. Evaluation Metrics: Numerous metrics may be determined depending on the characteristics of the issue at hand and the suggested model to assess how well various methods for developing a tag-aware recommendation system operate. The performance evaluation measures are reviewed in this section in the manner listed below:

$$recall@N = \frac{\# \text{ of recommended resources @N that are relevant}}{\text{total \# of relevant resources}} \quad (1)$$

By recommending tags to users, tag suggestion systems make it easier for users to tag items without having to manually remark on them. Tags are advantageous as they provide valuable supplementary information to RS as a flexible and efficient approach to information management by summarizing the properties of items and reflecting user preferences. Tags act as a bridge to create an implicit relationship between users and items by assigning several personal tags (Huang et al., 2020). Equation (1) is recall@N, representing the proportion of relevant resources found in the top-N recommendations (Pan et al., 2021).

$$precision@N = \frac{\# \text{ of recommended resources @N that are relevant}}{\# \text{ of recommended resources @N}} \quad (2)$$

Equation (2) is precision@N which is the proportion of recommended resources in the top-N set that are relevant (Pan et al., 2021).

$$F1@N = \frac{2 \cdot precision@N \cdot recall@N}{precision@N + recall@N} \quad (3)$$

Equation (3) is F1- measure@N, which is a harmonic mean of recall@N and precision@N and becomes a comprehensive indicator (Pan et al., 2021).

$$MRR = \max_{q \in Q} \frac{1}{C_q} \quad (4)$$

Equation (4) is The system's capacity to return relevant tags at the top of the ranking (or the quality of top suggested tags) is demonstrated by Mean Reciprocal Rank (MRR), where C_q indicates the rank attained by relevant tag q (Mauro and Ardissono, 2019).

$$RK(u)@k = \sum_{i \in Test(u) \cap Top-k(u)} \frac{1}{rank(i)} \quad (5)$$

Equation (5) is the Ranking accuracy of user u at top-k ranking, $RK(u)@k$, is a metric that is used to demonstrate

if a tag with a better rank is actually more relevant, where rank(i) denotes the rank of item i in top-k list (Kim et al., 2011).

$$S@k = \begin{cases} 1 & \text{if } Q \cap C_k \neq \emptyset \\ 0 & \text{otherwise} \end{cases} \quad (6)$$

Equation (6) is Success at Rank k (S@k) is the probability of finding a relevant tag, $q \in Q$, in a set of top-k recommended tags, C_k (Cagliero, Fiori and Grimaudo, 2014).

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (t_i - p_i)^2} \quad (7)$$

Equation (7) is Root Mean Squared Error (RMSE), Where t_i is the test rating value and p_i is the predicted rating value (Kim and Kim, 2014).

$$MSE = \frac{1}{n} \sum_{i=1}^n |t_i - p_i| \quad (8)$$

Equation (8) is Mean Absolute Error (MSE). To return recommendations as important evaluation metrics when facing a real-time application problem or when there is a large amount of data to computation, it considers the computation time and cost for a system. (Font, Serra and Serra, 2015).

Quantitative Assessment

This section will present a comprehensive evaluation of the scientific papers in the field of the Tag-Aware Recommender System, which were collected in a certain period of years, from 2004 to 2022. The number of collected papers reached 33 scientific papers. We will display the papers and evaluate them according to different categories, including the domain, the type of publication, a journal, a conference, or periodicals. Also, the dataset, the technology used in each paper, and another category. Table 1 presents the papers and assessments for each paper in detail for all categories. We started by presenting the types of papers over the years in Fig. 1.

Through the assessment, we note that the actual increase in the publication of scientific papers starts from 2014, and before this year the publication of papers is considered very few. Most papers have been published in the journal type, with the fewest being periodicals. Where the percentage of journal papers reaches 73%, and the percentage of papers published from the conference type reaches 23%, and the percentage of periodical papers is 4%, and this is illustrated in Fig. 2. Next, we examined the papers according to the techniques used in the papers. Several techniques appeared through our analysis in Table 1, which are Deep Learning, Collaborative Filtering, Machine Learning, Deep Reinforcement Learning, and Hybrid approach.

The result showed that the most used techniques in

scientific papers are three techniques, which there is a slight percentage among them, they are Collaborative Filtering, where the percentage reaches 29%, followed by Deep Learning by 25%, and then Machine Learning by 21%.

The other techniques are little in use compared to the three mentioned techniques shown in Fig. 3 as a pie chart. After that, we examined the papers in different fields. The number of fields has reached 12 different fields covered by scientific papers. The most covered fields are music with 26%, followed by movies with 19%, then social media with 17%. Fig. 4 illustrates this with the other percentages of other fields as a pie chart. Finally, we examined the papers in terms of the databases used. Fig. 5 illustrates the distribution of the datasets used as a pie chart. It appears that the frequently used datasets are Last.FM and MovieLens, as it appears that 20% of the papers use Last.FM, and 17% use MovieLens.

Figure 1: Types of Publications Over Years

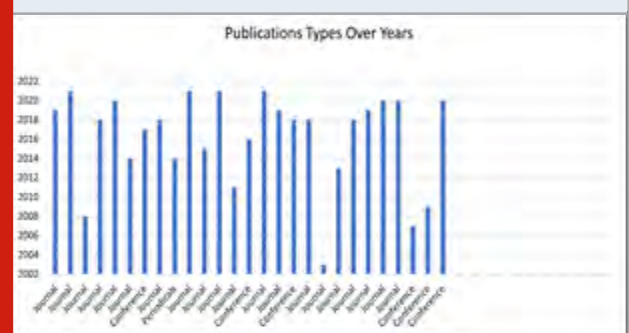


Figure 2: Distribution of Publications by Their Types

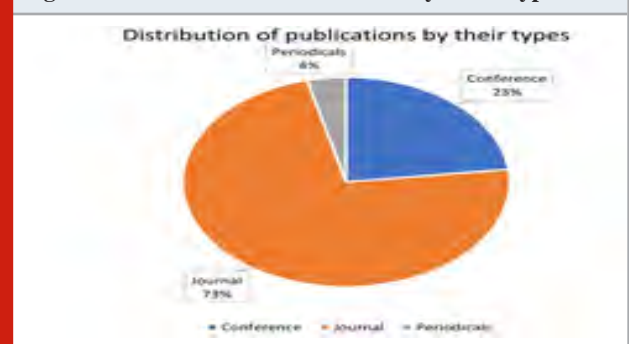


Figure 3: Distribution of Techniques

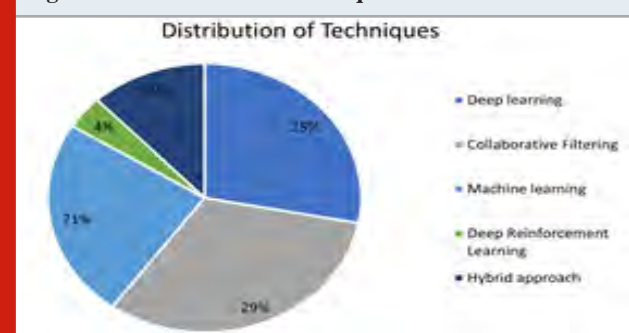


Figure 4: Distribution of Domain

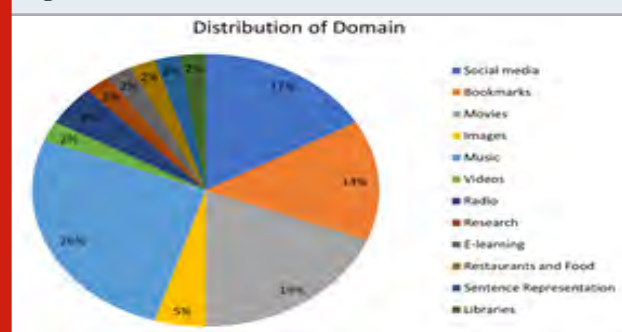
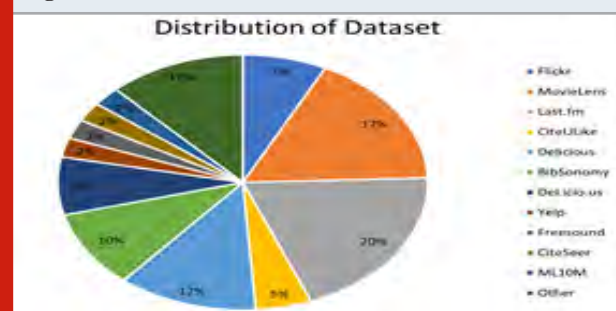


Figure 5: Distribution of Datasets



Insights And Discussions

The approaches used for tag-aware recommendation systems varied in different fields and on various data sets.

This section discusses our findings and conclusions and provides the reader with insights based on the general analysis of the tag-aware recommendation systems throughout the study period.

Table 1. Comprehensive Literature

Ref	Type of Paper	Domain	Techniques	Model	Dataset used	FINDING/ RESULTS
(Malmström, 2019)	Journal	Videos Images	Deep Learning	Deep learning model (a deep learning hybrid (content- and tag cooccurrence-based) tag recommender system) and a baseline model (a hybrid model combining content and tag cooccurrence.)	Flickr	Deep learning can be used to successfully model tag co-occurrence both separately and jointly together with content information.
(Zhao et al., 2021)	Journal	Movies	Deep Reinforcement Learning	Proposed a tag-aware recommender system based on deep reinforcement learning without complex function design	MovieLens	The experiment proves that the recommendation algorithm used in this study has smaller errors, and it also has a beneficial effect on the overfit problem
(Tso, Marinho and Schmidt-Thieme, 2008)	Journal	Radio Music	Collaborative Filtering	Propose a generic method that allows tags to be incorporated into standard CF algorithms	Last fm	Adapted fusion method has successfully captured the relationships between users, items, and tags
(Liang et al., 2018)	Journal	Movies	Deep Learning	Tag-aware recommender system based on deep learning (TRSDL) for rating prediction task	MovieLens	TRSDL is effective and competitive for rating prediction tasks. It improves traditional collaborative filtering methods and performs better than the state-of-the-art models on this dataset.
(A. Mohamed Hassan, Sansonetti and Micarelli, 2020)	Journal	Libraries	Deep Learning	Propose a hybrid approach that leverages deep semantic representation of research papers based on social tags assigned by users.	CiteULike	The proposed approach outperforms state-of-the-art collaborative filtering-based tech- proposed model shows the effectiveness of integrating deep semantic representation of research papers based on social tags with collaborative filtering.
(Kim and Kim, 2014)	Journal	Movies	Hybrid Approach	Hybrid item recommendation and a recommendation framework for social tagging systems	MovieLens	For less active users, as we expected, the hybrid approach performs better than other methods.
(Zhang et al., 2017)	Conference	Social media	Machine Learning	Proposed two tag ranking algorithms, Document Frequency-Weights from regression and Folk Popularity Rank	Flickr	1- FP-Rank makes better recommendations with a higher level of influence on popularity boosting over the other three tag recommendation methods. 2- FP-Rank has better effect on popularity boosting in the unpopular test set.

Continue Table 1

(H. Liu, 2018)	Journal	Social media	Collaborative Filtering	Propose a tag-based recommender system framework, a unified profile model (UPM) for social bookmarking websites	Delicious, BibSonomy	The experiment results show that the proposed recommender framework achieves higher performances than the baselines and it is more flexible and scalable.
(Movahedian and Khayyambashi, 2014)	Conference	Bookmarks	Collaborative Filtering	a new recommender system is proposed based on the similarities between user and item profiles	Delicious	Experimental results demonstrate that the proposed approach provides a better representation of user interests and achieves better recommendation results in terms of precision and ranking accuracy as compared to existing methods
(Chen <i>et al.</i> , 2021)	Journal	Radio Music		Propose a novel tag-aware top-n recommendation model AIRec	Last.Fm, Delicious	The result shows significant improvements of AIRec over state-of-the-art methods for tag-aware top-n recommendation.
(Font, Serra and Serra, 2015)	Periodicals	Music	Collaborative Filtering	Deeply analyze the impact of a tag recommendation system in the folksonomy of Freesound	Freesound	The results are that tag recommendation effectively increases vocabulary sharing among users of the platform. - tag recommendation is shown to contribute to the convergence of the vocabulary as well as to a partial increase in the quality of annotations.
(Xu <i>et al.</i> , 2021)	Journal	Sentence Representation	Deep Learning	Novel neural network model (TagHyperTreeLSTM)	Stanford Sentiment Treebank (SST2), Movie Reviews (MR), Sentences grouped as being either subjective or objective (SUBJ), TREC, SICK	The experiment results show that the proposed recommender framework achieves higher performances than the baselines and it is more flexible and scalable.
(Kim <i>et al.</i> , 2011)	Journal	Movies	Collaborative Filtering	Propose a new collaborative approach to user modeling that can be exploited to recommender systems.	The Internet Movie Database (IMDb)	Experimental results show that the proposed model achieves superior or competitive performance in text classification and text semantic matching based on six benchmark datasets when compared against previous tree-structured models.
(Bang and Lee, 2016)	Journal	Movies	Collaborative Filtering	Collective Matrix Factorization using Tag Embedding	MovieLens	The experimental results have shown the proposed model provides a better representation in user interests and achieves better recommendation results in terms of accuracy and ranking.
(Pan <i>et al.</i> , 2021)	Journal	Movie Bookmarks Music	Machine Learning	New social tag expansion model (STEM)	MovieLens, Delicious, Last fm, BibSonomy	The analysis and experimental results showed that the new STEM technique was able to correctly find a sufficient set of tags and to improve the recommendation accuracy by solving the tag sparsity problem. At this point, this technique has consistently outperformed state-of-art tag-aware recommendation methods in these extensive experiments.
(Mauro and Ardissono, 2019)	Journal	Restaurants and Food	Collaborative Filtering	Propose the Extended Category-based Collaborative Filtering (ECCF) recommender	Yelp	The evaluation showed that ECCF outperforms User-to-User Collaborative Filtering in accuracy, MRR, intra-list diversity and user coverage. - ECCS also obtains higher accuracy and diversity than the SVD++ recommender system, based on Matrix Factorization
(Li, Huang and Zhong, 2018)	Conference	Social media	Deep Learning	Propose a reconstruction method of tag-based profiles of users and items to enhance tag-aware recommendations	Delicious, Last.fm	The results show our method can achieve improvement of recommendation performance by leveraging reconstructive profiles of users and items.
(Xu <i>et al.</i> , 2018)	Journal	Social Media Bookmarking	Machine Learning	Proposed an effective ontological similarity measure that uses ontologies to solve the tag ambiguity problem and to semantically measure the similarity between user and document profiles.	Delicious	The experiments show that the proposed ontological similarity is semantically more accurate than the state-of-the-art similarity metrics
(Tang and McCalla, 2005)	Journal	E-learning	Collaborative Filtering	propose an evolving web-based learning system which can adapt itself not only to its users, but also to the open Web in response to the usage of its learning materials	CiteSeer	The system can retrieve relevant information related to users and their situated learning characteristics.

Continue Table 1

(Cagliero, Fiori and Grimaudo, 2014)	Journal	Images	Collaborative Filtering	A novel personalized tag recommendation system that discovers and exploits generalized association rules, that is, tag correlations held at different abstraction levels, to identify additional pertinent tags to suggest.	MIR Flickr 2008	The effectiveness of the proposed approach has been validated against a recently proposed tag recommendation system. Experiments show that the use of the generalizations in rule-based tag recommendation yields significant performance improvements.
(Zheng <i>et al.</i> , 2018)	Journal	Music	Hybrid Approach	A Gaussian state-space model coupled with low-rank matrix factorization	Last.fm	Experiments have been conducted over a large-scale real-world music data set and demonstrate the effectiveness of the proposed music recommendation framework.
(Li <i>et al.</i> , 2019)	Journal	Music	Collaborative Filtering	Propose a novel tag-aware recommendation framework by incorporating tag mapping scheme into ranking-based collaborative filtering model.	Lastfm, Citeulike	Experiments on real-world recommendation datasets show that the proposed recommendation method outperformed competing methods on ranking-oriented recommendation performance.
(B. Chen <i>et al.</i> , 2020)	Journal	Movies, music	Deep Learning	Propose a novel tag-aware recommendation model named Tag Graph Convolutional Network (TGCN)	MovieLens, Last.fm, Delicious	Extensive experiments demonstrate that TGCN achieves remarkable performance improvement compared with state-of-the-art models.
(Huang <i>et al.</i> , 2020)	Journal	Bookmarks Music Movies	Deep Learning	Tag-aware Neural Attention Model	Del.icio.us, Last.fm, Movie Lens	Experiment results demonstrate that TNAM significantly outperforms the state-of-the-art baselines in Top-N recommendation on the evaluation metrics of HR and NDCG.
(Jäschke <i>et al.</i> , 2007)	Conference	Bookmarks Music	Collaborative Filtering	-	Del.icio.us, Last.fm, BibSonomy	The straightforward collaborative filtering adaptation based on projections and an adaptation of the well-known PageRank algorithm named FolkRank.
(Gemmell <i>et al.</i> , 2009)	Conference	Bookmarks	Hybrid Approach	The hybrid recommender can surpass the effective graph-based approaches while retaining the efficiency of its parts.	Bibsonomy	Alone these recommenders perform poorly; together they achieve a cooperation which proves to be as effective as state-of-the-art tag recommenders. The hybrid recommender can surpass the effective graph-based approaches while retaining the efficiency of its parts.
(X. Chen <i>et al.</i> , 2020)	Conference	Social media	Deep Learning	Proposed a graph neural networks boosted personalized tag recommendation model (GNN-PTR)	Last.fm, ML10M	Experimental results show that our proposed method outperforms the state-of-the-art personalized tag recommendation methods.

- There has been an increase in studies from 2014 until now, but it is still not noticeable and fast. Therefore, the field still needs more attention from researchers.
- We noticed no diversity in the type of publication, as most papers are published in journals or conferences, while other types are almost non-existent or non-existent.
- The domains of application of the tag recommendations systems varied, but some domains witnessed more bias than others, such as music, movies, and social media.
- Collaborative filtering is one of the most used methods until now in tag recommendation systems. Enriching the user profile by collaborating with user profiles and other similar tags contributes to recommending new items.
- In recent years, tag-aware recommendation systems have witnessed great interest in developing deep and machine learning models to overcome the problems and challenges facing traditional approaches and improve accuracy.
- Deep learning techniques deal with cold start problems of tag recommender systems by extracting features from profile information and integrating them into the user's item preferences.
- Neural networks are a deep learning technique that has recently emerged in tag-recommendation systems by using tag-based profiles of users and objects to improve tag-aware recommendations. In neural network training, neural network methods need to be measured more effectively to balance tag-based profiles and abstract representations to improve the item recommendation further.
- One of the challenges facing tag-recommendation systems is users' unwillingness to share tags, leading to tag scattering. Therefore, the accuracy of recommendations is significantly at risk when few tags are attached to users or resources. Creating a dynamic user profile is a solution to improve the performance of the recommendation.

CONCLUSION

This survey aims to present the scientific papers related to TRS. 33 scientific papers were evaluated based on the field, type of publication, dataset, techniques, model, and results. As a result, the papers began to increase in 2014. 73% of the papers were published as a journal, and 29% of papers used collaborative filtering. The most covered area being music with 26%. And the most used dataset is Last.FM with 20%. The research related to TRS is few, and the number of publications has been few over the years. Also, scientific publications do not vary; it focus on a specific field more than others.

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Cucurbitacin Compounds Against Estrogen Receptor: Literature Review

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ABSTRACT

Over the past few decades, extensive research in the field of carcinogenesis has been the toughest challenge in finding newer drugs. One of the leading causes of death in women worldwide is breast cancer. Cucurbitacin is one such compound identified to suppress the oncogenic signalling pathways for survival. JAK/STAT pathways were identified for tumour growth as one of the key targets for cucurbitacin. Mainly, the compound cucurbitacin Q against estrogen receptors could be a target of concern among researchers around the globe. The structured review of cucurbitacin was documented by retrieving the data from various literature reports, review articles and research papers published on the PMC platform. In context with the fascinating role of cucurbitacin Q against estrogen receptors, it inhibits the tumour progression by blocking the STAT3 pathway. Cucurbitacin Q induces apoptosis in the tumour that activates the STAT3 gene when compared to other genes, which were found to be susceptible to breast cancer cell lines. Therefore, Cuc Q finds itself a new way of intervening with the JAK/STAT3 pathway by suppressing the progression of the tumour. Increased production of Cuc Q if proved to be active against oncogenes by blocking the STAT3 pathway. This article discusses the background, chemical structure and biological mechanism of cucurbitacin Q compound against estrogen receptors for breast cancer treatment.

KEY WORDS: CARCINOGENESIS, CUCURBITACIN, ESTROGEN RECEPTOR, MECHANISM AND ONCOGENE.

INTRODUCTION

Medicinal plants are found all over the globe for the benefit of mankind. The plant consists of various secondary metabolites found with different compositions in various parts of the plant. Mainly potential secondary metabolites from the traditional medicinal plant are responsible for different disorders, ailments and other treatments. Natural products are familiar for exerting anti-tumour activities partly based on their ability to lessen ROS (Reactive Oxygen Species) and to defend critical cellular components like DNA, proteins and lipids from oxidative damage (Rafter 2002). The pharmaceutical market needs to be updated with newer drugs for developing effective treatments against deadly diseases (Gupta and Kohli 2019). Developing treatment plans for cancer is an unending struggle, but relapses and treatment-related complications continue as the main impediment (Miladiyah et al. 2020).

To eliminate the possible failures in the drug development stage several approaches such as *in silico*, *in vitro*, *in vivo*, and cell lines are being practised (Umar et al. 2020). Estrogen receptor alpha

(ER α) and progesterone receptor (PR) are found in cancer cells of the breast. If the breast cancer cells have estrogen receptors, the cancer is called ER-positive breast cancer (Altwegg and Vadlamudi 2021). ER, signalling is a key driver of ER + breast carcinogenesis and inhibition of ER signalling is the mainstay of ER + BC therapy and has enhanced the patient's survival rate (Scabia et al. 2022). This review focuses on E2 binding to membrane-bound ER α and ER β receptors that swiftly stimulate nuclear transcription factors via the MAPK pathway and other pathways involved. The rationale of the study includes molecular targets, especially like JAK2/STAT3 pathway for tumorigenesis where such cucurbitacin compounds could prove to inhibit these pathways (Scabia et al. 2022).

Cancer: Cancer is a disease resulting in abnormal growth of cells and uncontrolled multiplication of cells within the body (Pushpalatha et al. 2017). According to World Health Organization (WHO), cancer is one of the second main reasons of mortality around the globe with about 9.6 million death in 2018 (Bray et al. 2018). Mostly the cancers are treated through chemotherapy. Drugs from herbal sources are low in toxicity, low cost and bioavailable. The most common cancers are lung, liver, colorectal, stomach and breast cancer (WHO 2017; Scabia et al. 2022).

Article Information:*Corresponding Author: gandepallipratap@gmail.com
Received 18/09/2022 Accepted after revision 25/11/2022
Published: Dec 2022 Pp- 494-500
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Available at: <https://bbrc.in/> DOI: <http://dx.doi.org/10.21786/bbrc/15.4.2>

The International Agency for Research on Cancer (IARC), a unit of WHO reported that 28 types of cancer are found in 184 countries and this is alarmingly increasing (Kumar et al. 2020). Breast cancer (BC) is the most common sort of tumour mostly in females, yet metastases are the key reason for deaths (Cava and Castiglioni 2020). Breast cancer chemotherapy is marked by pointing to the role of receptors such as ER α (Estrogen Receptor alpha), PR (Progesterone Receptor), EGFR (Epidermal Growth Factor Receptor) (Acharya et al. 2019). Sahayarayan et al. (2021) reported that over 60% of breast cancer cases are diagnosed as estrogen receptor alpha positive (ER α) cancers mainly in Asian countries. In humans, both alpha and beta estrogen receptors are revealed and many studies are focused on these ER receptors (McDonnell et al. 2015).

Regarding the issue, many researchers have focused on finding highly sensitive and specific markers for the initial detection of breast cancer (Yan et al. 2015). Moreover, the anticancer effects of cucurbitacin compound on different tumour types like neuroblastoma, breast cancer, lung cancer, endometrial cancer and hepatocellular carcinoma have been well studied and documented (Si et al. 2019). We provide a framework of the main estrogen receptor and various oncogenic pathways which regulates the processes of human tumorigenesis (Scabia et al. 2022).

Estrogen Receptor (ER): In humans, alpha and beta estrogen receptors were described, and many studies focused on these receptors (McDonnell et al. 2015). The increased production of estrogen is one of the main foremost causes of breast cancer (Sahayarayan et al. 2021). In women, ER (Figure 1) plays a vital part in apoptosis, inflammation, homeostasis, differentiation, maturation, metabolism and proliferation in breast cancer (Bai and Gust 2009). Several studies have reported that estrogen, in specific 17 β -estradiol, has been reported to up-regulate the expression. Also, the purpose of c-Myc and cyclin D1 genes is to lead the promotion of the cell cycle from G1 phase to S phase in the epithelial cells of mammary glands (Acharya et al. 2019; Scabia et al. 2022).

Figure 1: Three-dimensional (3D) structure of Estrogen receptor protein (PDB ID: 3ERT)



The hyperactivity of ER- α in the mammalian cells leads to the conservation and growth of types of breast cancers and also holds many molecular targets for the study of cancer drugs (Sahayarayan

et al. 2021). Peng et al. (2009) reported that nearly 60% of premenopausal women and about 75% of post-menopausal women have suffered from estrogen-dependent breast cancer, and ER- α activity was efficiently inhibited using cancer therapy. Even though a lot of anticancer drugs and potential inhibitors against various targets are available, the effective surge in resistance along with side effects indicates that there is an urgent need for novel tumour therapy (Sahayarayan et al. 2021).

Women with breast cancer who took tamoxifen treatment are at higher risk. They also have an increased occurrence of endometrial cancer but a reduced amount of certain bone fractures and a dramatic 45% diminution in the incidence of breast cancer (Shaiu et al. 1998). Still, a lot more research is to be done, and is tremendous progress toward finding a cure for ER- α of breast cancer. Bernards (2012) reported that the vital issue is the lesser dependability on cell lines that predicts the efficacy of drugs since cell lines didn't confirm to be a perfect model. Therefore, computational methods are in demand to expand the potential role of a drug in the context of the pathway (Cava et al. 2018; Scabia et al. 2022).

Molecular Mechanism of Cucurbitacin and Cucurbitacin Q:

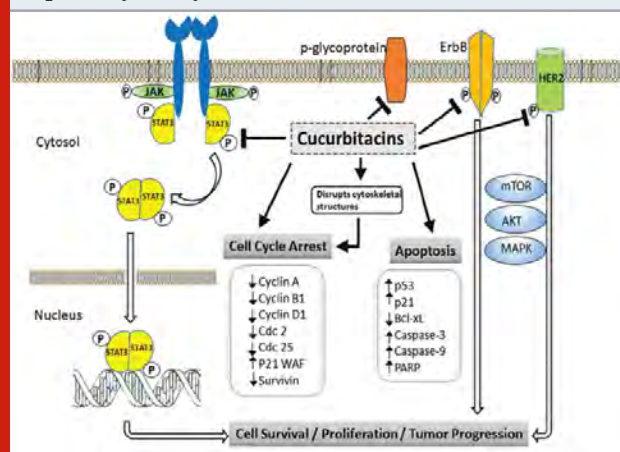
There are many oncogenic signaling pathways that are frequently included in cancer cell proliferation and survival. Recent studies have uncovered that several molecular targets of cucurbitacin such as the JAK2/STAT3 pathway, cofilin, cyclins, cdc2, COX-2, TYR and EcR among which actin cytoskeleton appears to be a prime target (Blaskovich et al. 2003; Chen et al. 2012). The JAK/STAT (Signal Transducers and Activators of Transcription) pathway (Figure 2), Akt-PKB pathway and MAPK Pathway are significant pathways in cancer cells and are also targets of the *Cucurbitaceae* family (Lee et al. 2010). In many cancer cells, activation of STAT3 and STAT5 has been known to play key roles in tumorigenesis (Yu and Jove 2004). During the initial findings, reports revealed that Cuc I is a dual inhibitor of STAT3 and JAK2 pathways but didn't affect any other oncogenic signaling pathways such as Akt-PKB or MAPK (Blaskovich et al. 2003; Scabia et al. 2022).

In cancer cells, cucurbitacin compounds labour as STAT3 inhibitors and make cells prone to the attack of reactive oxygen species (ROS) and free radicals during inflammation (Jayaprakasam et al. 2003). Inhibition of the IKK/NF- κ B pathway by cucurbitacin relies on the inhibition of key inflammatory enzymes, like cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) whose overproduction leads to tumorigenesis (Jayaprakasam et al. 2003; Park et al. 2004; Escandell et al. 2007). Although other mechanisms like activation of the MAPK pathway leading to cancer cell proliferation and survival, another study revealed that only STAT3, but not the MAPK pathway which was impaired in breast cancer cells when treated with cucurbitacin E compound (Lan et al. 2013; Alsayari et al. 2018).

However, cucurbitacin F, O, P, and Q and their derivatives were identified to have the finest anticancer activity (Chen et al. 2005). Alghasham (2013) reported that cucurbitacin Q induces apoptosis more effectively in human and murine tumours. It selectively

blocks the activation of STAT3 and induces apoptosis without inhibiting JAK2, Src, Akt, Erk or JNK. A study showed that in nude mouse tumour xenograft model, Cuc Q, but not Cuc A suppresses tumour growth signifying that JAK2 blockage alone is not enough but suggests the competence of Cuc Q to impede tumour growth that is linked to anti-STAT3 activity (Sun et al. 2005). Among the two, Cuc A was shown to be an inhibitor of the JAK2 pathway, whereas Cuc Q induces apoptosis and inhibits tumour growth that contains activated STAT3 (Sun et al. 2005; Scabia et al. 2022).

Figure 2: The mechanistic inhibitory activity of cucurbitacin in pathway (Alsayari et al. 2018)



Bernard et al. (2010) reported that conversion of the C3 carbonyl of the cucurbitacin to a hydroxyl result in loss of anti-JAK2 activity, whereas the addition of a hydroxyl group to C11 of cucurbitacin results in loss of anti-STAT3 activity. Also, Cuc Q persuades cell death more potently in human and murine tumours which constitutively activates STAT3 (A549, MDA-MB-435 and v-Src/NIH3T3) when compared to other (H-Ras/NIH 3T3, MDA-MB-453 and NIH 3T3 cells) (Bernard et al. 2010). Therefore, suppression of oncogene STAT3 seems to be related to blocking the tumour which doesn't eliminate alternate mechanisms (Zhang et al. 2004; Chan et al. 2010b; Yasuda et al. 2010; Scabia et al. 2022).

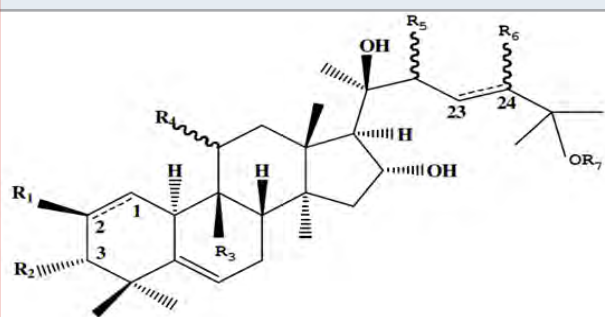
Cucurbitacin Q: Cucurbitacin (Figure 3) are one synthesized chemically by tetracyclic cucurbitane (triterpene hydrocarbon) nucleus skeleton 19-(10 α 9 β)-abeo-5 α -lanostane base, altered by the positional replacement of oxygen atom (Sharpless et al. 2002; Kaushik et al. 2015). Overall, there are notably 40 identified species of cucurbitacin and their derivatives, that are classified into 12 groups namely A, B, C, D, E, I, H, Q, R and dihydrocucurbitacin B (Alghasham 2013). Other variations of cucurbitacin are now under exploration for their potential as anticancer drugs (Cai et al. 2015; Garg et al. 2017).

Different studies have revealed by examining the effects of these compounds in several cell lines including *in vitro* and *in vivo* against diverse malignant subtypes. Figure 4 shows the compound cucurbitacin Q (formula: C₃₂H₄₈O₈, molecular weight: 560.7 g/mol) identified in the plants of *Cucurbitaceae* and other families with peculiar biological properties (Garg et al. 2017).

Jayaprakasam and co-researchers revealed the cytotoxic properties of cucurbitacin B, D, E and I identified from the fruits of *Cucurbita andreana* against colon, breast, lung and CNS cancer cell lines (Jayaprakasam et al. 2003). Interests in cucurbitacin have developed in recent years and countless studies have demonstrated that analogues of Cus have a wide variety of therapeutic activity that includes, hepatoprotective, anti-cancer and anti-inflammatory activities (Rios et al. 2012). Yet, thorough molecular mechanisms underlying their biological activity remain indescribable (Zhong et al. 2019). A recent study confirmed that *Cissampelos pareira* contains a substantial amount of Cuc Q compound with antiproliferative activity (Amresh et al. 2007; Thavamain et al. 2014). Ali et al. (2019) reported that it doesn't signify that the anticancer potential of the *C. pareira* plant is only due to the existence of a large amount of cucurbitacin Q compound as it requires further research (Ali et al. 2019).

Cucurbitacin Q – Sources: In general, other families of *Scrophulariaceae*, *Begoniaceae*, *Primulaceae*, *Liliaceae*, *Tropaecolaceae* and *Rosaceae* contain cucurbitacin apart from the *Cucurbitaceae* family (Ali et al. 2019). The seeds of certain cruciferous plants such as *Iberis* species and *Lepidium sativum* to comprise the cucurbitacin compound (Teuscher and Lindequist 1994; Ali et al. 2019). *Cucurbitacin* Q has been isolated from plants of different families and genera around the globe for research findings (Table 1). The bioactivity of cucurbitacin Q showed its activity on cancer cells in lung A549 human and murine cancer A549, MDA-MB-435 and v-SRV/NIH 3T3 isolated from *Cayaponia tayuya* (Hernandez et al. 2015). Sun et al. (2005) reported that cucurbitacin Q is susceptible to breast cancer cell lines: MDA-MB-435, MDA-MB-453 (Sun et al. 2005; Ali et al. 2019).

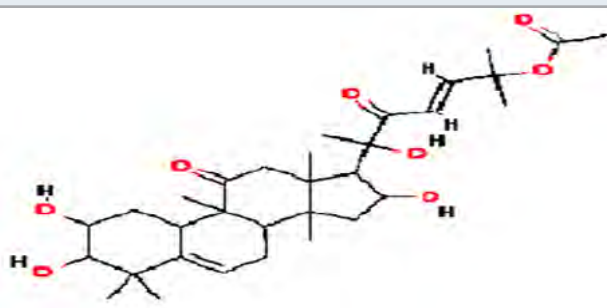
Figure 3: The chemical structure skeleton of main cucurbitacin (Cai et al. 2015)



Cucurbitacin Q – Toxicity: Cucurbitacin belongs to the terpenoids class of various compounds found in the plants of the *Cucurbitaceae* family which has both medicinal and toxic properties (Ali et al. 2019). Gry et al. (2006) reported that the main reason for cucurbitacin being cytotoxic *in vitro* might be due to the compounds influencing cell adhesion to culture vessels. In addition, giving rise to the cytotoxicity of the compound and receptor interaction, isolated cucurbitacin or extracts comprising cucurbitacin have proved to contain biological effects *in vitro* (Gry et al. 2006; Ali et al. 2019). Especially, very minute concentrations of Cuc E and Cuc B, less than 1 μ M was shown to inhibit the

adhesion of transformed B cells (Musza et al. 1994; Ali et al. 2019). As Raikhlin-Eisenkraft and Bentur (2000) point that various factors can affect the toxicity of the cucurbitacin compounds. Most of the cucurbitacin-containing plants have been documented where the plants or extracts were screened for cytotoxicity in a battery of human tumour cell lines (Gry et al. 2006; Ali et al. 2019).

Figure 4: Structure of cucurbitacin Q compound



Cucurbitacin Q – Biological Supply Chain and Future Scope:

Due to the extreme bitterness of cucurbitacin, plants comprising these compounds would usually not be consumed. The biological activity of cucurbitacin including its pharmacological effect has been analysed from traditional medicinal plants as an active principle (Gry et al. 2006; Ali et al. 2019). The existence and production of cucurbitacin Q have to be extensively studied in future and also about the efficiency of the drug. Over the last few decades, the Cuc Q compound has been shown to block the STAT3

pathway which inhibits tumour progression. Another prominent modification was that cucurbitacin researchers began to examine the biological mechanism of action of cucurbitacin at the molecular level (Lee et al. 2010; Ali et al. 2019).

In silico Analysis of Compounds on Breast Cancer Cell

Receptors: A study on molecular docking of compounds that reported its presence in fungal endophytes of *Chaetomium* sp as one of the cytotoxic agents against breast cancer protein (HER α – 1G50). The results revealed that 2 compounds bearing xanthone and benzonaphthyridinedione scaffolds as hit ligands (Hariono and Rollando 2016).

Tamoxifen is an antagonist of ER- α and commercially available as a medicine to inhibit the growth of breast cancer (Jordan 1992). It binds with ARG 394 and blocks the role of ER (Desai et al. 2012). Recently, screening of bioactive compounds from *Phyllanthus emblica* namely quercetin, kaempferol, kaempferol 3-beta-D-glucopyranoside, isocorilagin and 1,1-diphenyl-2-picrylhydrazyl which showed good binding affinity of -7.57 kJ/mol, -7.66 kJ/mol, -6.77 kJ/mol, -7.90 kJ/mol and -5.06 kJ/mol respectively against ER (3ERT) protein (Afrin et al. 2018). A recent study showed that among the phytochemicals like anthocyanins, isoflavone and carnosol with ER, carnosol compound revealed to inhibit with higher binding affinity of -12.1 kJ/mol (Pandian et al. 2014). Among the compounds, SANDB_11243993 has the highest binding affinity of -14.253 kcal/mol against 3ERT protein (Sahayarayan et al. 2021). Paclitaxel, a compound showed good binding interactions with the target proteins in the order ER > PARP 1 > AKT 2 > CDK 6 > HER 2 (Kumar et al. 2020).

Table 1. Potency of cucurbitacin Q against cancer

S. No	Plant sources	Presenting condition	Activity explored	References
1.	<i>Helicteres isora</i>	anticancer	Anti-tumour/	Cai et al. 2015
2.	<i>Anagallis arvensis</i>		STAT3 pathway	
3.	<i>Gurania Subumbellata</i>			
4.	<i>Picrorhiza Kurrooa</i>			
5.	<i>Cissampelos pareira</i>	anticancer	Anti-tumour	Bala et al. 2015, Bala et al. 2019
6.	<i>Citrullus colocynthis</i>	anticancer	Anti-tumour	Al-Snafi 2016, Hussain et al. 2014
7.	<i>Wilbrandia species</i>	anticancer	Anti-tumour	Matos et al. 1991
8.	<i>Ecballium elaterium</i>	anticancer	Anti-tumour	Chen et al. 2005

In our current study, molecular docking of protein with ligands were studied to analyze the interactions and among the cucurbitacin compounds, cucurbitacin Q has shown to inhibit the ER- α protein (3ERT) which showed maximum docking score of -9.3 kcal/mol. Among the compounds, cucurbitacin paved a way much into the development of drug in near future. Predicting the drug candidates for pharmacokinetic and dynamic profile early in the drug development preparation which is the key aspect of ADME was tested using SWISS software (Daina et al. 2017). Molecular dynamic simulation studies of the protein-ligand complex are under process using the NAMD software (Phillips

et al. 2005) to predict the stability of the molecule or compound (Ali et al. 2019).

Cucurbitacin Q – *In vitro* Production Studies: To increase the biomass and yield of cucurbitacin Q, *in vitro* production studies have to be implemented from traditional medicinal plants using plant tissue culture technology as an alternative source. Enhanced accumulation of total cucurbitacin content was shown to be higher than the parent plant. This *in vitro* secondary metabolite production from medicinal plants was considered a suitable alternative method compared to whole plant extraction (Devendra

et al. 2012). Till now up to date, there is no report on increased production of cucurbitacin Q compound from medicinal plants. Further, investigation of the effect of plant growth regulators and elicitors plays a vital role in the biosynthesis of cucurbitacin Q and their intermediates (Ali et al. 2019).

Biosynthetic Pathway of Cucurbitacin: Balliano et al. (1983) have explored the biosynthesis of cucurbitacin glycosides from squalene-2,3-epoxide to the final cucurbitacin, aiming at the possible routes for biosynthesis (Figure 5). The occurrence of 10 α -cucurbita-5,24-dien-3 β -ol in many seeds of food plants has been revealed. This compound is now considered a primary intermediate in the biogenesis of cucurbitacin (Akihisa et al. 1986; Ali et al. 2019). The most probable change of lanostane C-9 carbonium ion (3) to cucurbita-5,24-dienol (5) was examined as the precursor. The fact that two pentacyclic compounds, glutinol and simiarenol are frequently found together with cucurbita-5,24-dienol is taken as support for the biosynthetic route of triterpenoid compounds (Balliano et al. 1983; Ali et al. 2019).

CONCLUSION

The findings of the present review has shown that over several decades, this neglected compound is gaining attention as a potential anticancer drug. Cucurbitacin Q is an under-explored compound which can be verified and worth due to its cytotoxic potential activity against cancer and other activities. Through this study, the suppression of STAT3 and JAK2 in the JAK/STAT pathway can be deactivated by inhibiting its process using Cuc Q and Cuc A and is an excellent candidate for clinical investigation. Moreover, studies related to the genetic mice tumour model should be considered to assess the active anticancer activities of cucurbitacin Q in the STAT3 pathway which inhibits tumour progression. Subsequently, it caused growth arrest, apoptosis, cellular differentiation and blockage of proliferation in cancer cells. Finally, clinical trials for Cuc Q as the targeted compound for the anticancer agent as an independent effector.

Conflicts of Interests: Authors declare no conflict of interests to disclose.

Data Availability Statement: The authors declare that the information provided in this paper is available and can be shared when required based on the request made to the corresponding author.

ACKNOWLEDGEMENTS

Required support to complete this study was provided by students and Dr. Saravanan N / Head, Department of Biotechnology, Muthayammal Engineering College, Rasipuram.

Funding: This research work did not have any particular funding.

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Effect of Phytohormones on *In vitro* Bud and Root Formation of Water Hyacinth (*Eichhornia crassipes*) [Mart.] Solms)

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ABSTRACT

Water hyacinth is an aquatic plant, of which proliferation rate is extremely rapid as a weed, causing great economic, social and environmental damage. On the other hand, the water hyacinth also has potential economic value, because it is used as food for livestock, gas, fertilizer, environmental treatment, art crafts, decoration items, as well as herbal medicine. This research investigated the effects of concentrations of BA (Benzyl adenine) and NAA (Naphthylacetic acid) on *in vitro* bud and root formation of *Eichhornia crassipes* [Mart.] Solms to create *in vitro* sample source which is initially used in subsequent researches of water hyacinth. After 4 weeks of *in vitro* culture, the results showed that the two-layer MS (Murashige Skoog) medium - the lower solid agar medium and the upper liquid medium - accompanied by aerobic culture conditions supplemented with 0.5 mg/L BA were suitable for bud proliferation. Next, these explants after destroying shoot apical meristem were transferred to MS medium supplemented with 0.75 mg/L BA suitable for bud development. The mature buds were transferred to MS medium supplemented with 0.25 mg/L NAA, suitable for rooting of water hyacinth and gave high survival rate (83.00%) when planted in the garden on hydroponic nutrient medium Howard of 600ppm.

KEY WORDS: BA, NAA, IN VITRO, WATER HYACINTH (EICHHORNIA CRASSIPES [MART.] SOLMS).

INTRODUCTION

The dense development of water hyacinth has caused serious economic, social and environmental problems for many countries around the world, especially riverine communities (Howard, 2003). Water hyacinth has become a major weed in more than 50 countries in the tropics and subtropics with strong and lasting effects (Holm et al, 1991). According to the most recent reports in 2017, the major effects of this weed include: Impact on the irrigation system; increase in epidemics such as malaria, flukes, worms, cholera; lack of clean water, effects to waterway traffic; loss of aesthetic value of water bodies, affecting tourism; reduction of capture fisheries and fishing; reduction of biodiversity; difficulties in the hydropower sector; floods; increase in snake and crocodile attacks (Gebregiorgis, 2017).

Regardless of its limitations, the water hyacinth also has advantages. Water hyacinth has potential economic, social and environmental values. Water hyacinth grows widely with a yield of 400 tons/ha, which will meet the requirements of

biogas production for cooking, silage for ruminant cattle, mushroom cultivation or fuel (coal briquettes) (Suthar, 2022). In the alkaline soil, water hyacinth juice is put into the pond to raise the pH from 3.2 to 4.5, creating more nutrients in the water, especially creating a good environment for *Chlorella* algae - which is the food of fry, especially flounder and marble goby (Jimenez, 2020). In its natural form, water hyacinth has the effect of absorbing heavy metals (such as lead, mercury, strontium) and thus, can be used to eliminate environmental pollution (Mary P.N, 2011). According to Dan Viet newspaper, the current saltwater intrusion has caused death for water hyacinth, dozens of poor and disadvantaged households in Hoa Tien commune (Vi Thanh city, Hau Giang province) who live on this plant have lost their jobs.

The whole water hyacinth contains 92.6% water; 2.9% protein; 0.9% sugar; 2.2% fiber; 1.4% ash - with 40.8% calcium; 0.8% phosphorus; 0.86% carotenoids; 20% vitamin C and inorganic components such as SiO₂, Mg, K, Na, Cl, Cu, Mn, Fe. Besides, it also contains vitamins such as B1, B2, B6, B12 and E. Its flower contains delphinidin diglucoside (Cheng, 2015). With a chemical composition rich in vitamins and fiber, the water hyacinth is also used as a green vegetable in the family's diet. Young buds, petioles and flowers of water hyacinth are washed, boiled or cooked in

Article Information:*Corresponding Author: thoatta@hufi.edu.vn
Received 10/09/2022 Accepted after revision 25/11/2022
Published: Dec 2022 Pp- 501-506
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Available at: <http://bbrc.in/> DOI: <http://dx.doi.org/10.21786/bbrc/15.4.3>

half-done soup, people can eat this refreshing meal without itchiness. In addition, the water hyacinth also works as a medicine, its extract has antibacterial properties, helping to inhibit the growth of gram-negative and gram-positive bacteria (Shanab, 2010). In addition, the scientists also indicated that some extracts of water hyacinth also have anti-candida albicans effects. Moreover, the antioxidant activity in water hyacinth has the effect of preventing liver and breast cancer cells (Aboul-Enein, 2014). However, due to its ability to absorb heavy metals, the water hyacinth can be harmful to users. Thus, it is necessary to cultivate clean water hyacinth for food and medicine.

Besides, the water hyacinth is also used as art crafts, decoration items, ornamental plants. Dried water hyacinth can be used to braid into ropes and then weave into mats, crafts, or tables and chairs. Water hyacinth is also grown as a decorative ornamental plant in gardens, fish tanks, etc (Villamagna, 2010).

Along with the advantages and disadvantages of water hyacinth, the problem is to research the growth and development of water hyacinth so that it can be adjusted when necessary. Specifically, it is possible to increase growth and development in case of using water hyacinth as food, gas, fertilizer, environmental treatment, art crafts, decoration items, etc., or inhibit the growth and development of water hyacinth in case water hyacinth spreads as fast as weeds..., or adjust the growth and development of water hyacinth in the direction of creating a source of clean plants, or creating a source of plants with small beautiful shapes as ornamental plants. To solve this problem, the plant tissue culture can be used to create *in vitro* plants. The tissue culture is the culture of material completely free of microorganisms on artificial nutrient medium under sterile conditions (Soumare, 2021).

In vitro plant plays very important role in the research of plant growth and development, in understanding the physiological mechanisms of plants. In addition, the *in vitro* plant also makes a fast, abundant and clean sample source. However, in the *in vitro* plant researches, water hyacinth is most unconcerned because of its untapped economic value and difficulties in *in vitro* culture of group of aquatic plants. In this research, we focus on describing the process of creating *in vitro* samples of water hyacinth, *Eichhornia crassipes* [Mart.] Solms through the investigation of the effects of phytohormones on *in vitro* bud and root formation of water hyacinth.

RESEARCH MATERIALS AND METHODS

Research Materials: The research materials were 2-week-old water hyacinth buds grown in the experimental garden, the buds were removed from the leaves and cut into 3 cm long segments bearing the apical meristem, trimmed off the roots.

Cultivation conditions: Lighting of 12 hours/day, light intensity of 3000 lux; temperature of 25 °C ± 2 °C at the Cell Technology Laboratory, Ho Chi Minh City University

of Food Industry.

2.2. Research Methods

2.2.1. Investigation of suitable *in vitro* culture mediums for bud viability of water hyacinth: Water hyacinth buds grown in the experimental garden with all leaves removed were disinfected by washing with soap for 5 minutes and putting under running water for 3 hours, disinfecting with alcohol 700 2 min and HgCl₂ 0.1% for 3 minutes. Then they are cultured on MS basic medium supplemented with 30g/l sucrose and solid MS medium conditions (4g/ml agar added), 2-layer MS medium- solid bottom and liquid top, and liquid MS medium with cotton wool. They are cultivated under *in vitro* conditions with lighting of 3000 Lux, temperature of 25 °C. The experiment consisted of 3 treatments, each treatment had 5 samples, each culture flask had 1 sample. The development of *in vitro* water hyacinth was monitored by imaging at 10, 20, 30, 40 days after culture.

2.2.2. Investigation of the effect of BA concentration on bud formation and bud proliferation for *in vitro* *Eichhornia crassipes* [Mart.] Solms: The experiment was designed to determine the optimal BA concentration for bud formation and bud proliferation of *Eichhornia crassipes* [Mart.] Solms. The *in vitro* buds are 2-2.5 cm in size, are not destroyed shoot apical meristem and subcultured to two-layer MS medium supplemented with BA with different concentrations of 0.0; 0.5; 1.0; 1.5; 2.0 mg/L for the purpose of investigating bud proliferation. The *in vitro* buds are 2-2.5 cm in size, after destroying the apex of the mother buds, subcultured to two-layer MS medium supplemented with BA with different concentrations of 0.0; 0.25; 0.5; 0.75; 1.0 mg/L for the purpose of investigating bud formation (new bud formation). Each treatment was repeated 3 times, with 5 flasks each replicate, and 1 sample each flask. After 4 weeks of culture, the parameters including number of buds (buds/sample), bud morphology under the *in vitro* conditions are monitored.

2.2.3. Investigation of the effect of NAA concentration on rooting ability for *in vitro* *Eichhornia crassipes* [Mart.] Solms: The experiment was designed to determine the optimal concentration of NAA for *in vitro* rooting of Water hyacinth *Eichhornia crassipes* [Mart.] Solms. The *in vitro* buds are 5-6 cm in size, 2-3 leaves were cultured on two-layer MS medium supplemented with 30g/L sucrose, 4g/L agar, supplemented with NAA with different concentrations of 0.0; 0.25; 0.50; 0.75; 1.0 mg/L respectively for rooting investigation purposes. Each treatment was repeated 3 times, with 5 flasks each replicate, and 1 sample each flask. After 4 weeks of culture, the parameters including number of roots (roots/sample), root morphology under the *in vitro* conditions are monitored.

2.2.4. Investigation of the effect of nutrient environment on the survival of seedlings in the nursery garden: The experiment was designed to investigate the appropriate nutrient environment for the survival of seedlings in the nursery garden. The *in vitro* water hyacinth samples are 8-10 cm in size, have healthy roots and buds. The *in vitro* water hyacinths are domesticated in glass bottles for 15 days, then cultured in 3 medium types including water,

Howard Resh hydroponic solution 300ppm, and 600ppm Howard Resh hydroponic solution (Resh, 2022). After 10 days, the survival rate (%) of water hyacinth is monitored in the nursery garden.

Statistical processing: All experiments were repeated 3 times, data were recorded and statistically processed by Statgraphics Centurion XV software, the significant difference was at $p \leq 0.05$.

RESULTS AND DISCUSSION

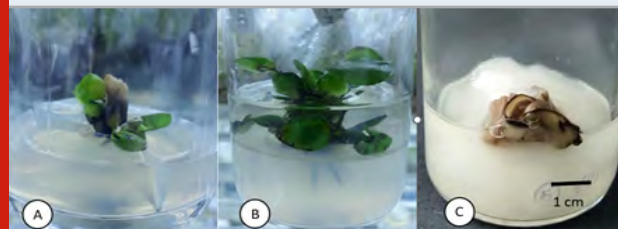
3.1 Investigation of suitable *in vitro* culture mediums for bud viability of water hyacinth.: Water hyacinth grown in the experimental garden removed all the leaves, were disinfected and cultured on the conditions of solid MS medium (A), 2-layer MS medium- solid bottom and liquid top (B), and liquid MS medium with cotton wool (C). In the 2-layer medium, plants had the highest survival rate and well developed bud morphology. Meanwhile, in the solid MS medium, the new leaves of plants were formed in the early stage, but quickly absorbed and drained water, leading the drying of the agar medium and the yellowing of leaves after 15 days of culture. In liquid medium, cotton and *in vitro* buds are completely undeveloped and blackened at the 15th day (Table 3.1, Figure 3.1).

Table 3.1 . Survival rate of *in vitro* water hyacinth in three culture conditions

Treatment	Survival rate (%)
A medium	44.44 b \pm 11.11
B medium	77.78 c \pm 11.11
C medium	0.00 a \pm 0.00

Figure 3.1. *in vitro* water hyacinth after 15 days of culture.

A: solid MS medium, the buds form 3-4 leaves, have no stolon, the plant turns yellow and begins to die after 15 days. **B:** 2-layer MS medium, the buds form 4-5 leaves, have stolon, develop well. **C:** liquid MS medium with cotton wool, the plants do not produce buds, turn black and die after 15 days of culture.



However, under culture conditions in medium B, *in vitro* plants have yellowing phenomenon and die after 15 days. Subsequent observations on airtight and aerobic culture conditions resulted in the following: In an airtight culture, the *in vitro* water hyacinth formed buds from axillary buds after 15 days of culture and roots after 30 days of culture in semi-solid MS medium. After 30 days, the stolon extends

from the lateral bud, the first leaf of the seedling also has a reduced leaf blade, the second and third leaves grow linearly embracing each other, roots form just below the base of the first leaf, the outer spongy petioles embrace the entire leaf and the buds inside, the top of the leaf sheath is a thin membrane embracing the entire inside. The *in vitro* complete plant has 4-5 young green leaves with spongy petioles. The rootlets arise in the upper position of the stolon extending from the axillary bud, the roots are dark gray in color. The *in vitro* plants live up to 30 days, then the leaves begin to show signs of yellowing. On the 45th day, the leaves turned yellow and died (Figure 3.2).

Figure 3.2. The *in vitro* water hyacinth after 15,30,45 days of culture in semi-solid MS medium under airtight condition
A: The *in vitro* water hyacinth after 15 days of culture, with 3 leaves and stolon extending from the lateral bud of the explant, **B:** Water hyacinth leaves with spongy petioles after 15 days of culture.
C: The *in vitro* water hyacinth after 30 days of culture with leaves turning yellow
D: The *in vitro* water hyacinth died on the 45th day of culture.

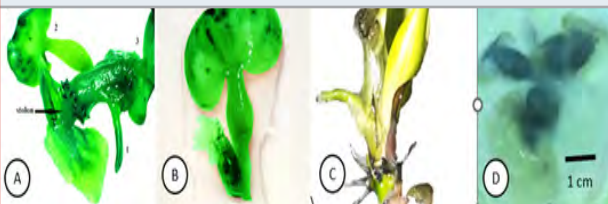
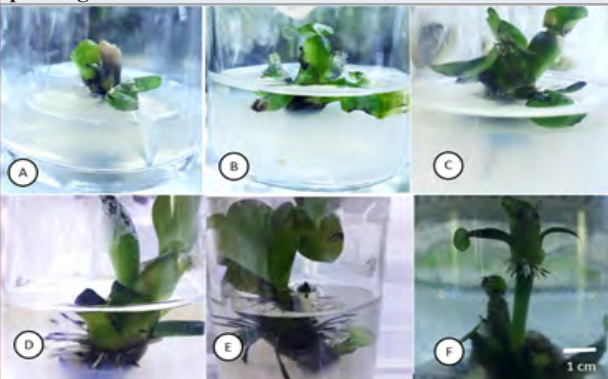


Figure 3.3. The *in vitro* water hyacinth and days of culture in MS medium under aerated conditions

A: The *in vitro* water hyacinth after 7 days of culture, there were two lateral buds and the medium was dry and cracked.
B: The *in vitro* water hyacinth in liquid MS medium after 15 days of culture.
C: The *in vitro* water hyacinth after 20 days of culture
D: The *in vitro* water hyacinth after 30 days of culture.
E: The *in vitro* water hyacinth after 40 days of culture, stolon appeared
F: The *in vitro* water hyacinth after 45 days of culture with prolonged stolon



Under aerated culture conditions, water hyacinth plants absorb water and transpire strongly, causing the drying and cracking medium at the 7th day (Figure 3.3 A). When transplanted to 2-layer MS medium, the plants thrived with two lateral buds, young green leaves, and spongy petioles (Figure 3.3 B). The *in vitro* water hyacinth continued to absorb water and transpire strongly, causing the water level to dry up quickly on the 20th day (Figure 3.3 C). On the 30th day of culture, the *in vitro* water hyacinth increased leaf size and increased number of roots (Figure 3.3D).

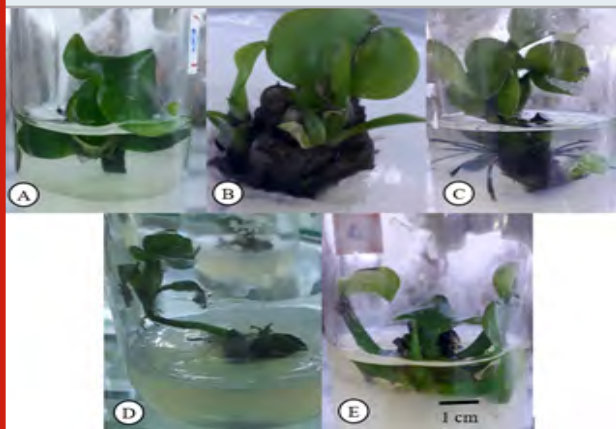
From the 40th day onwards, new buds appeared at the roots and leaf axils. The stolon also stretches in the horizontal and vertical directions (Figure 3.3 E, F).

Table

BA concentration (mg/L)	number of buds	Length of buds (cm)
0,00	1.00 ^a ± 0.00	5.20 ^b ± 0.97
0,50	2.33 ^b ± 0.53	6.80 ^d ± 1.31
1,00	1.66 ^{ab} ± 0.33	6.20 ^c ± 0.73
1,50	1.00 ^a ± 0.00	4.80 ^a ± 1.54
2,00	1.00 ^a ± 0.00	5.30 ^b ± 0.72

a, b, c: show differences in significant columns at confidence level $p \leq 0.05$ in the Duncan test.

Figure 3.4: Effect of BA concentration on bud formation in *in vitro* *Eichhornia crassipes* [Mart.] Solms



3.2 Investigation of the effect of BA concentration on bud formation and bud proliferation for *in vitro* *Eichhornia crassipes* [Mart.] Solms: The *in vitro* buds are 2-2.5 cm in size, are not destroyed shoot apical meristem and subcultured to two-layer MS medium under aerobic conditions supplemented with BA with different concentrations of 0.0; 0.5; 1.0; 1.5; 2.0 mg/L for the purpose of investigating bud proliferation. At a concentration of 0.5 mg/L, the highest number of buds were formed with a well-developed bud morphology with 2.33 buds/sample. Meanwhile, at concentrations of 1; 1.5; 2 mg/L BA the

number of buds formed was lower than that of 0.5 mg/L BA and there was no statistical difference compared to the control (Table 3.2, Figure 3.4).

Table 3.3. Effect of BA concentration on bud proliferation for *in vitro* *Eichhornia crassipes* [Mart.] Solms

BA concentration (mg/L)	number of buds	Length of buds (cm)
0,00	2.00 ^a ± 0.29	5.20 ^{ab} ± 0.55
0,25	2.33 ^a ± 0.23	5.00 ^a ± 0.95
0,50	4.33 ^b ± 0.23	6.20 ^b ± 0.29
0,75	7.66 ^c ± 0.20	6.80 ^{bc} ± 1.33
1,00	3.66 ^b ± 0.29	5.10 ^a ± 0.84

a, b, c: show differences in significant columns at confidence level $p \leq 0.05$ in the Duncan test.

Figure 3.5. Effect of BA concentration on bud proliferation for *in vitro* *Eichhornia crassipes* [Mart.] Solms

A: 0,00 mg/L BA concentration, 2 baby buds . B: 0,25 mg/L, 2-3 baby buds. C: 0,50 mg/L, 4-5 baby buds. D: 0,75 mg/L, 7-8 baby buds. E: 1,00 mg/L, 3-4 baby buds.

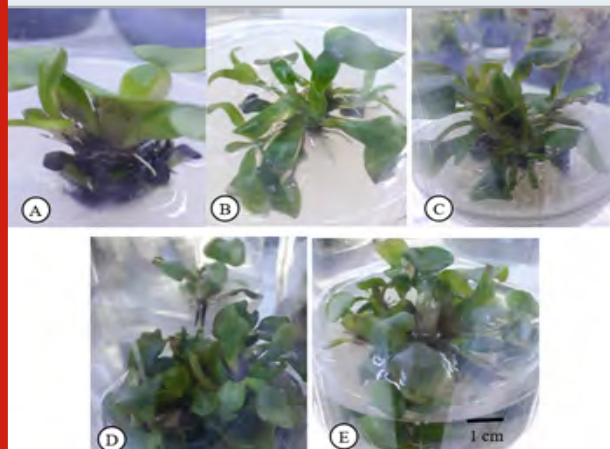


Table 3.4. Effect of NAA concentration on rooting ability for *in vitro* *Eichhornia crassipes* [Mart.] Solms

NAA concentration (mg/L)	number of roots	Length of roots (cm)
0,00	10.00 ^a ± 0.00	1.20 ^b ± 0.31
0,25	17.44 ^b ± 0.20	2.90 ^d ± 0.98
0,50	11.00 ^a ± 0.88	0.70 ^a ± 0.33
0,75	12.00 ^a ± 2.18	1.50 ^c ± 0.48
1,00	11.78 ^a ± 0.51	1.00 ^a ± 0.24

a, b, c: show differences in significant columns at confidence level $p \leq 0.05$ in the Duncan test.

Cytokinin has a strong stimulant effect on bud differentiation, so under the basic MS medium supplemented with BA, all samples were induced increased biomass. According to Phillips, a high concentration of BA is suitable to stimulate bud formation, however, lower BA concentration is required at the bud proliferation stage because BA at high concentrations can inhibit the absorption of nutrients, creating abnormal buds. Experimental results of Jordan et al. showed that when using MS medium supplemented with BA for plant bud proliferation at a concentration of 0.5 μ M, this result is similar to the research experiment. Thus, subculture to MS medium supplemented with 0.5 mg/L BA is suitable for bud proliferation.

A: 0.00 mg/L BA concentration, mother bud grows, no baby bud. B: 0.50 mg/L, mother bud grows, has a baby bud. C: 1.00 mg/L, mother bud grows, has a baby bud. D: 1.50 mg/L, mother bud grows, no baby bud. E: 2.00 mg/L, mother bud grows, no baby bud. The in vitro buds are 2-2.5 cm in size, after destroying the apex of the mother buds, subcultured to two-layer MS medium supplemented with BA with different concentrations of 0.0; 0.25; 0.5; 0.75; 1.0 mg/L for the purpose of investigating bud formation. Under the condition of destroying shoot apical meristem, the number of buds formed increased and reached the highest number of buds at the concentration of 0.75 mg/L BA with 7.6 buds/mother plant (Table 3.3, Figure 3.5).

Figure 3.6. Effect of NAA concentration on rooting ability for in vitro *Eichhornia crassipes* [Mart.] Solms

A: 0,00 mg/L NAA concentration, black, small and weak. B: 0,25 mg/L, black, big. C: 0,50 mg/L, light brown, small and weak. D: 0,75 mg/L, light brown, small and weak. E: 1,00 mg/L, light brown, small and weak



Table 3.5. Effect of nutrient environment on the survival of seedlings in the nursery garden

Nutrient environment	Survival rate (%)
Water	10.00a \pm 2.00
300ppm hydroponic solution	50.44 b \pm 8.30
600ppm hydroponic solution	83.00 c \pm 9.87

Cytokinin has a strong stimulant effect on bud differentiation, corrects apical dominance, and releases lateral buds from

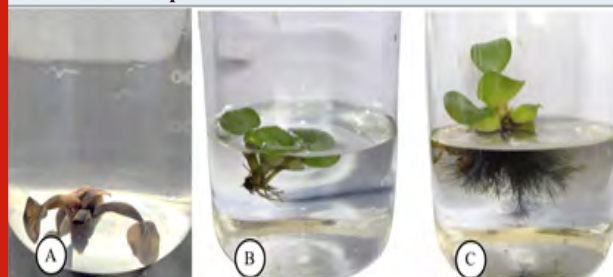
the relative inhibition of apical buds. Therefore, cytokinins are often used to induce bud proliferation and increase the propagation coefficient in tissue culture. On MS medium supplemented with BA, the samples were induced and bud sprouted. However, when used at high concentrations, it often has an inhibitory effect (Phillips, 2019). In this experiment, the obtained results also correspond to the proven theory. The ability to sprout new buds increased in the treatments from 0.00-0.75 mg/L BA and reached the highest concentration at 0.75 mg/L with 7.66 buds/sample and then the number of new buds decreased at a concentration of 1.00 mg/L, this proves that it inhibits bud proliferation at high concentrations of Cytokinin.

3.3. Investigation of the effect of NAA concentration on rooting ability for in vitro *Eichhornia crassipes* [Mart.] Solms:

The in vitro buds are 5-6 cm in size, 2-3 leaves were cultured on two-layer MS medium supplemented with 30g/L sucrose, 4g/L agar, supplemented with NAA with different concentrations of 0.0; 0.25; 0.50; 0.75; 1.0 mg/L respectively for rooting investigation purposes. After 4 weeks of culture, at the concentration of 0.25 mg/L NAA, the highest number of roots were formed with stiff, black root morphology (Table 3.4, Figure 3.6).

Figure 3.7. Effect of nutrient environment on the survival of seedlings in the nursery garden water hyacinth *Eichhornia crassipes* [Mart.] Solms

A: water solution, leaves turn yellow. B: 300ppm hydroponic solution, roots develop weakly, stems are submerged in water. C: 600ppm hydroponic solution, leaves and roots are well developed



Auxin stimulates root formation, especially minor roots (Kumari, 2022). In tissue culture, auxin stimulates root differentiation and branching root development. At high concentrations, the auxin inhibited primary root elongation but initiated lateral root and indeterminate root formation (Bui Trang Viet, 2020). The MS medium supplemented with NAA stimulated root formation in water hyacinth buds. At the concentration of NAA 0.00-0.25 mg/L, the number of roots/sample increased, the roots were strong, black with hairy suckers. After that, at the concentration of 0.5 mg/L to 1.00 mg/L NAA, the rooting efficiency is not high, the number of roots is small. At a concentration of 1 mg/L NAA, the number of roots per sample decreased, and the roots were short and brittle. Thus, the best concentration is NAA 0.25 mg/L for roots with hairy suckers, with many black, strong roots.

3.4. Investigation of the effect of nutrient environment on the survival of seedlings in the nursery garden: We

can clearly see that in water without adding nutrients, water hyacinth has browning and dies after 2 weeks. In contrast, in hydroponic solution with concentrations of 300 ppm and 600 ppm of dissolved inorganic substances, the plants tend to grow well, have long roots and generate new buds. However, in a hydroponic solution of 300 ppm, the plants have few buds and roots and do not grow as much as the plants in the 600 ppm hydroponic solution.

CONCLUSION

After 4 weeks of culture, the results have determined that two-layer MS (Murashige Skoog) medium - the lower solid agar medium and the upper liquid medium - under aerobic conditions with the addition of BA 0.5 mg/L is suitable for the growth of water hyacinth buds, then destroying shoot apical meristem and transferring to a medium supplemented with 0.75 mg/L BA suitable for bud growth, and supplemented with 0.25 mg/L NAA suitable for rooting. The survival rate at the nursery garden was 83 % on the hydroponic nutrient medium with TDS of 600ppm. The results of the research determined that exogenous sources of cytokinin and auxin have an impact on the micropropagation of water hyacinth, increasing the ability to create healthy buds and roots, creating a premise for the plant to have a high survival rate when transferred to nursery garden in hydroponic nutrient medium. This is the basis for continuing to research the effect of auxin and cytokinin coordination ratio on bud and root formation in order to improve the efficiency of the micropropagation process of *Eichhornia crassipes* [Mart.] Solms.

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Bacteriological and Physicochemical Evaluation of Selected Bottled Water Brands in Nasarawa, Kano State, Nigeria

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ABSTRACT

Twenty-six different brands of bottled water were tested for bacteriological and physicochemical qualities in Nasarawa Local Government Area of Kano State, Nigeria. Bacteriological assessments have been used to outline the wide varieties of heterotrophic bacteria, overall coliform and faecal coliform. The chloride content, pH, temperature, conductivity, fluoride content, total hardness and suspended particles have been additionally investigated. The coliform count ranged from 0 to 1000 coliform/100ml of the sample while the heterotrophic bacterial count ranged from 0 to 118 cfu/ml. In the bottled water samples, there was no faecal coliform and fluoride contents. The pH, temperature, conductivity, Chloride content, total hardness, and total suspended particles were all within acceptable limits between 6.50 and 8.30, 19.8 and 21.00C, 63.10 and 86.92 μ S/cm, 3.58 and 4.64 mg/l, 1 and 86 mg/l and 1.0 x 10⁻³ to 3.0 x 10⁻³ g/100ml, respectively. *Staphylococcus*, *Aeromonas*, *Klebsiella*, *Micrococcus* and *Bacillus* were among the bacterial species found. For heterotrophic bacteria, total coliform and faecal coliform, 25 (96%), 24 (92%) and 0 (100%) of the 26 different bottled water samples met the standard respectively. For pH, temperature, conductivity, fluoride content, chloride content, total hardness and total suspended particles, all of the bottled water samples also met the criteria. According to the results of this study, 88.46 percent of the bottled waters tested were fit for consumption based on bacteriological and physicochemical factors. When producing bottled water, it is recommended that stringent criteria should be followed to ensure that the bottles are well capped, and that statistics including the producing date, expiration date and consignment number be imprinted on the bottles.

KEY WORDS: SUSPENDED PARTICLES, HETEROTROPHIC BACTERIA, PHYSICOCHEMICAL, BOTTLED WATER.

INTRODUCTION

Despite decades of government efforts, safe and clean water supply in Nigeria's urban areas remains objectionable. In most developing countries, the common method for delivering municipal-level, safe drinking water via treated pipe-borne water is woefully inadequate. (Othmani et al 2021), The importance of water quality to public health cannot be overstated. Water is responsible for the transmission of many infectious illnesses via the faecal-oral pathway. Drinking water-borne diseases claim the lives of approximately five million youngsters every years and sicken one-sixth of the world's population (Yang et al 2021 Amanda et al 2022).

The significance and contribution of neighborhood produce low-fee opportunity ingesting water schemes

to accomplishing sustainable get entry to in growing countries' rural and urban settings can't be over emphasized, particularly in the light of renewed global responsibilities to the Millennium Development Goals (MDGs) set for 2015. Bottled water is widely accessible, although it is out of reach for the majority of Nigerians. It is sold all over the country, and the best quality varies depending on the producer, raising concerns about the safety of certain of these items. The National Agency for Food, Drug Administration, and Control (NAFDAC) took the ingenuity to free Nigeria of soiled bottled and sachet water by mandating companies to chronicle with it so that it could monitor their activities, (Vanessa et al., 2021).

Given the lack of clean drinking water in Nigeria and the hot weather, sachet and bottled water sales persisted prominent in 2015 (Ugochukwu et al., 2017). In both urban and rural areas of Nigeria, a large percentage of households who do not have admittance to potable water. It is assumed that at least half of the populace purchases water on a day-to-day basis (Richard et al., 2021).

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Received 17/10/2022 Accepted after revision 25/11/2022

Published: Dec 2022 Pp- 507-512

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Available at: <https://bbrc.in/> DOI: <http://dx.doi.org/10.21786/bbrc/15.4.4>

The increased availability of bottled water in the country necessitates an examination into the operations of the producers to evaluate the magnitude to which they adhere to the established standards. The Federal Government of Nigeria has spent a significant amount of money on basic health care in the last decade since many health workers have expressed concern about the increasing symmetry of dangerous water-borne infections like typhoid fever, diarrhea and measles (Moremi 2012). As a result, a small number of people have taken precautions by boiling and filtering their drinking water at home. In some families, however, drinking bottled water is a safer strategy to avoid contact with these infections.

The product was launched in Nigeria to give safe drinking water free of waterborne infections. However, with the growth of bottled water firms, the original purpose of supplying safe drinking water has been compromised. The purpose of this study was to scrutinize the quality of bottled water in light of the increasing demand for secured and drinkable potable water, as well as the economic expansion of small scale entrepreneurs in the bottled water industry. The study's goal is to assess the bacteriological and physicochemical strengths of bottled water, depict and recognize the bacterial isolates found in the bottled water, define the incidence of bacteria isolated in the bottled water, and develop research-based initiatives to ensure the quality of bottled water sold in the Nasarawa area of Kano State, Nigeria.

MATERIAL AND METHODS

Sample Collection: Twenty-six different brands of bottled water had been acquired from shops in Nasarawa and coded A to Z so that it will disguise the identification of the producers' identities. The samples had been gathered and transported to the laboratory in smooth coolers, wherein they had been preserved at ambient temperature until they were needed.

Bacteriological counts of bottled water samples: The number of heterotrophic bacteria was estimated using a standard plate count (SPC). Bacterial cultures were grown on nutrient agar (Nawzat 2021 & Karissa et al., 2021). Using the 3-3-3 multiple tube fermentation regime, the total coliform count was determined. The culture was done in MacConkey broth. The number of significant tubes with acid and gas generation was recorded after incubation, and the most probable number (MPN) of coliform per 100ml of sample taken was calculated using the MPN index table (Karissa et al., 2021). Using the spread plate approach, the faecal coliform (*E. coli*) of the water samples was determined by Eosin methylene blue (EMB) agar. The number of *E. coli* colonies was reckoned and expressed as colony forming units per milliliter (cfu/ml) (Aneja 2014).

Characterization and Identification of bacterial isolates:

The colony morphology, cellular features, and biochemical reactions of the bacteria were used to identify them (Veronica et al., 2020 & Shima et al., 2021). To acquire pure culture of the isolates, all colonies were sub-cultured from the mixed culture. The genuine isolates were then

conveyed to sterilize nutrient agar slants and stowed in the refrigerator as stock cultures (Karissa et al., 2021).

Determination of physicochemical parameters: The pH of the sampled bottled water was evaluated using a pre-calibrated Denver pH meter model 20 (Karissa et al., 2021). The total suspended particles were estimated by weighing a pyrex beaker after it had been dried up in an oven at 105°C for 60 seconds (Nawzat 2021).

Determination of Temperature: All temperature of the bottle water samples had been measured using a simple mercury-in-glass thermometer calibrated in degrees Celsius, as defined by (Siti et al., 2021, (Muhammad et al., 2021) and (Sheshe & Magashi 2014).

Table 1. Showing the Bacteriological Counts of Bottled Water Samples

Samples	FC (cfu/ml)	TC (MPN/100ml)	HBC (cfu/ml)
A	0	0	2
B	0	0	0
C	0	0	0
D	0	0	68
E	0	0	1
F	0	0	1
G	0	0	2
H	0	1000	118
I	0	0	3
J	0	0	10
K	0	0	3
L	0	0	7
M	0	0	0
N	0	0	0
O	0	0	0
P	0	220	7
Q	0	0	1
R	0	0	0
S	0	0	0
T	0	0	0
U	0	3	61
V	0	0	0
W	0	0	0
X	0	0	2
Y	0	0	1
Z	0	0	10

Key:FC= Faecal Coliform, TC = Total Coliform HBC= Heterotrophic Bacterial Count.

Determination of Conductivity: It was done using the conductivity meter. The conductivity meter was connected to a power source. 25ml of the sample was collected in a flat bottom flasks, followed by 25ml of deionized water in

a second flat bottom flasks. The conductivity of deionized water was read as the control, and then the conductivity probe was placed in the flasks containing the water sample, and its conductivity was read from the conductivity meter (Amanda et al., 2022).

Determination of Fluoride: In a dry square sample cell, 10 ml of each water sample were placed, and 2cm³ of SPADNS reagent was added and mixed. After 30 seconds, the absorbance of the samples was calculated with a spectrophotometer (Nor Azlida et al., 2020).

Determination of Total Chloride: The total chloride was determined using an argentometric titration, which entailed titrating 100 ml of distilled water with water was added of 5% potassium chromate as an indicator and 0.1 N AgNO₃ as a standard. At the end, the solution changed the colour from yellow to pale pink (Sule et al., 2016).

Table 2. Showing the Bacterial Isolates' Characterization and Identification

Isolated bacteria	Gram reaction	Cell shape	Cell arrangement	Motility	Oxidase	Catalase	Acetoin	Starch hydrolysis	Inulin	Chitin	Lactose	Urea	Sucrose	Glucose	Butyrate	Mannitol	VP	Nitrate	Indole	Hydrolysis	Hydrolysis		
1	+	Co	CL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Staphylococcus sp.
2	-	R	CH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Aeromonas sp.
3	+	Co	CL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Micrococcus sp.
4	-	R	CL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Klebsiella sp.
5	-	R	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Bacillus sp.

Key: + = Reaction; - = Non- reaction; Co= Cocci, CL = Cluster, CH = Chain; R= Rod, S= Single, F = Fermentative.; OX = Oxidative; OXF = Oxidation-Fermentation, A= Acid production; VP = Voges Proskauer

Determination of Total Hardness: In a complexometric titration, total hardness was determined by titrating 100 ml of water with 0.1 N EDTA using Erichrome black-T as an indicator. The water sample was treated with 10 drops of 25% ammonia before the titration began. The purple color changed to a faint blue color at the end (Mica et al., 2021).

RESULTS AND DISCUSSION

Bacterial Counts: The bottled water's total heterotrophic bacterial counts oscillated from 0 to 118 cfu/ml while total coliform counts ranged from 0 to 1000 coliform/100ml. There was no faecal coliform in all the bottled water samples (Table 1). In this investigation, 96.15 % of the bottled water samples had heterotrophic bacterial counts of fewer than 100 cfu/ml. In reality, 88.46% of the bottled water samples had heterotrophic bacterial counts of less than 10 cfu/ml.

Table 3. Showing the Bacterial Isolates' presence in the Bottled Water Samples

Sample	S1	S2	S3	S4	S5
A	+	+	-	+	-
B	-	-	-	-	-
C	-	-	-	-	-
D	-	-	-	+	-
E	+	-	-	+	-
F	-	+	-	-	-
G	-	+	-	+	-
H	-	+	-	+	-
I	+	+	+	+	-
J	-	+	+	-	+
K	+	-	+	-	-
L	-	+	-	+	-
M	+	-	+	-	+
N	+	+	+	+	-
O	-	-	-	-	-
P	-	-	-	-	-
Q	-	-	+	-	-
R	+	-	-	+	+
S	+	-	-	+	-
T	-	+	-	-	-
U	+	-	+	-	-
V	-	+	+	-	-
W	-	+	-	+	-
X	+	-	-	-	+
Y	-	+	-	-	+
Z	-	-	+	+	+

Key: + = Isolated; - = Not isolated; S1 = *Staphylococcus sp.*; S2 = *Proteus sp.*; S3 = *Micrococcus sp.*; S4 = *Klebsiella sp.*; S5 = *Aeromonas sp.*

Isolated Bacteria from Bottled Water Samples: The bacteria *Staphylococcus sp.*, *Bacillus sp.*, *Micrococcus sp.*, *Klebsiella sp* and *Aeromonas sp.* were identified and characterized in the bottled water samples (Table 2). Table 3 shows where they were found in the bottled water samples.

Bottled Water Samples' Physicochemical Properties: The Hydrogen ion concentration of the bottled water samples ranged from 6.50 to 8.30, and the total hardness ranged from 1 to 86 mg/l. The quantities of chloride and general suspended solids was ranged from 3.58 and 4.64 mg/l and 1.0×10^{-3} to 3.0×10^{-3} g/100ml, correspondingly. (Table 4).

In terms of public health, the significance of safe and quality drinking water cannot be overstated. Some water-borne diseases such as dysentery, cholera, typhoid etc, have

become far less common since the introduction of bottled water. All of the producers were successful in maintaining a faecal coliform count of zero in their goods. Furthermore, 24 bottled water brands (92.30 %) surpassed the maximum coliform limit for drinkable water of 10 per 100 mL of water WHO (2022). However, for 23 of the 26 varieties of bottled water, zero total coliform count was indorsed for emergency use, accounting for 88.46 percent of the entire coliform count (Bukar et al., 2015).

The bacteria identified in the bottled water might have come from the manufacturing machinery or faulty capping and

post-production contamination in bottled water distribution and sales. Many studies have observed that the many heterotrophic bacteria, total coliform and the number of bacterial species found in this study was substantially lower than that seen in sachet water. In five types of sachet water sold in Maiduguri, Bukar et al. (Sasikaran et al., 2012) found heterotrophic bacterial counts ranging from 15400 to 19600 cfu/ml. These figures are higher than the maximum heterotrophic bacterial count discovered in this study, which was 118 cfu/ml in a bottled water brand. Aerobic bacterial counts varying from 0–800 cfu/ml were observed in 22 brands of bottled water obtainable in the Jaffna Peninsula (Omalu et al., 2012).

Table 4. Showing the Physicochemical Potentials of Bottled Water Samples

Sample	pH	Temperature (°C)	TH(mg/l)	CC(mg/l)	Conductivity (µS/cm)	TSS(g/100ml)	FCo (mg/l)
A	6.70	20.4	33	4.20	63.10	0.003	0
B	6.60	20.3	18	4.64	66.20	0.001	0
C	8.30	19.6	10	3.85	63.47	0.002	0
D	7.50	20.1	14	4.64	73.13	0.001	0
E	6.80	20.6	19	3.91	72.40	0.003	0
F	6.50	20.8	7	4.16	72.40	0.001	0
G	6.70	20.4	15	3.91	80.40	0.001	0
H	7.50	20.3	86	4.64	81.33	0.002	0
I	7.50	20.1	8	4.64	86.41	0.003	0
J	6.70	20.2	1	3.93	63.95	0.003	0
K	7.40	20.4	7	4.19	64.00	0.001	0
L	6.70	20.6	9	3.91	64.30	0.001	0
M	7.50	20.1	4	4.44	74.79	0.002	0
N	7.50	19.9	9	3.91	70.20	0.002	0
O	7.90	21.0	11	4.62	79.60	0.003	0
P	8.00	20.0	15	3.91	84.30	0.001	0
Q	7.90	20.9	2	3.58	86.90	0.002	0
R	6.50	20.2	4	3.75	65.30	0.001	0
S	7.50	19.8	24	4.62	72.40	0.002	0
T	6.80	20.0	2	3.91	72.40	0.003	0
U	6.70	20.1	7	4.42	80.40	0.003	0
V	7.50	20.0	9	3.91	72.40	0.001	0
W	7.90	20.6	4	3.84	63.47	0.001	0
X	6.50	20.1	1	3.94	72.10	0.002	0
Y	7.60	20.3	8	3.91	80.40	0.001	0
Z	6.50	19.8	11	3.93	63.56	0.003	0

Key: TH = Total Hardness, CC = Chloride content, TSS = Total Suspended Solid, FCo = Flouride Content

Klebsiella, *Pseudomonas*, *Proteus* sp., and *Chromobacterium violaceum* reported found from sachet water marketed in Samaru, Nigeria, according to (Ugochukwu et al. 2017). Coliform, *Staphylococcus*, and *Pseudomonas* were found in many kinds of bottled water sold in Ile-Ife, South Western Nigeria, by Igbeneghu and Lamikanra (Bukar et al., 2015). Non-carbonated bottled drinking water sold in Sri Lanka

contained *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Pasteurella haemolyticus* (Igbeneghu and Lamikanra 2014). However, the three bacteria genera found in this study, *Staphylococcus*, *Micrococcus* and *Aeromonas*, suggest that bottled water is healthier than sachet water. *Staphylococci* and *Micrococci* are

commensals that can be found on human skin, soil and other surfaces. As a result, they conquered the bacterial species isolated from the bottled water samples used in this study. Both water and soil have been shown to contain *Aeromonas* species (Herath et al., 2012).

In order for bacteria to survive in water, pH is critical. The pH values, temperature and conductivity of the bottled water samples used in this investigation were between 6.5 and 8.5, 20-210C, 5 μ S/cm - 199 μ S/cm which is the range allowed in potable water (WHO (2022 & Ojosipe 2007) kinds of bottled water sold in the Jaffna Peninsula have pH values ranging from 4.11 to 7.58 (Omalu et al., 2012).

The pH of all the samples, as well as their temperature, chloride content and turbidity was quite well below their limits, similar to those reported by (Sule et al., 2017). The suspended solids in bottled water were less than 30 mg/l (corresponding to 0.003 g/100ml in home water) (Willey et al., 2008). The pH of water is crucial for bacteria to live. The pH of the bottled water samples utilized in this research ranged from 6.5 to 8.5, which is the range permitted in potable water (Amanda et al., 2022). The pH of 22 types of bottled water offered on the Jaffna Peninsula ranges from 4.11 to 7.58 (Omalu et al., 2012). Total suspended solids in bottled water were less than 30 mg/l (0.003 g/100ml in household water) (Willey et al., 2008).

CONCLUSION

Data of the present study strongly suggest that the government should step up its efforts to monitor the fast growing water bottling industry in order to ensure that the public has access to safe and hygienic water. During the manufacturing process, manufacturers should strictly follow all good manufacturing practices (GMP) standards. Bottled water should also be explicitly labeled with an expiration date, authentic manufacturing date, batch number and bottles ought to be closed appropriately to avoid contagion. If any batch of brand fails to gratify regulatory criteria, regulatory organizations should then adequately notify the public. In such cases, the product should be withdrawn from the public. According to the findings of this study, In terms of bacteriological and physicochemical properties, twenty-four of the twenty-six diversities of bottled water samples assessed, 92% of the samples met the approved drinking water quality standards. As a result, the vast majority of bottled water sold in Nasarawa, Kano State is perfectly safe to drink.

Conflict of Interest statement: The authors have stated that there are no competing interest.

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Effect of Multi-Functional Therapeutic Active Extract of Marine *Asterias rubens* against Tooth Decaying Pathogens

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ABSTRACT

Recently, childhood caries have become one of the main health related diseases, especially developed by lactic acid bacteria, affecting millions of school going children worldwide. In the present work, we have isolated about 50 lactic acid bacteria samples from different tooth decay affected children from Fen Dental Clinic, Tirupur India. The emerging bacteria were confirmed by phenotype and genotype characterization. After, the pathogens were screened for the production of biofilm it was checked for the resistant mechanism of the *Lactobacillus acidophilus*. In this study the MTLA1-50 isolates produced strong biofilms which were confirmed for the *Lactobacillus acidophilus* carrying resistant capability. In particular, the maximum strong biofilm producing strains were MTLA 32 and MTLA 50, followed by other isolates. As star fish extract has been reported to play a vital role in eliminating the bacterial biofilm for this motive in this study we have chosen the star fish, *Asterias rubens* extract in the concentration of 50, 100, 150 µl. The analysis was performed and executed using well diffusion standard techniques. The results showed that the strain No. MTLA 32 and MTLA 50 got the maximum zone of inhibition at 150 µl (52mm and 38 mm) of the star fish extract. The findings demonstrate that the star fish extract has a powerful anti-biotic ability to treat the dental pathogens particularly, caused by *Lactobacillus acidophilus*.

KEY WORDS: STAR FISH EXTRACT, DENTAL CARIES, BIOFILM PRODUCTION.

INTRODUCTION

Human plaque is a prevalent disease in the world. A universal, 36% of the population affected approximately has decay in their permanent teeth. WHO estimates that virtually all human beings have tooth decay at some point in their lifetimes. Infant teeth distresses about 9% of the residents (Vos et al., 2012, Bagramian et al., 2009). The main reason is the formation of tooth decay causing destruction of oral tissues by lactic acid from bacterial fermentation of carbohydrates, resulting in the caries development. *Streptococcus* and *Lactobacillus* play major role in establishment of tooth decay (Chen and Wang, 2010, Al-Zahrani et al., 2022).

In ancient times, the development of tooth decay was mainly exhibited by primary microbes especially *Lactobacillus* spp. (Owen et al, 1949) and the pathogen appeared throughout the foremost years of infant's life causing root caries and periodontal diseases (Badet and Thebaud, 2008, Austin et al., 1999). Currently, some anti-drugs prescribed for the

treatment of plaque disease. But the distress pathogenic dental microbe fully oppose towards presently updated antibiotic medicine. One of the techniques to decrease the drug resistant is by using antibiotic resistant inhibitor from starfish *Asterias rubens* in the way of extract containing the therapeutic compound. Starfish extracts can eliminate the target sites of the emerging dental pathogens because of their antibacterial potential ability as earlier, *Asterias rubens*, from numerous tissues like coelomocyte and body wall have shown to inhibit the pathogens, (Haug et al., 2002, Zahrani et al., 2022).

Hence the present study has been attempted to highlight role of the extract of the marine echinoderm, *Asterias rubens* which can be use as a prospective source for derivation of extraction of therapeutic compound, which can have anti-caries activity, as it can breakdown the biofilm formation this can be a possible treatment for caries infection in future.

MATERIAL AND METHODS

Collection of Plaque Sample: In this study childhood decay samples were collected from 50 patients attending the outdoor patients in Fen Dental Clinic, Tirupur District

Article Information:*Corresponding Author: drvijayalakshmi@vicas.org
Received 15/10/2022 Accepted after revision 17/11/2022
Published: Dec 2022 Pp- 513-515
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Available at: <http://bbrc.in/> DOI: <http://dx.doi.org/10.21786/bbrc/15.4.5>

using sterile forceps. In this research 10% concentration of NaCl saline is used as a transport medium for enumerating the lactic acid bacteria.

Isolation of bacteria: After collection of dental sample, the decay sample serially diluted with peptone broth for minimizing bacterial growth for extracting 0.1ml culture plated over the Man Rogosa Sharpe agar for isolating child major dental pathogen *Lactobacillus acidophilus*.

Identification of Bacterial Isolates: In this research, all plaque pathogenic isolates identified by various biochemical character and 16Sr-RNA gene sequencing method.

Biofilm Assay: The decay isolates were grown on nutrient broth with and without 5% glucose, incubated overnight in microtiter plates at 37°C. End of incubation, the micro titer plate finally treat with crystal violet solution for exactly verify the ring of dental biofilm as visible as purple ring over the microtiter plate. The clinical cariogenic isolate showing maximum biofilm formation was chosen for antibacterial activity.

Collection of Star Fish: Sea star - *Asterias rubens* specimen were collected from Mandapam Coast, Chinnapalam - Thoppukadu Theevu, Rameshwaram Dt. The specimen were cleaned with sea water at the sampling site of Thoppukadu to remove sediments and contamination, then it was put in sterile polythene bags and stored in ice box suddenly transported to the laboratory for isolation of bio-medically active extract for treatment of dental pathogen.

Preparation of the crude extracts: The samples of star fish *Asterias rubens* were grinded by mortar and pestle instrument. Then, the extracts were filtered using Whatman No. 1 filter paper and it was mixed with dimethyl sulphoxide solution (10ml). In this study the antagonistic activity of starfish with DMSO extract was screened against dental pathogen.

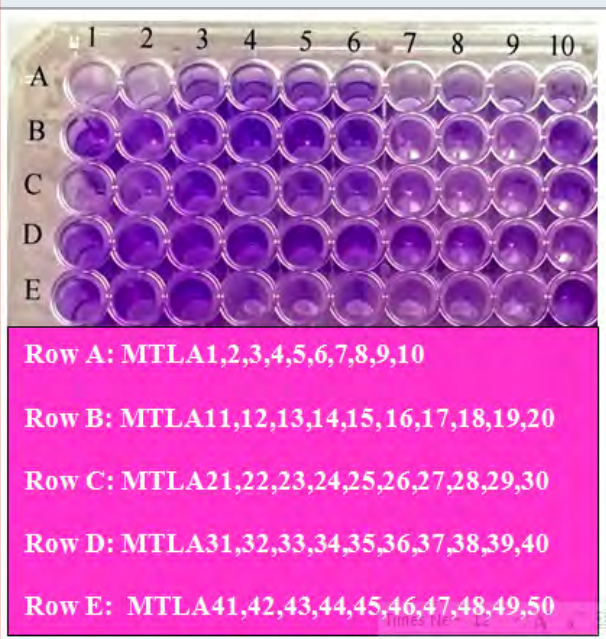
Antibacterial bioassay: Pharmaceutical activity of 50, 100 and 150µl concentration of starfish extract was performed by well assay against lactic acid bacteria and the results were detected by measuring the diameter of inhibition zone around the dental bacterial colony and the measurement expressed in mm in diameter.

RESULT AND DISCUSSION

Totally, 50 *L. acidophilus* lactic acid acidic strains isolated from 50 dental samples were identified by biochemical, biofilm production and 16SrRNA Gene Sequencing. In this study screening of cariogenic dental biofilm was analyzed with 5% concentration of glucose in the nutrient medium at 37°C. The micro titer plates filed with 5% con. of glucose concentrated medium with cariogenic grown culture were inoculated in each well. After incubation the plates were analyzed for the production of biofilm around the well of micro titer plate. Among 50 isolates the utmost strong effect of biofilm was observed in the strain no. MTLA32, MTLA50 followed by the strain no. MTLA2, MTLA3, MTLA4, MTLA5, MTLA6, MTLA7, MTLA8, MTLA9,

MTLA10, MTLA11, MTLA12, MTLA13, MTLA14, MTLA15, MTLA16, MTLA17, MTLA18, MTLA19, MTLA20, MTLA21, MTLA22, MTLA23, MTLA24, MTLA25, MTLA26, MTLA27, MTLA28, MTLA29, MTLA30, MTLA31, MTLA33, MTLA34, MTLA35, MTLA36, MTLA37, MTLA38, MTLA39, MTLA40, MTLA41, MTLA42, MTLA43, MTLA44, MTLA45, MTLA46, MTLA47, MTLA48 and MTLA49 (Fig. 1).

Figure 1: Biofilm Production from Dental Pathogen *Lactobacillus acidophilus*



In the present study marine star fish (Fig. 2) was collected from Thoppu Kaadu , Rameshwaram, Mandapam Coastal area with the help of sea shore community peoples and the marine star fish identified at CMFRI.

Figure 2: Marine Star Fish



In this research anti-cariogenic activity of marine star fish *Asterias rubens* extract was performed by well assay against dental acidic pathogen. Result indicated that this study the utmost zone of inhibition 32mm, 48mm and 52mm was observed in strain no. MTLA32 followed by the zone of inhibition 25mm, 28mm and 38mm was observed in strain

no. MTLA50 at the 50, 100 and 150 μ l concentration of novel star fish extracts (Fig.3).

Figure 3: Antibacterial activity of the starfish against biofilm producing *Lactobacillus acidophilus*

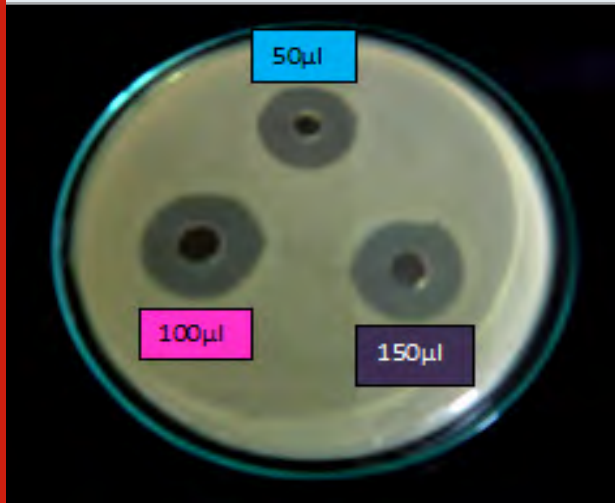


Table 2. Antibacterial activity of the starfish against biofilm producing *Lactobacillus acidophilus*

S. No	Strain. No	Sponge concentration (50 μ g)		
		50 μ l	100 μ l	150 μ l
1	MTLA32	32	48	52
2	MTLA50	25	28	38

In this study examination of cariogenic strain *Lactobacilli* in 50 deep caries samples the strain No. MTLA32 and MTLA50 is predominantly exhibit strong the biofilm by tooth decay isolates. This is due to the presence of acidogenic microbe *Lactobacillus* in the oral cavity it depends on various factors such as the existence of biological niches (Loesche et al., 1984).

In present research the utmost biofilm creation has induced in the presence of 5% concentration of glucose by the dental pathogen *L. acidophilus*. This result is in agreement with that of Christensen et al., (1985) who found that the glycocalyx development and bacterial observance both can improve the strong biofilm creation due to the presence of glucose absorption in bacterial growth media. Remarkably, Phylum Echinodermata - Marine starfishes, have a huge number of therapeutic drug molecules in their body, therefore, our research was to focus on the therapeutic properties of bioactive substances in the form of crude extracts of star fish species, *Asterias rubens* collected from the Rameshwaram Sea, Thoppukadu Theevu, Tamil Nadu India.

The medical activity of the extract was done by well assay techniques. In the present investigation, a well-defined antimicrobial activity of sea star crude extracts has been observed besides a strong biofilm producing dental strains such as MTLA 32 and MTLA 50. However, the values

of the antibacterial activity ranged between 25 mm to 52 mm as inhibition zones. This potential ability of the star fish extracts therapeutic activity is in agreement with that of Layson et al., (2014) who showed that the extracted pharmaceutically active drug from marine sea star *Linckia laevigata* and *Oreaster nodosus*, had significant biomedical activity against the pathogenic *Escherichia coli*.

CONCLUSION

The present research concluded that the marine *Asterias Rubens* – star fish extract is drastically effective against tooth decay organism *Lactobacillus acidophilus* at 50, 100 and 150 μ l. Hence these therapeutic extracts act as gold standard multi functional broad spectrum activity to kill the dental pathogen around the oral surroundings of infants and this paper assure that there is no any side effect at the time of treatment of dental caries in future. So this research paper proved to exhibit various kind of biomedical applications of the phylum Echinodermata make it a viable option for use in all dental emergencies in childhood caries in future.

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Quality Enhancement of Multifocus & Medical Images using Hybrid Wavelets based Fusion for Bioengineering Applications

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ABSTRACT

Multifocus image fusion employs fusion principles to integrate many focused images of the same scene. All-in-focus images are instructive and valuable for visual perception. Maintaining shift-invariant and directional selectivity in a fused image is crucial. Traditional wavelet-based fusion methods are hindered their performance due to a lack of invariant shift and reduced directionality. In this paper, a classical multifocus hybrid wavelet-based approach with principal component analysis (PCA) is proposed. At the first level of decomposition, stationary wavelet transformation (SWT) is used to perform the fusion process with the given source images. In the next level, approximation coefficients of source images are selected for decomposition as well as fusion using dual-tree complex wavelet transformation (DTCWT) and finally, PCA is applied to generate a final fused image. Analysis of the proposed method has been accomplished by evaluating various objective parameters.

KEY WORDS: DIRECTIONAL SELECTIVITY, FUSION RULES, IMAGE QUALITY METRICS, INVARIANT SHIFT, PRINCIPAL COMPONENT ANALYSIS.

INTRODUCTION

The crucial stage involved in various applications of image processing is an acquisition of images. The limitation involved in capturing an image is to focus all the objects. The optical lenses used in the acquisition of images have limited depth of field (DOF). The distance from the objects appeared in the image to optical lens usually creates multifocus images. To improve the DOF and to focus all the objects of the image and to improve their sharpness, an inexpensive methodology/algorithm needs to be developed. The developed technique should appreciably improve the focus of all objects by integrating the multiple images captured using different focal planes. Image fusion is a low-cost methodology used by various researchers in the last decade. The main objective of the different algorithms used in the image fusion process is to combine two or more

images acquired in different focal planes. The obtained fused image should invariably show superior performance in the detection of objects than the objects that appeared in the multifocus images (Chai and 2011; Shah and 2013; Zhang et al. 2014).

Since the social optical arrangement handles multiresolution knowledge under the transform field method computing principle, transform field algorithms, or more precisely, multiresolution algorithms, are superior. Numerous multiresolution approaches, including pyramid approaches, have been advanced in the literature [Petrovic and Xydeas 2004; Wahyuni and Sabre 2016], including distinct wavelet transformation [Wang and 2003], stationary transform wavelet [Borwonwatanadelok and 2009; Li and 2011; Sharma and Gulati 2017], multiresolution singular value decomposition [Naidu 2011], DCHWT, lifting schemes of WT, DDDWT, and ST [Li and 2012; Shreyamsha Kumar 2013; Zou and 2013; Liu and 2014; Pujar and Itkarkar 2016]. In pyramid domain orientation blocking effect is a major problem, which causes spatial distortion in fused

Article Information:*Corresponding Author: ramamohanchinnem@gmail.com
Received 18/09/2022 Accepted after revision 25/11/2022
Published: Dec 2022 Pp- 516-524
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Available at: <http://bbrc.in/> DOI: <http://dx.doi.org/10.21786/bbrc/15.4.6>

images. The traditional wavelet-based algorithms cause quality distortions due to ringing artifacts in the image. The DTCWT [Yang et al. 2014; Radha and Babu 2019] and SWT [Aymaz Samet and Köse 2019] are two important wavelet transformation methods precise elimination of major problems like sensitivity and invariance and also maintains better selectivity, well prediction of image details in fusion process. Being implementation of these transformations little bit complicated due to their satisfaction conditions. The DTCWT is available with various levels and methods, which indicates its superiority in the transformation-based algorithms. With the above reasons the proposed methodology is used to choose these two wavelets in fusion process.

Many methods of multifocus image fusion have been proposed over the years. An efficient image fusion model using improved adaptive PCNN was proposed, for instance, by (Panigrahy and 2020) for the NSCT domain. The suggested method for image fusion makes use of the sub-bands of the source images obtained by the NSCT algorithm. This new FDFM algorithm is used to determine the adaptive linking strength. Based on their categorization of region-based fusion methods, (Meher et al. 2019) offered an overview of the state-of-the-art in this field. The importance of fusion objective assessment indicators for comparing the aforementioned available techniques is highlighted. To better image processing, (He et al. 2020) formulated a multi-focus image fusion method. For the purpose of estimating the impact of fusion rules, multi-focus image fusion has used the cascade-forest. In a recent study (Aymaz and Köse 2019), the authors developed a novel multi-focus image fusion strategy that makes use of a super-resolution hybrid technique.

With the use of a convolutional neural network, (Wang et al. 2019) suggested a new method of multifocus image fusion for the DWT field. The advantages of both spatial and transform domain techniques are merged in the CNN algorithm. The CNN is used to enhance features and construct separate decision maps for different frequency subbands in place of traditional image blocks or source pictures. Furthermore, the CNN method's use of an adaptive fusion rule is an added bonus. Laplacian energy and variance were two of the most important measures derived by (Amin-Naji and Aghagolzadeh 2018). (Aymaz and 2020) present a novel method for multi-focus image fusion, use focus measures to assess the degree to which the source blocks and the artificially blurred blocks correlate in the DCT domain.

Super-resolution techniques, namely the SWT with the dmey (Discrete Meyer) filter added for decomposition, are of interest for improving contrast. Using a gradient-based method and a new fusion rule, a better final image can be achieved. To separate out the high and low frequency components, by (Li and 2019) to extract high and low frequency coefficients. Additionally, deep convolution

neural networks are utilized to build a high-quality fused image by directly mapping between the learning of high-frequency and low-frequency components of the source images. The encircling technique was used by (Nejati et al. 2017) to offer a novel focus metric based on the surface area of regions. It is shown that using this metric, fusion methods can distinguish between hazy regions. Using MST and CSR, (Zhang 2021) suggested a new fusion approach that addresses both MST and SR fundamental flaws.

The low-frequency and detailed directional components of each source image are initially extracted using MST. After that, CSR is employed for the low-pass fusion, while the max-absolute condition was used for high-pass fusion. A classical MIF system that uses the qshiftN-DTCWT and MPCA in the LP domain was proposed by (Mohan et al. 2022) to extract the focused image from a set of input images. The fused image has the potential to enhance sharpness, directional accuracy, and shift invariance. Using the CRF-minimization, labels, and ICA-transform coefficients, (Bouzos and 2022) proposed the CRF-Guided fusion, which guides high-frequency fusion after low-frequency fusion.

Through coefficient shrinkage, CRF-Guided fusion makes it possible to perform image denoising while the fusion process occurs. (Zhang and Feng 2022) use the CAOL framework to propose a method for fusing images from several focal images. This technique uses a sharp BPEG-M to learn several convolution filters, solve memory issues, and facilitate parallel computation. This research contributes new knowledge by applying CAOL-learned filters to three distinct methods of multifocus image fusion. (Ma et al. 2022) proposed a MsGAN (i.e., a multi-scale generative adversarial network) for end-to-end MIF, which maximizes the utility of image features through the fusion of multi-scale decomposition and CNN. The outcome of this method has produced a final fused image with a sharp focus. The rest of the paper follows this structure; Section 2 describes the material and methods. The result and discussions are presented in Section 3. Finally, conclusions are given in Section 4.

MATERIAL AND METHODS

Stationary Wavelet Transform (SWT): Nason and Silverman introduced the SWT category of wavelet transforms, which has special shift-invariant and redundancy properties (Nason and Silverman 1995). Shift-invariance plays a vital role in denoising and image processing. SWT changes the filter component by adding zeros for each level of decomposition through the use of upsampling. As a result, SWT maintains the initial input signal. The redundancy of SWT increases the speed of processing. Multifocus input images are decomposed into four sub-bands by exploiting SWT. SWT crates four sub-bands, such as LL, LH, HL, and HH, for the original multifocus image. LL is the

low-frequency sub-band that consists of approximation information of the input source image. The high-frequency sub-bands LH, HL, and HH, comprise the input source image's detail coefficients (Ayman and Köse 2019).

Dual-Tree Complex Wavelet Transform: Wavelets are superior concerning transformation, which is represented by various dimensions. These dimensions have issues with directional sensitivity and shift invariants. Since DTCWT is an advanced wavelet transformation version, it also suffers from invariance and sensitivity problems. The DTCWT is implemented internally with filter banks, which distributes the image information into two parts, i.e., real and imaginary. With the reflection of filter banks, the fused images of DTCWT acquire smooth and continuous edges. The advantages of DTCWT include directional sensitivity and scaling of real and imaginary parts at various angles. The detailed information is preserved due to the representation of six sub-bands. However, a few problems are resolved in the DTCWT filter approach; it minimizes issues like frequency responses and energy preservation.

Principal Component Analysis: In most cases, the PCA approach establishes correlations between previously unrelated variables. This technique helps examine data and select the most relevant aspects of a dataset. Following image fusion, the PCA [Suhail et al. 2014; Naidu and Raol 2008] method is used to find the most optimal value for the fused image. Next, multiply these by the corresponding fused image to get the all-in-focus image. Information is converted into eigenspace as part of the principal component analysis process. Data variance increases while the covariance decreases by keeping the components with the biggest eigenvector. In particular, this approach eliminates extraneous information and extracts the most crucial parts of the fused image. In addition, PCA gives weight to elements resilient to noise and impacts. In this way, the PCA reduces spatial blurring and distortions. Our earlier studies [Mohan and 2020; Mohan et al. 2020] detail the PCA algorithm's phases.

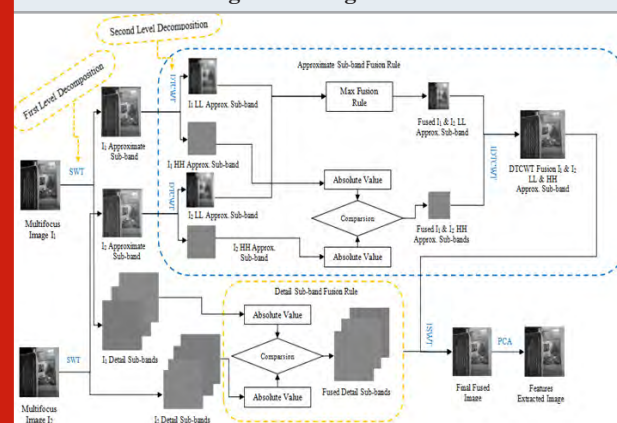
Flow Diagram of Proposed Approach: The flow diagram of the proposed work is shown in Figure 1, which consists of two layered processes such as hybrid wavelet-based image fusion (i.e., SWT and DTCWT) and PCA. In the first stage decomposition and fusion have been done by using hybrid wavelets i.e., a combination of SWT and DTCWT which eliminates spatial distortions and blurring artifacts. In the second stage, to extract information efficiently, the PCA algorithm is implemented. The description of the process steps is given below.

Read multi-focus images. Read the images with multiple focuses. The second step is to divide the input multi-focus images into approximate and detail sub-bands using SWT to perform the initial level of decomposition. Take

advantage of DCTWT for approximating sub-bands that decompose image data into LL and HH components at the second level of decomposition. Apply maximum fusion to the DCTWT-obtained approximate sub-bands of the LL components, and the resulting sub-band will contain all of the LL components. After comparing the intensities of these two sub-bands, the remaining HH approximate sub-bands of DCTWT are determined by taking the sub-band with the highest intensity into account. Use IDTCWT to combine the approximate subbands of LL and HH to form a fused subband. On the other hand, the first-level subbands are evaluated independently to determine which intensity is the highest before settling on a final subband. Create the final fused image using inverse SWT for the DCTWT fused image and the final detailed sub-band. Use principal component analysis to derive features from the fused image.

Performance analysis of proposed method: The proposed method is tested using two-fold performance measures such as subjective assessment and objective evaluation which is further compared with the state-of-the-art technologically advanced methods reported recently. The objective analysis is based on quantitative methods of comparing the values of the image fusion measures. It compares the fused image to the input images in terms of spectral and spatial similarity. Quantitative analysis can be done in two ways: with or without a reference image (Jagalingam and Hegde 2015).

Figure 1: The flow diagram of the proposed hybrid wavelet and PCA-based image fusion algorithm



Evaluation of quality measures: In this paper, eleven popular metrics, such as $Q^{AB/F}$ (Total Fusion Performance), $E(F)$ (Entropy), Q_E (Edge-dependent Fusion Quality), AG_F (Average Gradient), GM (Gray Mean Value), CC (Correlation Coefficient), $SSIM$ (Structural Similarity), SD (Standard Deviation), EI (Edge Intensity), ID (Image Definition), and Q^0 (Universal Image Quality Index) are employed to quantitatively evaluate the performances of different fusion methods (Wang et al. 2004; Li and 2011; Wang and Bovik 2002; Yang 2014).

RESULTS AND DISCUSSION

In this paper, a hybrid wavelet (i.e., SWT and DTCWT) is utilized to perform image fusion and a PCA is proposed to evaluate the image features. Quality measures such as $Q^{AB/F}$, $SSIM$, $E(F)$, AG_F , EI , CC , Q^0 , QE , SD , GM , and ID were employed to assess the performance of the proposed algorithm. The obtained quality measures of the fused image are compared with state-of-the-art existing methods. The resemblance and robustness of the fused pictures against distortions are measured using these criteria. The choice of multifocus source images is very particular in the case of multi-focus image fusion. Experimental results are available on many multi-focus images for different areas like biomedical, remote sensing, etc. with different data sets. Clock, Desk/Book-Shelf, Book, Flower, Lab, Leopard, Pepsi, Craft, Balloon, Calendar, Wine/Bottle, Plant-Clock/Corner, Grass, Medical, and Remote Sensing images are utilized in this work and the obtained results are compared with the existing methodologies reported in the literature (Panigrahy and 2020; Zhang 2021).

Figure 2: (Clock): a. Original Image; b, c. Multifocus input images; d. Proposed fusion.



Table 1. Comparison of quality metrics of the proposed scheme with ‘(Panigrahy and 2020)’

Evaluation Metric	Chinmaya Panigrahy et al. 2020	Proposed
$E(F)$	7.3854	7.4042
AG_F	6.0719	5.2238
CC	0.9808	0.9812
$Q^{AB/F}$	0.8968	0.9996
$SSIM$	0.9031	0.9857
Q_E	0.8538	0.9058

Figure 3: (Desk): a. Original Image; b, c. Multifocus input images; d. Proposed fusion.



Comparison for multi-focus clock image: Figure 2 depicts the assessment of the first multifocus image using the clock. The images shown in Figures 2(a), (b), and (c)

are the original, the left, and the right-focused images, correspondingly. Figure 2(d) depicts the final, sharp image that results from using the method. In order to evaluate the efficacy of the proposed approach, several metrics are computed, including $Q^{AB/F}$, $SSIM$, $E(F)$, AG_F , SF , CC , and Q_E . Finally, the effectiveness of the proposed strategy is evaluated in light of other approaches already present in the literature. In Table 1, we see how the quality metrics for the proposed hybrid wavelet approach compare to those for (Panigrahy and 2020). The comparison between the suggested hybrid strategy and the stated methodology demonstrates its superiority, with the top-performing methods highlighted in bold.

Table 2. Quantitative analysis of proposed and existing image fusion scheme (Panigrahy and 2020).

Evaluation Metric	Chinmaya Panigrahy et al. 2020	Proposed
$E(F)$	7.346	7.3550
AG_F	8.215	8.2089
CC	0.9644	0.9627
$Q^{AB/F}$	0.8958	0.9996
$SSIM$	0.8693	0.9887
Q_E	0.8669	0.9280

Figure 4: (Book): a. Original Image; b, c. Multifocus input images; d. Proposed fusion.



Table 3. Quantitative analysis of proposed and existing image fusion scheme (Panigrahy and 2020).

Evaluation Metric	Chinmaya Panigrahy et al. 2020	Proposed
$E(F)$	7.2957	7.3999
AG_F	13.7059	12.3623
CC	0.9825	0.9898
$Q^{AB/F}$	0.9145	0.9996
$SSIM$	0.9539	0.9227
Q_E	0.8838	0.9195

Comparison for multi-focus desk image: The assessment of the second multifocus image is the desk, which is displayed in figure 3, which comprises the original, multifocus, and result image after the image fusion approach. The following parameters— $SSIM$, Q_E , CC , AG_F , $Q^{AB/F}$, and, $E(F)$ —are computed to evaluate the proposed methodology's performance. In the end, the performance of

the recommended strategy is assessed and contrasted with that of other methods previously published in scholarly research. Table 2 presents the results of a comparison of the quality indicators used by each of these methods. According to the study that has been published (Panigrahy and 2020), the hybrid wavelet strategy that was proposed was found to be more effective than those other approaches. The results of the methods that were found to be the most effective are highlighted in bold.

Figure 5: (Flower): a. Original Image; b, c. Multifocus input images; d. Proposed fusion

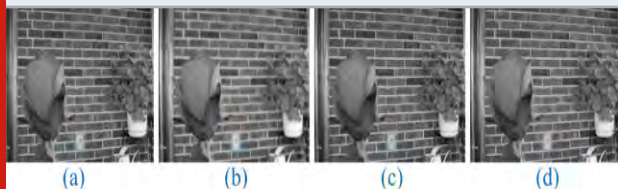


Table 4. Quantitative analysis of proposed and existing image fusion scheme (Panigrahy and 2020)

Evaluation Metric	Chinmaya Panigrahy et al. 2020	Proposed
$E(F)$	7.2212	7.1982
AG_F	14.3156	14.3329
CC	0.9687	0.96529
$Q^{AB/F}$	0.8869	0.9996
$SSIM$	0.9477	0.9890
Q_E	0.8617	0.9066

Figure 6: (Lab): a. Original Image; b, c. Multifocus input images; d. Proposed fusion.



Table 5. Quantitative analysis of proposed and existing image fusion scheme (Panigrahy and 2020).

Evaluation Metric	Chinmaya Panigrahy et al. 2020	Proposed
$E(F)$	7.1178	7.1292
AG_F	6.6468	6.6797
CC	0.9791	0.9774
$Q^{AB/F}$	0.8996	0.9996
$SSIM$	0.9122	0.9913
Q_E	0.8675	0.9258

Comparison for multi-focus book image: Figure 4 is an illustration of the assessment of the third multifocus image, which is the book. The multifocus image, as well as the original image, are depicted in Figures 4 (a), (b), and (c), respectively. After the procedure has been successfully applied, the process of creating an entirely focused image is depicted in Figure 4(d), which may be found below. The following parameters— Q_E , $Q^{AB/F}$, CC , AG_p , $SSIM$, and $E(F)$ —are computed so that the suggested methodology's performance can be evaluated. In the end, the performance of the recommended strategy is assessed and contrasted with that of other methods earlier published in scholarly research. Table 3 of the study contains the comparison's findings, which can be viewed here. The proposed strategy appears to be more effective than the study described by (Panigrahy and 2020), and the results that appear to be the most favorable are highlighted in bold.

Figure 7: Some multifocus image pairs.

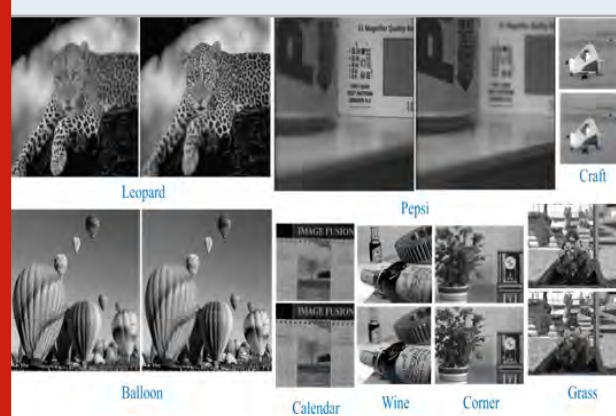
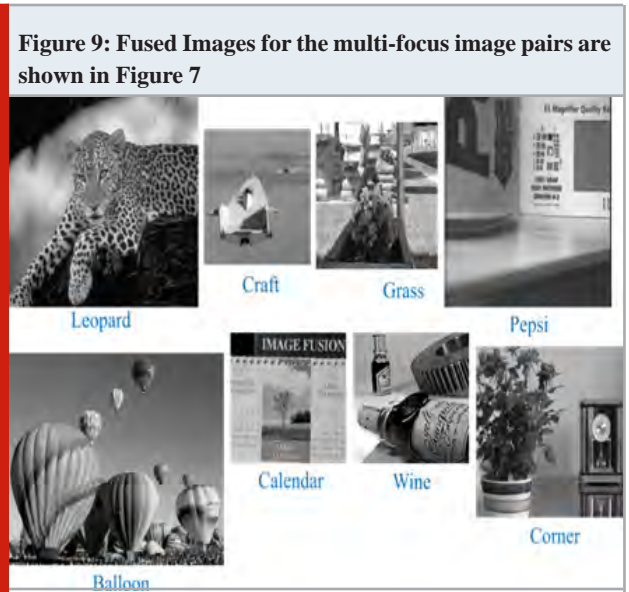


Figure 8: Some multi-focus image pairs.



Comparison of multi-focus flower image: Figure 5 depicts the evaluation of the fourth multifocus image, a flower. The original image is shown in Figure 5(a). Multi-focus examples of flowers are shown in Figures 5(b) and (c), where the left and right focuses are, respectively. Figure 5(d) depicts the resultant sharp image that is achieved by using the method. The effectiveness of the proposed method is evaluated by calculating Q_E , $SSIM$, $Q^{AB/F}$, CC , AG_p , and

$E(F)$. Finally, the proposed strategy is considered in light of various approaches already in the literature. Table 4 displays the report's comparison results. Table data on quality measures demonstrate that the proposed method outperforms the approach proposed by (Panigrahy and 2020).



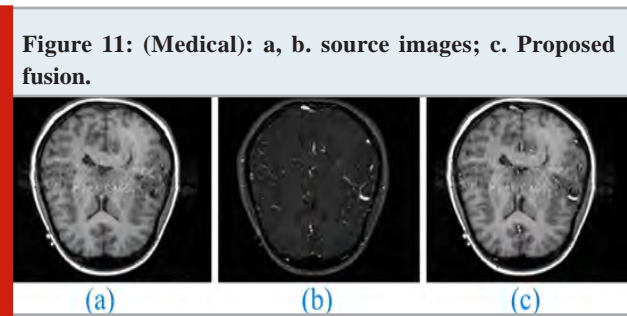
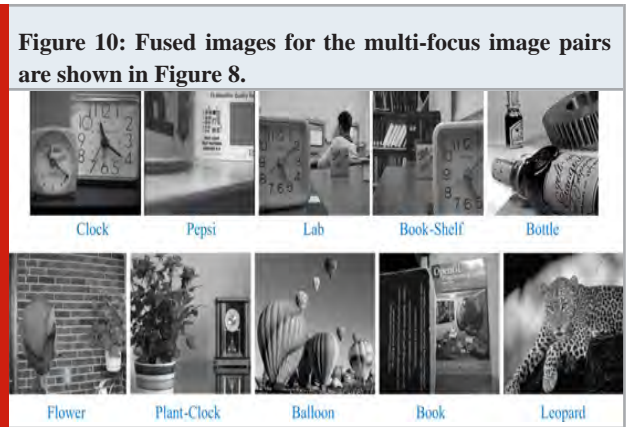
Comparison for multi-focus lab image: Figure 6 illustrates the assessment of the fifth multifocus image, i.e., lab. The following parameters— Q_E , $SSIM$, $Q^{AB/F}$, CC , AG_F , and $E(F)$ —are computed so that the suggested methodology's performance can be evaluated. In the end, the performance of the recommended strategy is assessed and contrasted with that of other methods previously published in scholarly research. Table 5 contains an analysis of the findings from the comparison. The results shown by the proposed approach are more effective than the methodologies reported by (Panigrahy and 2020), and the most successful outcomes of the methods are shown in bold.

Table 6. Comparative Analysis of quantitative measures (average value) using ‘(Panigrahy and 2020)’

Evaluation Metric	Chinmaya Panigrahy et al. 2020	Proposed
$E(F)$	7.2766	7.2530
AG_F	15.1203	16.0783
CC	0.9736	0.97029
$Q^{AB/F}$	0.898	0.9635
$SSIM$	0.872	0.9756
Q_E	0.8336	0.9102

Table 7. Comparative analysis of quantitative measures (average) using ‘(Zhang 2021)’.

Fusion Methods	AG	EI	GM	SD	ID	$Q^{AB/F}$	Q^0	QE
DWT - CSR	7.7793	78.6239	104.4005	52.3230	9.8798	0.7360	0.8168	0.6941
DTCWT - CSR	7.6828	77.7976	103.8605	52.0522	9.7117	0.7745	0.8152	0.7433
CVT - CSR	7.6924	77.8838	104.4969	52.1355	9.7320	0.7420	0.8051	0.7120
NSCT - CSR	7.6807	77.7429	104.6126	52.2404	9.7074	0.7571	0.8327	0.7186
Proposed	12.5994	67.5573	105.0181	52.8417	8.6535	0.9997	0.9232	0.9233



Analysis of a few more image pairs: No single strategy can guarantee optimal results regarding subjective and objective metrics for any image set. In light of this, in Figures 7 and 8, the current research presented several additional pairs of multi-focus images based on the current method. Figures 9 and 10 show the resulting fused images from these

multi-focus images. Results from applying the suggested fusion method to the tested image pairs are presented in Figures 9 and 10. Tables 6 and 7 indicate the average objective evaluation of multiple processes for the image pairs depicted in Figures 7 and 8. Tables 6 and 7 provide the comparison outcomes. The best results of the various methods are emphasized in bold. The proposed method is superior to those described in the literature (Panigrahy and 2020; Zhang 2021).

Table 8. Comparative analysis of Quantitative measures (Average) with '(Panigrahy and 2020)'.

Evaluation Metric	Chinmaya Panigrahy et al. 2020	Proposed
$E(F)$	6.4646	6.4741
AG_F	14.6353	14.4300
CC	0.9255	0.9204
$Q^{AB/F}$	0.8704	0.8636
$SSIM$	0.7027	0.9632
Q_E	0.813	0.8958

Figure 12: (Remote Sensing): a. Original Image; b, c. Multifocus input images; d. Proposed fusion.**Table 9. Comparative Analysis of quantitative measures (average) with '(Panigrahy and 2020)'.**

Evaluation Metric	Chinmaya Panigrahy et al. 2020	Proposed
$E(F)$	7.195	7.1433
AG_F	12.6765	12.4640
CC	0.5219	0.4751
$Q^{AB/F}$	0.8229	0.9996
$SSIM$	0.6679	0.8068
Q_E	0.6085	0.7351

Analysis of remote sensing and a medical image pair:

Finally, remote sensing and medical image pairs test the proposed methodology. With these image pairs as a test, it is possible to determine whether the proposed method performs well in various contexts. Tables 8 and 9 show the results achieved using multiple approaches for the "Remote sensing" and "Medical" picture pairings, respectively. In contrast, Figures 11 and 12 show the fusion results produced using various methods for the "remote sensing" and "medical" image pairs, respectively. The following parameters are calculated to assess how well the suggested approach performs: Q_E , $SSIM$, $Q^{AB/F}$, CC , AG_p and $E(F)$. The performance of the proposed strategy is then contrasted with that of other approaches previously reported in the literature. The evaluation findings are displayed in tables 8 and 9 of the study. According to the tables, the suggested methodology demonstrates its effectiveness for (Panigrahy and 2020), and the highest-quality metrics are denoted in bold.

CONCLUSION

Traditional wavelets based fusion algorithms are degraded due to edge loss and spatial distortions. The proposed methodology using SWT-DTCWT-based hybrid wavelet with PCA overcomes these severe limitations. The significant objectives of the image fusion process are to achieve better visual quality, the extraction of relevant information from the source images, and the preservation of edges and important regions with acceptable quality. The proposed work is validated with a wide range of data sets using statistical measures like AG , SD , $SSIM$, $Q^{AB/F}$, etc. It is evident from the results that the proposed method produces better visual perception with less distortion.

ACKNOWLEDGEMENTS

Funding: This research did not receive any specific grant from funding agencies in the public.

Conflict of Interests: The authors declare no conflict of interest.

Data Availability Statement: Data can be available on request.

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Diversity and Abundance of Beetles in Lakhimpur, Assam India

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ABSTRACT

This investigation is a short term assessment which is attempted to explore the diversity of beetles in Lakhimpur, Assam as the region is extensively familiar for its biodiversity enrichment. Lakhimpur, a district of Assam receives tropical wet evergreen forest and lies in northern bank of river Brahmaputra. The district is adobe to many valuable flora and fauna. Triggerred by our inquisitiveness, we planned an experiment and collected beetle samples from 4 distinct areas as paddy land, vegetable, garden and forest areas. Beetles were collected twice a day from 6.30-11am during morning and 3-5 pm during evening. Several approved collection techniques like Light trap, Pitfall trap, hand picking were applied. Beetles were then brought to the laboratory and stored in 4% formaldehyde in sterilised small glass vials. Identification was done followed by standard identification manuals. Remarkably, 25 species of beetles belonging to 13 different families have been identified in Lakhimpur as May beetle, *Derobrachus geminates*, Oriental beetle, Aphodine dung beetle, *Heteronychus arator*, *Gonocephalum simplex*, *Chrysochus cobaltinus*, *Compsosternus auratus*, *Gyrinus substriatus* and others. Weather parameters like temperature, relative humidity, rainfall may have influence over beetle abundance in Lakhimpur. Our beyond expectation findings clearly indicated that Lakhimpur, like other parts of Assam is heavily blessed with different varieties of insects

KEY WORDS: BEETLES, WEEVILS, INSECTS DIVERSITY, LAKHIMPUR, ASSAM.

INTRODUCTION

Beetles are contemplated as the largest among all insects making up around 30% of all the insect species described so far (Abou-Shaara et al, 2021). They extensively come under the order 'Coleoptera'. The word is derived from Greek word 'keleos' meaning 'sheathed wing' (Branco and Cardoso, 2020). Around 3,50,000 species of beetles have been identified universally (Gullan and Cranston, 2010). Sighted in all kinds of habitats like soil, dead plants, logs, dung, etc. Beetles have been described to feed on fungi, plants and animal matter. Beetles exhibit many interesting features like they are endopterygotes (undergoing complete metamorphosis); traps air bubbles for diving purpose (water beetle); chemical defense for protection (Tenebrionidae), parasitism (*Platypusyllus castoris*) (Das et al, 2016). From anatomical point of view beetles resembles to other class of insects excluding the elytra, which is formed by hardening of frontal pair of wings. They reveal many adaptations like camouflage (Chrysomelidae), mimicry (Cerambycidae),

aposematism, chemical defense (Tenebrionidae), parasitism (Castor sp.), pollination (Cantharidae), mutualism (ambrosia beetle), tolerance to extreme environment (*Stenocara gracilipes*), migration (Coccinellids), etc. Although some appear as serious agricultural pests and damage crops where as many beetles are proved helpful like ladybeetle acts as a predator against Colorado potato beetle in crop fields. Ground beetles feed on cutworms, caterpillars, snails, slugs and other soil dwelling insects. rove beetles takes shelter beneath stones or wooden blocks acts as good decomposers.

Lakhimpur, a district of Upper Assam is an administrative district of Assam. The district is bordered by Arunachal Pradesh, Majuli, Biswanath, Siang, etc. and by Subansiri river from all around. It covers a total area of 2,277 km² approx. The district is about 85m above sea level. Lakhimpur is geographically subdivided into 3 regions as- Narayanpur-Bihpuria, Dhakuakhana and North Lakhimpur. Biodiversity is enriched with tropical wet evergreen forest. Some popular forest reserves of Assam like Ranga Reserve, Dulung Reserve, Kakoi Reserve and Bardoibum-Beelmukh Wildlife Sanctuary are located in Lakhimpur and marks the biodiversity enrichment of this part of Assam.

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Received 15/09/2022 Accepted after revision 25/11/2022

Published: Dec 2022 Pp- 525-531

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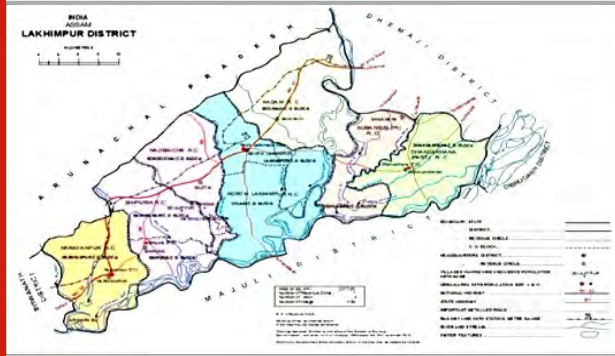
<https://creativecommons.org/licenses/by/4.0/>.

Available at: <http://bbrc.in/> DOI: <http://dx.doi.org/10.21786/bbrc/15.4.7>

Lakhimpur (Location, Constitution and Area) :- The rural area of Narayanpur is located about 7.2km from Tatibahar station, Lakhimpur, which comes under Bihpuria constituency. Our assessment was conducted specifically in various parts of Narayanpur.

Longitude - 94.20/ Latitude - 27.53/
 Average rainfall -1227mm approx. Humidity- 57%-63%.
 Temperature - 9°C to 24°C during summer.

Figure 1: Geographical location of Lakhimpur



MATERIAL AND METHODS

Collection and assemblage of beetles were carried out randomly from four different sites. Beetle sampling was initiated with the onset of pre-monsoon season as this season marks the peak time for beetle emergence (Bhattacharyya et al, 2015; Pathania M, and Chandel RS , 2016) and continued for 3 consecutive months from March to May, 2022 . Beetles were spotted twice on daily basis from 6.30am-11am during morning and 3pm-5pm during evening. Hand picking and insect catching nets were preferred. Collected beetles were killed by vapour of killing agents and brought to the laboratory with delicacy and later stored in 4% formaldehyde. Spotting was brought about based on physiological characteristics followed by the protocol of (Neog and Rajkhowa , 2016).

To monitor the abundance of beetles Light Trap method was also applied followed by the protocol of Reissing et al, 1986. Materials used for making Light Trap included bamboo/wooden poles, string/rope, kerosine lamp/electric blub and basin full of water/ jute sack. Light trap was installed in the target areas to trap beetles. The poles of the trap were firmly set on the ground and the bulb was mounted on the trap frame which was 5mtrs from the ground. To avoid electrocution , proper care was taken while using electric bulb. Next the jute sack was carefully set under the light chamber. The device was left overnight for 3 consecutive months and trapped insects were collected during morning hours. Another effective and proved technique known as ‘Pitfall trap’ was also applied to encounter the abundance of beetles in Lakhimpur (Gist &Crossley, 1973). There are two of its kind as ‘dry pitfall trap’ and ‘wet pitfall trap’. We preferred wet pitfall trap where big wholes were made in

the ground and filled with 4% formaldehyde so that insects fall over there and can be trapped easily.

Community structure analysis: Diversity of beetles were analysed using Shannon-Weiner Diversity Index (H). It is a renowned metric system used in ecological analysis. The index value rises with number of species and evenness of abundance. The more is diversity of species in habitat, the higher will be the index.

Figure 2: Relative abundance beetles recorded in Lakhimpur from March to May, 2022.

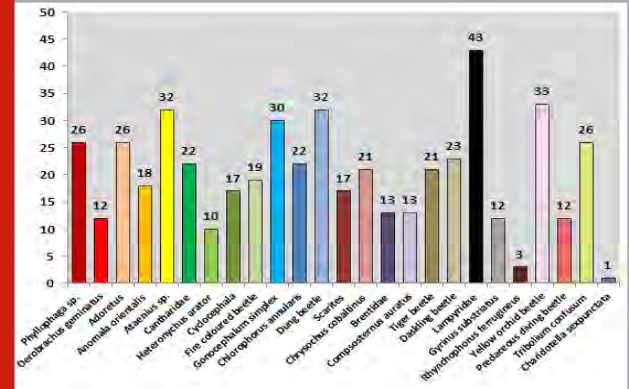
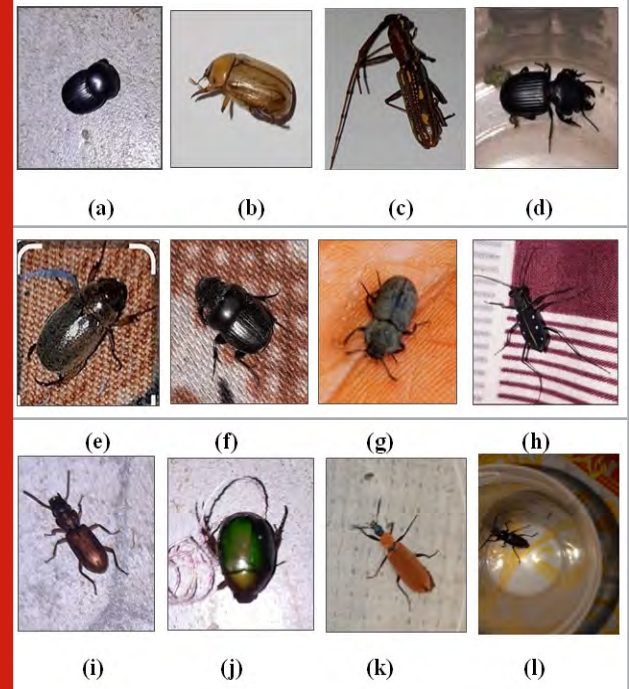


Figure 3: Pictures of some spotted beetles- (a) Ataenius sp,(b) Cyclocephala,(c) Brentidae, (d) Scarites, (e) Phyllophaga sp, (f) Heteronychus arator, (g) Gonocephalum simplex (l) Darkling beetle, (h) Tiger beetle, (j) Oriental beetle, (k) Fire coloured beetle, (l) Derobrachus geminatus



Shannon-Weiner Diversity Index (H)=-∑[pi *log(pi)]

Where,
 H= Shannon Diversity Index; pi=Proportion of individuals

of i-th species in a community; $p_i = n/N$,

Where,

n =Individuals of a given species and N =Total number of individuals of a community.

RESULTS AND DISCUSSION

More than 500 beetles were captured from March to May, 2022. A total of 25 species of beetles belonging to 13 different families were spotted. Scarabaeidae family beetles took lead followed by Chrysomelidae and Cerambycidae. Among the dominant species were *Lampyridae* (Firefly) (8.53%); Yellow orchid beetle(6.54%); *Ataenius* sp. (Aphodine dung

beetle), Dung beetle (6.34%); *Gonocephalum simplex* (Dusty brown beetle) (5.95%); *Phyllophaga* sp.(May Beetles), *Adoretus*, *Tribolium confusum* (Confused flour beetle) (5.15%). Whereas Darkling beetle (4.56%); Cantharidae (Soldier beetle), *Chlorophorus annularis* (4.36%); *Chrysochus cobaltinus* (blue milk weed beetle), Tiger beetle (4.16%); Fire coloured beetle (3.76%); *Anomala orientalis* (Oriental beetle) (3.57%); *Cyclocephala*, *Scarites* (ground beetle)(3.37%); *Brentidae* (straight snouted weevils) , *Compsosternus auratus* (2.57%); *Derobrachus geminatus*, *Gyrinus substriatus*, Predaceous diving beetle (2.38%) showed noticeable gathering. Only *Heteronychus arator* (African black beetle)(1.98%); *Rhynchophorus ferrugineus* (Red plan weevil) (0.59%); *Charidotella sexpunctata* (0.19%) turned up in less or very less quantity.

Table 1: Beetles spotted in Lakhimpur from March to May, 2022.

SL NO.	NAME OF SPECIES	SCIENTIFIC CLASSIFICATION						
		KINGDOM	PHYLUM	CLASS	ORDER	FAMILY	GENUS	SPECIES
1	<i>Phyllophaga</i> sp.(May beetles)	Animalia	Arthropoda	Insecta	Coleoptera	Scarabaeidae	<i>Phyllophaga</i>	--
2	<i>Derobrachus geminatus</i>	Animalia	Arthropoda	Insecta	Coleoptera	Cerambycidae	<i>Derobrachus</i>	<i>geminatus</i>
3	<i>Adoretus</i>	Animalia	Arthropoda	Insecta	Coleoptera	Scarabaeidae	<i>Adoretus</i>	--
4	<i>Anomala orientalis</i> (Oriental beetle)	Animalia	Arthropoda	Insecta	Coleoptera	Scarabaeidae	<i>Anomala</i>	<i>orientalis</i>
5	<i>Ataenius</i> sp. (Aphodine dung beetle)	Animalia	Arthropoda	Insecta	Coleoptera	Scarabaeidae	<i>Ataenius</i>	--
6	Cantharidae (Soldier beetle)	Animalia	Arthropoda	Insecta	Coleoptera	Cantharidae	Cantharidae	--
7	<i>Heteronychus arator</i> (African black beetle)	Animalia	Arthropoda	Insecta	Coleoptera	Scarabaeidae	<i>Heteronychus</i>	<i>arator</i>
8	<i>Cyclocephala</i>	Animalia	Arthropoda	Insecta	Coleoptera	Scarabaeidae	<i>Cyclocephala</i>	--
9	Fire coloured beetle	Animalia	Arthropoda	Insecta	Coleoptera	Pyrochroidae	--	--
10	<i>Gonocephalum simplex</i> (Dusty brown beetle)	Animalia	Arthropoda	Insecta	Coleoptera	Tenebrioninae	<i>Gonocephalum</i>	<i>simplex</i>
11	<i>Chlorophorus annularis</i>	Animalia	Arthropoda	Insecta	Coleoptera	Cerambycidae	<i>Chlorophorus</i>	<i>annularis</i>
12	Dung beetle	Animalia	Arthropoda	Insecta	Coleoptera	Scarabaeidae	--	--
13	Scarites (ground beetle)	Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	Scarites	--
14	<i>Chrysochus cobaltinus</i> (blue milk weed beetle)	Animalia	Arthropoda	Insecta	Coleoptera	Chrysomelidae	<i>Chrysochus</i>	<i>cobaltinus</i>
15	<i>Brentidae</i> (straight snouted weevils)	Animalia	Arthropoda	Insecta	Coleoptera	Brentidae	<i>Brentidae</i>	--
16	<i>Compsosternus auratus</i>	Animalia	Arthropoda	Insecta	Coleoptera	Elateridae	<i>Compsosternus</i>	<i>auratus</i>
17	Tiger beetle	Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	--	--
18	Darkling beetle	Animalia	Arthropoda	Insecta	Coleoptera	Tenebrionidae	<i>Stenocara</i>	<i>gracilipes</i>
19	<i>Lampyridae</i> (Firefly)	Animalia	Arthropoda	Insecta	Coleoptera	Lampyridae	<i>Lampyridae</i>	--
20	<i>Gyrinus substriatus</i>	Animalia	Arthropoda	Insecta	Coleoptera	Gyrinidae	<i>Gyrinus</i>	--
21	<i>Rhynchophorus ferrugineus</i> (Red plan weevil)	Animalia	Arthropoda	Insecta	Coleoptera	Curculionidae	<i>Rhynchophorus</i>	<i>ferrugineus</i>
22	Yellow orchid beetle	Animalia	Arthropoda	Insecta	Coleoptera	Chrysomelidae	--	--
23	Predaceous diving beetle	Animalia	Arthropoda	Insecta	Coleoptera	Dytiscidae	--	--
24	<i>Tribolium confusum</i> (Confused flour beetle)	Animalia	Arthropoda	Insecta	Coleoptera	Tenebrionidae	<i>Tribolium</i>	<i>confusum</i>
25	<i>Charidotella sexpunctata</i>	Animalia	Arthropoda	Insecta	Coleoptera	Chrysomelidae	<i>Charidotella</i>	<i>sexpunctata</i>

Lampyridae, the light emitting firefly belonging to Suborder Polyphaga and Superfamily Elateroidea administered as most dominant which might be due to its preference of temperate and tropical climatic conditions. Fire flies have been reported to disappear day by day due to excessive use of pesticides, fungicides, human traffic, light pollution and many more (Patole, 2019). Dung beetle has good role in agriculture, improving soil fertility, seed dispersal,

protection of livestock, etc. But in our study their dominance indicated proper management of ecosystem by the people of Assam which is a good remark as fire flies are human friendly (Latha and Sabu, 2018). *Ataenius* sp. and Yellow orchid beetles are considered as harmful for damaging crops and flowering plants, hence their huge presence might be seriously noted (Sruthi and Roopavathy, 2021).

Table 2. Comparative analysis of physiological features about beetles detected in Lakhimpur

SL NO.	NAME OF SPECIES	SPECIAL FEATURES	BODY STRUCTURE	COLOUR	HOST PLANTS	ECONOMIC IMPORTANCE
1	<i>Phyllophaga</i> sp. (May Beetles)	Nocturnal	12-35 mm	Blackish/reddish brown.	Turf grass, ornamental christmas trees. Cranberries and certain vegetables and field crops.	Injury to roots and rootstock causes small sapling and tender tap rooted plants.
2	<i>Derobrachus geminatus</i>	Have collar of spines on thorax. They fly at dusk.	3-3.5/4 inches. Sometimes 6-8 inches.	Black/brown	Paloverde trees, rose and olive plants.	Helps in pollination.
3	<i>Adoretus</i>	It is a small elongated oval beetle	10-12 mm	Brown covered with numerous white creamy setae	Lichu, rice, etc.	They damage wide variety of agricultural crops and several other plants.
4	<i>Anomala orientalis</i> (Oriental beetle)	Adults are weak fliers. Overwintering occurs in the final instars.	Body length 0.7-11 cm or 8-13 cm. Shape oval.	Metallic brown and black coloured elytra.	Daisies, roses, hollyhock, phlox and petunias.	Causing damage to horticultural species such as Dahlia sp., corn, crossberry.
5	<i>Ataenius</i> sp. (Aphodine dung beetle)	Male dung beetle have distinct horns, shows symbiotic relationship with mites.	1.5-4.5 cm in length.	Brown/black/bright metallic green in colour	This species breeds and feeds on the dung of carnivores. Eat mushrooms, carrion and decaying leaves and fruits.	They help in maintaining nutrient cycle. They present population of parasitic flies from breeding in the fresh faeces.
6	<i>Cantharidae</i> (Soldier beetle)	Also known as catherwings because of their soft elytra.	5-10 mm (0.2-0.6 inches)	Brown and black and trimmed like a soldiers uniform with red, yellow and orange.	Roses and other flowers. Goldenred.	Soldiers beetles are important pollinators.
7	<i>Heteronychus arator</i> (African black beetle)	Antennae 10 segmented, with a 3 segmented club.	12-15 mm long.	Shiny black oval shaped beetle (black to dark reddish brown)	Crop plants, garden flowers trees and shrubs.	Damage turf and under ground crops notably potato tubers.
8	<i>Cyclocephala</i>	Usually attracted to lights	11-14 mm long; 6-7 mm wide	Dull yellow brown with dark markings on head and legs	Plants roots and other matter near the soft surface	Damage crop lands.
9	Fire coloured beetle	They have 2 luminiscent spots at the posterior corners of the pronotum.	4-20 mm	Orange/red in color.	Feeds on pollen, nectar, fungus and rooting wood.	Not harmful in nature.
10	<i>Ganocephalum simplex</i> (Dusty brown beetle)	They may become cannibalistic if too crowded.	1.5 inches	Dusty brown in colour	Feeds on coffee and rice	Pest of a wide range of crops and poor plant stands. eg- cereals.
11	<i>Chlorophorus annularis</i>	Mostly found in bamboo forest.	0.6 inches (15 mm) long and 0.2 (5 mm) wide.	Yellow with black tiger like markings.	Bamboo, cotton, sugar cane	It is a serious and damaging pest of bamboo.
12	Dung beetle	Dung beetles are known to bring ecological balance and economic advantage to environment.	5-30 mm	Brown/black in colour.	Dung beetles eat liquid from animal dung.	Beetles are important for the breakdown and recycling of dung into the soil.
13	Scarites (ground beetle)	Found mostly in terrestrial habitat	25-35 mm long	Attractive purple/multicoloured dark brown to black	Fungi, decaying wood	Act as biological control agents in agro ecosystem.
14	<i>Chrysochus cobaltinus</i> (blue milk weed beetle)	It rarely has an exposing pygidium.	6.9 mm (0.24-0.35 inches) in length.	Blue in colour.	Feeds on foliage and flowers of newly developed plants.	Pest for many plants, causes damage to plants.
15	<i>Brentidae</i> (straight snouted weevils)	They are identified by their distinctive long	0.5-50 mm long	Dark in colour with orange markings in	Fungi, seeds, dead wood	Damages and kills crops

Table 2 Continue

		snouts and antennae .		wings		
16	<i>Composternus auratus</i>	Slender body	Approx 4.5 cm in length	Metallic green	Nector, pollen grains, sometimes soft insects like aphids	Helps to avoid predation.
17	Tiger beetle	Have large bulging eyes, long slender legs and large curve mandible.	10-20 mm long (2/5-4/5 inches) long	Uniform black in colour, greyish brown to black with white spots	Feeds upon spiders	Considered as indicators of healthy environmental conditions.
18	Darkling beetle	Only identifiable with lens/microscope	1-1.5 cm		Feeds on dead plants , fungal material, seeing flying around in gardens and woodland areas.	They can be vectors of diseases and source of several diseases like acute leucosis. They are served as bird food known as mealurosms.
19	<i>Lampyridae</i> (Firefly)	In firefly bioluminescence organs are found	5-25 mm	Vivid yellowish orange, gree, yellowish green	Soft bodies insects that live on ground like snails, slung worms, etc.	Fireflies contribute to the food web stability, playing important role as both predators and prey
20	<i>Gyrinus substriatus</i> (whirligig beetle)	It is an aquatic beetle, swims underwater. Boat shaped.	6mm (0.24 inches) long and 4.5 mm (0.18 inches) wide.	Black in colour with a metallic shine	Whirligig beetles feed by capturing and eating dead/decaying plants.	This beetle will scavenge on decaying plant material and detritus in fresh bodies of water.
21	<i>Rhynchophorus ferrugineus</i> (Red plan weevil)	Weevil larva can excavate holes in trunks of palm trees. Adult insect is an excellent flier	35 mm long and 10 mm wide and are characterised by a long curved rostrum	They have brown head and white body , dark spots are visible on the upperside of the middle part of the body.	They mostly feed on red plam, date and coconut	It is an invasive pest that causes severe economic loss in plam plant cultivation.
21	<i>Rhynchophorus ferrugineus</i> (Red plan weevil)	Weevil larva can excavate holes in trunks of palm trees. Adult insect is an excellent flier	35 mm long and 10 mm wide and are characterised by a long curved rostrum	They have brown head and white body , dark spots are visible on the upperside of the middle part of the body.	They mostly feed on red plam, date and coconut	It is an invasive pest that causes severe economic loss in plam plant cultivation.
22	Yelloworchid beetle	Mostly found near waterfalls	They have well developed legs, wings and antennae	Striking yellow body with black eyes	Orchid plant	They destroy flowers, mostly orchids.
23	Predaceous diving beetle	Larva are called 'water tigers'. These beetles prefer quiet at the edges of ponds and streams, floating gently among weeds.	Larva are elongated ,flattened and can be 2 inches long.	Shiny black, brown to dive beetles, sometimes with yellowish marks.	Fierce predators, they donot hesitate to attack prey larger than themselves, including small fish, tadpoles and frogs.	Predaceous diving beetles are common and important members of freshwater ecosystem.
24	<i>Tribolium confusum</i> (Confused flour beetle)	Distinctive antenna with 4 segments which gradually thickens towards the tip; shape of thorax is also special.	3-6mm (1/8-1/4 inch) in length	Reddish brown in colour	Found in infested grains, feeding on broken grains and other food items like rice, dried fruits.	Considered as common model organism in science.
25	<i>Charidotella sexpunctata</i>	Their crystal margins are expanded and nearly transparent.	Adult measures 5-7mm in length.	Color varies from reddish brown with black spots to brilliant, mirror like gold	Consumes foliage of plants, sweet potato, morning glory	Common garden pests

Table 3. Result of Shannon-Weiner Diversity Index

SL NO.	SPECIES	pi * ln (pi)	H
1.	<i>Phyllophaga</i> sp.	-0.065	1.235 rounded off to 1.2
2	<i>Derobrachus geminatus</i>	-0.033	
3	<i>Adoretus</i>	-0.065	
4	<i>Anomala orientalis</i>	-0.045	
5	<i>Ataenius</i> sp.	-0.073	
6	<i>Cantharidae</i>	-0.055	
7	<i>Heteronychus arator</i>	-0.020	
8	<i>Cyclocephala</i>	-0.045	
9	Fire coloured beetle	-0.045	
10	<i>Gonocephalum simplex</i>	-0.065	
11	<i>Chlorophorus annularis</i>	-0.055	
12	Dung beetle	-0.073	
13	Scarites	-0.045	
14	<i>Chrysochus cobaltinus</i>	-0.055	
15	<i>Brentidae</i>	-0.033	
16	<i>Compsosternus auratus</i>	-0.033	
17	Tiger beetle	-0.055	
18	Darkling beetle	-0.055	
19	<i>Lampyridae</i>	-0.087	
20	<i>Gyrinus substriatus</i>	-0.033	
21	<i>Rhynchophorus ferrugineus</i>	-0.026	
22	Yellow orchid beetle	-0.073	
23	Predaceous diving beetle	-0.033	
24	<i>Tribolium confusum</i>	-0.065	
25	<i>Charidotella sexpunctata</i>	-0.003	

G. simplex also known as Dusty brown beetle, acts as soil dwelling beetle greatly contribute to agroecosystem including predation, decomposition, etc. Though Darkling beetles are good source of food for birds, lizards, rodents, spiders, etc. but they cause immense harm to poultry by transmitting diseases among flocks, decreasing growth and productivity. Cantharidae (Soldier beetle) are abundant visitors of firmland & known as important biocontrols prior to their contribution in agriculture, pollination, etc. As the name signifies, they feed on eggs and larva of insects which are injurious to crops. They secrete noxious chemicals in 'self defence' (Gupta et al., 2020).

For multiple reasons tiger beetle are beneficial as they feed upon pests including ants, caterpillars, spiders, etc. *Chrysochus cobaltinus* though are harmful to host plants as they consume new leaves and flowers in bulk when attack in groups (Kaud and Sharma, 2017). *Tribolium confusum* attacks stored grains where their dead bodies, fecal pellets causes pungent smell resulting in rejection of grains (Kalawate, 2019).

Predaceous diving beetle, most common among other aquatic beetles, is known as 'water tiger', feeds on aquatic insects,

tadpoles and sometimes beetles of its own kind (Morgan, 1992). They can be best collected by light trapping method at night where as can be easily collected in freshwater zones in day time. Apart from the rest of beetles captured, *Charidotella sexpunctata* was the least abundant during our survey. Weather parameters like temperature, rainfall, humidity, moisture might have positively influenced the abundance of beetles and other insect pests in Lakhimpur (Yumamura et al., 2006).

Insects are poikilothermic animals. Researches have proved that weather factors like temperature, relative humidity, rain fall, etc. remarkably influences the abundance, intensity, distribution, feeding behaviour of beetles and most of the insects in any area (Sima and Srivastava, 2012). Temperature interferes in the metabolic activities of insects and leads to increase their larval and pupal growth (Montgomery et al., 2020). Insects grow best in between temperature range of 15-38 °C Rainfall also affects insect dynamics. In researches it has been reported that insect seasonality is related to rainy season as in rainy season there is abundance of food resources which gives high peak to population (Smith et al., 2017). Results of Shannon-Weiner Diversity Index with index value (H=1.2) correlated with beetle abundance in Lakhimpur.

CONCLUSION

Scarabaeidae family under order coleoptera is the largest and most abundant family of insects. Maintaining resemblance with our previous successful attempt to explore the insect diversity in Silapathar district, this attempt also surprised us. 25 species of beetles were spotted during our short term initiative with limited resources. The diverse climatic conditions of Assam as well as Lakhimpur have huge contribution in enriching the diversity of beetles as well as other organisms in this very area. We are enthusiastic that long term surveys will explore more about beetles of Assam like their roles in ecosystem, feeding habits, life cycle, adaptations, predations, etc.

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Acute Toxicity Alterations in Oxidative Stress Enzymes and Biochemical Parameters in *Oreochromis mossambicus*, Induced by Cartap Hydrochloride And The Modulatory Effects of *Ocimum sanctum* Supplementation

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ABSTRACT

The present study was assessed to determine the acute toxicity and the changes in oxidative stress enzymes and some other biochemical parameters at the sublethal level of a thiocarbamate pesticide cartap hydrochloride on freshwater fish *Oreochromis mossambicus*. The study reveals that the 96h median lethal concentration (LC₅₀) value of cartap hydrochloride is 20.7 µg/l. Besides, the exposed fish also exhibited erratic behavioral responses at the acute level. The effects of cartap hydrochloride at the sublethal concentration (30% of 96h LC₅₀ value) after 15d and 30d exposure induces alterations in biochemical parameters of freshwater fish *Oreochromis mossambicus*. Moreover, the modulatory effects of *Ocimum sanctum* powder (20 gm/kg feed) on the toxicity of cartap hydrochloride were investigated. The investigation demonstrated that sublethal concentrations of cartap hydrochloride increased the levels of liver catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), malondialdehyde (MDA), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Additionally, the exposed fish treated with dietary *Ocimum sanctum* abridged the toxic effects of the pesticide. Moreover by using integrated biomarker response (IBR) and biomarker response index (BRI) the change in the health status of pesticide exposed fish upon addition of *Ocimum sanctum* supplemented diet over control diet was determined. These results indicate that cartap hydrochloride alters the survivability and behavioral responses of *Oreochromis mossambicus* at the acute level and changes the biochemical parameters at the sublethal level which was modulated by the additament of *Ocimum sanctum*.

KEY WORDS: CARTAP HYDROCHLORIDE, *OREOCHROMIS MOSSAMBICUS*, BIOMARKER RESPONSE INDEX, *OCIMUM SANCTUM*, OXIDATIVE STRESS ENZYMES, BIOCHEMICAL PARAMETERS.

INTRODUCTION

The increasing density of the human population over the past few decenniums, rapid urbanization and industrial development lead to pollution in the freshwater ecosystem. Every year 80 percent of residential wastewater and commercial wastewater are dumped in natural water bodies worldwide which disrupt the aquatic ecosystems (Carazo-Rojas et al., 2018). It is reported that agrochemicals were estimated at around US\$7.55 billion in 2017 and are anticipated to hit US\$ 9.8 billion by 2050 (Nishimoto 2019).

This indicates that pesticide utilization has been gradually increasing (Maurya et al., 2019). Extensive utilization of pesticides lingers in the soil and finally enters the aquatic ecosystem by agricultural runoff thereby inducing water pollution and adversely affecting several non-target aquatic organisms like fish (Özkara et al., 2016). The pesticides alter endocrine processes and cause developmental anomalies with subsequent death of organisms (Maurya 2019 and Zhang 2017, Ogunnupebi et al., 2020).

Amongst the pesticides, carbamates are water-soluble and actively utilized for both residential and agricultural applications (Tsagkaris et al., 2020). Cartap hydrochloride is a thiocarbamate pesticide and is widely utilized in agriculture in India (Costa 2015 & Gilden 2016). It has been

Article Information:*Corresponding Author: research.ncsaha@gmail.com

Received 15/10/2022 Accepted after revision 05/12/2022

Published: Dec 2022 Pp- 532-541

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Available at: <http://bbrc.in/> DOI: <http://dx.doi.org/10.21786/bbrc/15.4.8>

routinely used against pests of rice, sugarcane, fruit trees, vegetable crops, tea plantations, rice-fish farms and tea farms (Boorugu & Chrispal 2012). The WHO relegates it as a moderately hazardous (Class II) technical grade active pesticide. The maximal acceptable daily intake (ADI) level is 0.05 mg/kg but in severe poisoning, death can occur (Kalyaniwala 2016 & Vivek 2016).

Fish are considered as bioindicators for aquatic contamination and serve as a potential model for environmental monitoring as they accumulate contaminants water (Ali et al., 2020). Recent studies denoted that fish are consequential sources of proteins and lipids (Balami et al., 2020). In our investigation, *Oreochromis mossambicus* is selected as a model fish species due to its high growth rate, prosperous adaptation to different diets, susceptibility to diseases, and effective tolerance to a wide range of environmental conditions (Ghane et al., 2020). Several experiments have been carried out in apes, rabbits, plants, and humans regarding the adverse effects of cartap hydrochloride (Gupta et al., 2015). However, research regarding the toxic effects of cartap hydrochloride on aquatic organisms especially fish *Oreochromis mossambicus* is scarce.

Pesticide contaminants are toxic to non-target species in the aquatic ecosystem (Dar et al., 2015). Their propensity for the formation of free radicals and reactive oxygen species (ROS) is potent to cause oxidative stress in aquatic organisms, leading to a disruption of ROS and antioxidant defense, and therefore can contribute to organism stress (Bhattacharya et al., 2021; Chatterjee et al., 2021). In addition, ROS affects most bio-molecules, including DNA, proteins, and lipids (Bhattacharya et al., 2021). Thus the evaluation of alterations in biochemical enzyme activity is a paramount approach for the evaluation of stress and may, consequently, serve as a possible implementation for aquatic toxicology (Tan et al., 2018). Several studies have been carried out on alterations of stress enzymes in fish exposed to pesticides (Bhattacharjee 2020 & Yang 2020). However, evidence regarding the toxic effect of this pesticide on alterations of biochemical stress enzymes in *Oreochromis mossambicus* is limited.

Medicinal plant science has been gaining great interest globally in recent years. Tulsi (*Ocimum sanctum*) is a shrub of the Lamiaceae family that has been established in northern Central India and is now native to the Eastern tropics. In a variety of research trials, tulsi has been recorded to possess immunomodulatory and antioxidant properties and are significantly effective towards several diseases (Smita 2018 & Sethi 2004). Integrated biomarker response (IBR) provides a methodology that combines all the biomarker responses and plays a vital role in determining the toxicity of contaminants (Beliaeff and Burgeot, 2002; Serafim et al., 2012). Moreover, Biomarker Response Index (BRI) has been widely utilized in recent years to integrate multiple biomarker responses (Hagger et al., 2008). It is rudimentarily focussed on the alteration level (AL) of biomarker responses in contamination treatments relative to those in the control. The AL of each biomarker is graded into four stages. The score of each biomarker is subsequently multiplied by the corresponding weighting to compose an

integrated index for evaluating the general impact and health status of the organism (Hagger et al., 2008).

The objective of the present study is to evaluate the acute toxicity and alterations in oxidative stress enzymes and some other biochemical parameters in Tilapia (*Oreochromis mossambicus*) exposed to cartap hydrochloride and the modulatory effects of *Ocimum sanctum* supplementation to treated fish. The entire biomarker dataset was evaluated using integrated biomarker response (IBR) and biomarker response index (BRI) to assess and compare the health status of exposed fish fed with control diet and *Ocimum sanctum* supplemented diet.

MATERIAL AND METHODS

Ethical Approval: The experimental bioassay was conducted as per the guidelines approved by Institutional Animal Ethics Committee.

Test chemical: The test chemical cartap hydrochloride, used in the study was collected from the local market. Its stock solutions (1% w/v) and subsequent dilutions were made following a standard protocol (APHA. (2005).

Test organism: Adult *Oreochromis mossambicus* (Class: Actinopterygii, Family: Cichlidae) of mean length 7.2 ± 0.49 cm and mean weight 17.4 ± 0.68 g was used for acute and sublethal bioassay. The specimens were given prophylactic treatment by bathing them in 0.05% potassium permanganate (KMnO_4) solution for 2 min to prevent any dermal infections.

Maintenance condition: Fish of different sizes were placed in outdoor cement vats for acclimatization for 7 days and were provided with commercial feed. During this acclimation period, continuous aeration and daily water exchange were conducted for all the tanks. The values of the different physicochemical parameters of water used in the study were as follows: temperature $29.5 \pm 0.5^\circ\text{C}$, pH 7.1 ± 0.5 , free CO_2 18.3 ± 2.0 mg/l, dissolved oxygen 6.2 ± 1.5 mg/l, total alkalinity 164 ± 7.6 mg/l, and hardness 120 ± 4.5 mg/l as CaCO_3 .

Acute toxicity bioassay: The static replacement bioassays were conducted in 15l glass aquaria with 10l of non-chlorinated tap water each containing 10 fish. The values of the physicochemical parameters of water used in the study were as follows: temperature $29.7 \pm 0.8^\circ\text{C}$, pH 7.2 ± 0.3 , free CO_2 26.7 ± 2.4 mg/l, dissolved oxygen 5.3 ± 0.5 mg/l, total alkalinity 174 ± 13.9 mg/l as CaCO_3 , hardness 125 ± 3.8 mg/l as CaCO_3 . Each test was accompanied by three replicates with a control consisting of tap water without any toxicant.

The fish were not fed for 24h before the commencement of the test. Initial range-finding tests were conducted to estimate the spectrum of concentrations of the test chemical. Then the selected concentrations of cartap hydrochloride (00, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 $\mu\text{g/l}$) were used to estimate the 24, 48, 72, and 96 LC_{50} to *Oreochromis mossambicus*. The number of dead organisms was counted

at every 24h of exposure during the experiment and was removed immediately to avoid any organic decomposition. From each aquarium, 10% of water was removed every 24h and replaced with the desired quantity of cartap hydrochloride to assure a fixed concentration of the toxicant in the solution.

The safe level of cartap hydrochloride was calculated based on application factors (AF) using standard protocols (Burdick 1967 & Edwards et al., 1967). The ratio of the maximum harmless concentration of a toxicant to the concentration that is lethal, after a given exposure period, to 50 percent of test animals (median lethal concentration, LC_{50}) has been termed the "application factor. Application factors are used to establish acceptable toxicant concentration ranges depending on water, quality, species under study and life stage (Edwards & Brown, 1967).

Collection of plant material and preparation of experimental diet.: Fresh leaves of *Ocimum sanctum* were collected from the campus of The University of Burdwan, Golapbag, West Bengal. They were washed thoroughly in running tap water and subsequently dried for 10 days. After 10 days of sun drying, the leaves were crushed and ground using a mixture grinder. The powdered form of *Ocimum sanctum* was then added to the control diet (containing fish meal, wheat flour, wheat bran, fish oil, sunflower oil, vitamins, and minerals) at an amount of 20 g/kg of fish feed. The amount of 20 g of *Ocimum sanctum* powder/kg feed was selected in our investigation as this particular amount of *Ocimum sanctum* powder/kg feed was reported to stimulate growth rates in fish (Sikotariya & Yusufzai, 2019).

Experiments on biochemical parameters during the sublethal bioassay: Bioassays on biochemical parameters were also conducted in 20l glass aquaria, each containing 10 l of water and five fish. 6 $\mu\text{g/l}$ (30% of 96 h LC_{50} value)] was employed for the experiment along with a control. The design of the bioassay is depicted in Table 1.

Table 1. Experimental design

Group	Treatment
I	Control without any contaminant + control diet
II	Cartap hydrochloride (6 $\mu\text{g/l}$) + control diet
III	Cartap hydrochloride (6 $\mu\text{g/l}$) + 20 gm of <i>Ocimum sanctum</i> powder/ kg diet
IV	Control without any contaminant + 20 gm of <i>Ocimum sanctum</i> powder/ kg diet

Fish was fed at 10% of the bodyweight daily. Amid the experiment, 20% of the test medium was renewed and replaced with the required amount of pesticide. After 15 and 30 days respectively, fish was anesthetized by immersing them in 0.1% 2-phenoxyethanol. Then the fish were decapitated, and the liver was immediately collected for biochemical examination.

Tissue Homogenization and centrifugation: The liver tissue was homogenized in 2ml of phosphate buffer saline (PBS). The homogenized tissues were spun in a refrigerated centrifuge (REMI C Model, India) at 5000rpm for 15 minutes at 4°C. After centrifugation the supernatants were used directly as aliquots and were stored at -20°C for enzymatic analysis.

Table 2. Lethal concentration values and 95% confidence limits of cartap hydrochloride to *Oreochromis mossambicus*

Point	Exposure concentration ($\mu\text{g/l}$)			
	24h	48h	72h	96h
LC_{50}	38.6 (34.1-43.7)	33.4 (28.7-38.8)	26.8 (22.1-32.4)	20.7 (16.0-26.9)

Table 3. Safe concentrations of cartap hydrochloride to *Oreochromis mossambicus* at 96h exposure period

Pesticide	96h LC_{50} ($\mu\text{g/l}$)	Method	Application Factor (AF)	Safe Level ($\mu\text{g/l}$)
Cartap hydrochloride	20.7	Edwards & Brown, 1967	0.4	8.3
		Burdick, 1967	0.1	2.0

Protein estimation: The protein content in liver tissue was measured by using the method of Lowry et al. (1951). Bovine serum albumin (BSA, Sigma) was used as a standard.

Oxidative stress enzymes analysis: Catalase (CAT) activity was measured following the reduction of hydrogen peroxide to water and molecular oxygen using a standard protocol (Aebi 1984). The estimation of the superoxide dismutase enzyme (SOD) was carried out by the protocol of Kakkar et al. (1984). Glutathione S-transferase (GST) activity was measured through the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (Habig et al., 1974). Standard protocol was employed for the analysis of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Bergmeyer, 1965) was followed with some minor modifications. The colorimetric assay of lipid peroxidation (LPO) was performed following the standard protocol (Buege & Aust 1978). The units of CAT, SOD, GST AST, and ALT were expressed as units of activity per milligram of protein (U/mg protein). MDA level was expressed as nmol TBARS per min per milligram of protein (nmol TBARS/min/mg protein). All the parameters were measured using a UV-Vis spectrophotometer (Cecil Aquarius CE 7400) at room temperature (28°C). All assays were run in triplicate.

Determination of IBR and BRI: IBR was determined by utilizing standard protocol with minor modifications (Beliaeff & Burgeot 2002). The IBR analysis for each biomarker was performed as follows:

1. Estimation of the mean and standard deviation for each treatment
2. Standardization of the results for each treatment as $Fi' = (Fi - \text{mean } F)/S$, where Fi' is the standardized value of the biomarker, Fi is the mean value of a biomarker, F is the mean of the biomarker and S is the standard deviation (SD) calculated for the treatment-specific values of each biomarker
3. Using the standardized data, X was calculated as $+Fi'$ in the case of activation and $-Fi'$ in the case of suppression and the minimum value for each biomarker for all treatments was obtained and then added to X .
4. Eventually, the score S was measured as $B = |\text{min } Fi'| + Z$, where B is the actual value of the minimum Fi' and $|\text{min } Fi'|$ is the actual value of the minimum Fi' .
5. Finally, IBR was determined by multiplying the obtained value of each biomarker (Bi) by the value of the next biomarker and dividing each measurement by 2, and thereafter summing all the values.

Moreover, the biomarker response index (BRI) for determining the health status of the organism was performed using a standard protocol (APHA. 2005).

Statistical methods: Finney's probit analysis method was employed for estimating LC_{50} values. The Shapiro-Wilk test was used to assess normal distributions and Levene's test was employed to evaluate homogeneity. All data obtained from our study fulfilled the parametric criteria and were analyzed using One-way ANOVA followed by Tukey multiple comparisons test to compare the means among the different treatment groups within each exposure period. The correlation matrix and principal component analysis were performed using software Graphpad prism 9 and JMP Pro 14. $p < 0.05$, $p < 0.01$ and $p < 0.001$ and $p < 0.0001$ were accepted as levels of statistical significance. Data are presented as mean \pm SEM.

RESULTS AND DISCUSSION

Acute toxicity and Behavioural Responses: The 24, 48, 72, and 96 h LC_{50} values of cartap hydrochloride to *Oreochromis mossambicus* with 95% confidence limits are depicted in Table 1. Based on the 96h LC_{50} value, the safe permissible limit of cartap hydrochloride was determined which is depicted in Table 2 and is reported to be within the range of 2.0 – 8.3 $\mu\text{g/l}$.

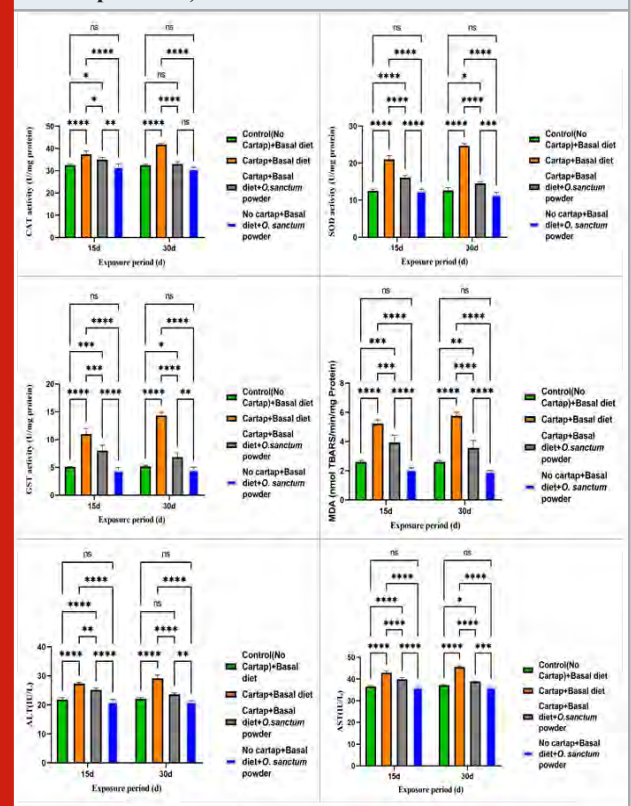
Oxidative stress and biochemical parameters: The effects of cartap hydrochloride on oxidative stress and biochemical parameters as well as their modulation by using *Ocimum sanctum* supplemented diet are depicted in Fig.1 respectively.

CAT activity: In 15d and 30d exposure period, catalase activity increased significantly ($p < 0.05$) in pesticide exposed fish supplemented with a control diet with respect

to control. But the activity was reduced significantly ($p < 0.05$) upon addition of *Ocimum sanctum* supplemented diet to the fish exposed to 6 $\mu\text{g/l}$ of pesticide on 15 and 30d exposure period.

SOD activity: SOD activity significantly increased ($p < 0.05$) in fish exposed to 6 $\mu\text{g/l}$ of pesticide and provided with control diet after 15d and 30d with respect to control. However, the activity was significantly reduced ($p < 0.05$) when the *Ocimum sanctum* supplemented diet was incorporated to pesticide fish exposed on both 15d and 30d exposure periods.

Figure 1: Effects of cartap hydrochloride on A) CAT, B) SOD, C) GST, D) MDA, E) AST and F) ALT levels in *Oreochromis mossambicus* at different exposure periods. CH indicates cartap hydrochloride and OS indicates *Ocimum sanctum*. The values are represented as mean \pm SEM, ns indicates non-significant and * indicates level of significance (* = $p < 0.05$, ** = $p < 0.01$, * = $p < 0.001$ and **** = $p < 0.0001$).**



GST activity: In 15d and 30d exposure period, the activity of GST increased significantly ($p < 0.05$) in fish exposed to 6 $\mu\text{g/l}$ of cartap hydrochloride provided with a control diet with respect to the control. However, the activity was reduced significantly ($p < 0.05$) upon addition of *Ocimum sanctum* supplemented diet to the fish exposed to 6 $\mu\text{g/l}$ of pesticide on both 15d and 30d exposure periods.

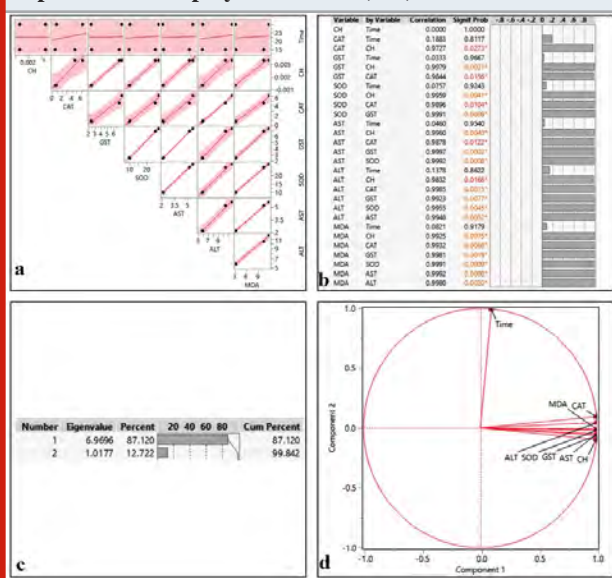
MDA activity: MDA activity increased significantly ($p < 0.05$) in fish exposed to 6 $\mu\text{g/l}$ of pesticide provided with a control diet in both 15d and 30d exposure periods with respect to the control. However, a significant reduction

in MDA activity was observed upon the addition of an *Ocimum sanctum* supplemented diet to the exposed fish on both exposure periods.

AST and ALT activity: ALT and AST activities in fish exposed to 6 µg/l of pesticide combined with a control diet increased significantly ($p < 0.05$) in 15d and 30d exposure periods compared to the control. However, adding an *Ocimum sanctum* enriched diet to the fish exposed to 6 µg/l of pesticide on both 15d and 30d treatment periods decreased the ALT and AST activities significantly ($p < 0.05$).

Chemometrics: The results of the correlation matrices between the concentration of cartap hydrochloride and all the tested parameters are depicted in Fig 2 a-b that was quantified by principal component analysis (Fig. 2 c-d). The statistical analysis demonstrated that CAT, SOD, MDA, AST, and ALT are positively and significantly correlated with cartap hydrochloride concentration (CH) ($p < 0.05$).

Figure 2. Correlation matrix (a), pairwise comparison (b) and ordination diagram of PCA (c-d) on biochemical parameters of the liver in *Oreochromis mossambicus*, after exposure to cartap hydrochloride (CH)



IBR and BRI: IBR was utilized to determine the overall stress of the exposed fish *Oreochromis mossambicus* upon addition of a control diet vs supplemented diet. Higher IBR values indicate high stressful conditions while lower IBR values indicate the low stressful condition to the organism. In our investigation, the IBR value of pesticide-exposed *Oreochromis mossambicus* provided with a control diet is greater than the IBR value of pesticide-exposed *Oreochromis mossambicus* provided with *Ocimum sanctum* supplemented diet (Fig. 3). BRI designated the health status of pesticide exposed fish upon addition of control diet vs *Ocimum sanctum* supplemented diet. According to BRI, there are four classifications: no or slight effect (BRI>3), moderate effect (BRI between 2.75 and 3.0), major effect (BRI between 2.5 and 2.75), and severe effect (BRI ≤ 2.5). Our results indicated that based on BRI values severe

adverse effect was observed in *Oreochromis mossambicus* exposed to cartap hydrochloride and supplemented with a control diet while the severity of the adverse effect was reduced in *Oreochromis mossambicus* exposed to cartap hydrochloride when provided with *Ocimum sanctum* supplemented diet (Fig. 4).

Figure 3: IBR values of cartap hydrochloride (CH) fish *Oreochromis mossambicus* upon addition of control diet vs *Ocimum sanctum* supplemented diet

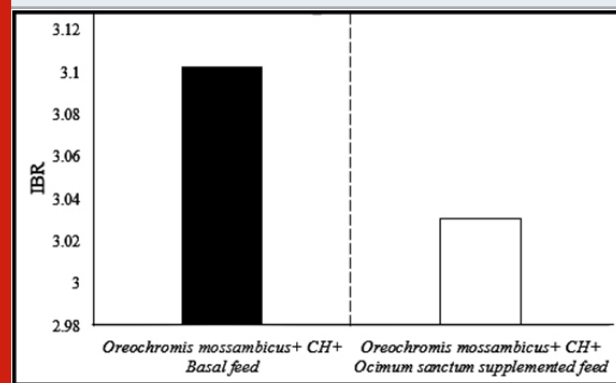
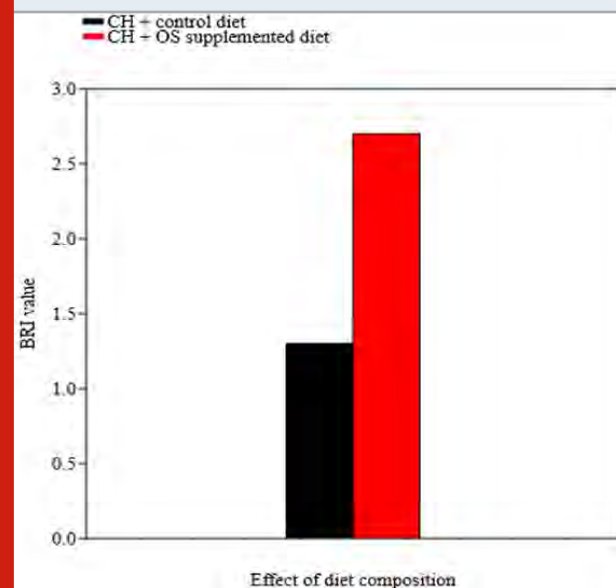


Figure 4: BRI values of cartap hydrochloride (CH) exposed *Oreochromis mossambicus* provided with a control diet vs *Ocimum sanctum* (OS) supplemented diet.



In the present analysis, the 96h LC₅₀ value of cartap hydrochloride to *Oreochromis mossambicus* is 20.7µg/l, suggesting that it is extremely toxic and is lower than the LC₅₀ value of other fish species such as 0.376 mg/l in *Cirrhinus mrigala* and 0.3551 mg/l in *Labeo rohita* (Ali 2020 & Vani 2020). These variations between different fish species in the LC₅₀ value of cartap hydrochloride depend on species, age, bodyweight water physiochemical parameters and duration of exposure (Saravanan et al., 2011). The result of the present study showed that the difference in species, water physiochemical parameters and duration of exposure

is responsible for variations in LC_{50} value reported in our study from the study of previous researchers.

Biochemical stress indices are considered as potential biomarkers and are utilized as diagnostic tools to quantify the effects of environmental stress (Faheem 2019 & Iheanacho 2020). Superoxide dismutases (SOD) are a group of metalloenzymes that initially protects the cell against injury mediated by reactive oxygen species (ROS) (Bhattacharya et al., 2021; Chatterjee et al., 2021). These enzymes catalyze the dismutation of superoxide anion free radical (O_2^-) into molecular oxygen and hydrogen peroxide (H_2O_2) thereby damaging the cells (Bafana et al., 2011).

In the present study, SOD activity in the liver of exposed fish increased significantly which is due to the induction of superoxide ion that prevents the cell against oxidative stress (Zhang et al., 2013). A similar increase in SOD activity was reported in *Pseudotroplus maculatus*, *Cyprinus carpio*, and *Ctenopharyngodon idellus*, upon addition to Chlorpyrifos (Raibeemol, Chitra 2018 & Kaur 2017). CAT is an significant enzyme in the antioxidant system that is primarily involved in ROS detoxification and degradation of H_2O_2 into molecular oxygen and water. In the current study, the increase in catalase activity in pesticide exposed fish is likely due to the neutralization of the inimical effect of the toxicant-induced increased ROS generation.

Moreover, the greater increase in CAT activity revealed its efficient scavenging capabilities in eliminating H_2O_2 caused by pesticide-induced oxidative damage. An increase in CAT activity has also been observed in the studies with *C. carpio* after quinalphos exposure (Hemalatha et al., 2016). This upregulation of enzyme production might be a defense mechanism, providing the first line of defense against pesticide toxicity. To make xenobiotic chemicals more hydrophilic for excretion, GST promotes the conjugation of electrophilic substances or groups into tripeptide glutathione (Pontes et al., 2016). In the present analysis increased GST level in the liver of exposed fish is possibly due to the high rate of formation of glutathione disulfide (Li et al., 2010). Native gel electrophoresis study revealed that a significant increase in GST activity occurred in *Mugil cephalus* and *Chanos chanos* upon the addition of chlorpyrifos (Marigoudar et al., 2018).

One of the principal processes caused by oxidative stress is lipid peroxidation. Lipid peroxides are produced from the oxidative degradation of polyunsaturated lipids in the membranes of cells and organelles (Grotto et al., 2009). Bi-products of lipid peroxidation, such as malondialdehyde (MDA), are utilized as markers of incremented cellular reactive oxygen species concentration and symptoms of cellular injury (Grotto 2009 & Ayala 2014). Increased MDA in pesticide-exposed fish in the present study was likely due to induced oxidative cell injury and increased ROS generation (Faheem et al., 2019). In integration to chlorpyrifos and dichlorvos, similar induction of the MDA level has additionally been reported in *Ctenopharyngodon idellus* and juvenile *Clarias gariepinus* (Kaur 2017 & ON 2018).

Increased hepatic aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity as examined in the current study is indicative of active amino acid catabolism to slake immediate energy demand under toxicant stress. Hence, cellular damage caused by the toxicant was accompanied by increasing cell membrane permeability and enzyme leakage (Majumder & Kaviraj 2017). A high level of MDA coupled with the increase in the activities of hepatic enzymes in the liver was observed in our investigation. This positive correlation between MDA concentration as well as ALT and AST activities, suggests that the enhanced lipid peroxidation may be linked to hepatic damage caused by cartap hydrochloride. A similar induction in hepatic AST and ALT was reported when *Oreochromis niloticus* and *Cyprinus carpio* was exposed to chlorpyrifos (Stoyanova 2020 & Majumder 2017).

One of the oldest aromatic herbs, the leaves of tulsi (*Ocimum sanctum*) have great medicinal value, which keeps our body safe and averts the toxic effects of different environmental and chemical-induced injuries and damage by modulating the levels of anti-oxidant bio-molecules in the body (Sah et al., 2018). The leaf of tulsi, contains many bioactive compounds, including eugenol, ursolic acid, β -caryophyllene, linalool, and 1,8-cineole that might act as a potential immunostimulant. (Yang et al., 2020).

Several scientific studies reported that tulsi is a paramount remedy for chronic lifestyle-cognate diseases such as diabetes, metabolic syndrome, and psychological stress (Jamshidi & Cohen 2017). In our research, the *Ocimum sanctum* supplemented diet (20 g/kg diet) provided to cartap hydrochloride exposed *Oreochromis mossambicus* resulted in the substantial restoration of biochemical stress biomarkers by minimizing stress and thereby improving the health status of the fish as revealed from our IBR and BRI values. This shows that *Ocimum sanctum* possesses intrinsic antioxidant activity that resulted in the suppression of pesticide-induced oxidative stress.

Administration of *Ocimum sanctum* supplemented diet (20 g/kg diet) in the pesticide-exposed group not only restored oxidative stress biomarkers but also hepatic enzymes. The increased activity of ALT and AST was ameliorated in the group that received *Ocimum sanctum* supplemented diet (20 g/kg diet), suggesting that *Ocimum sanctum* can reduce hepatic enzyme activities after exposure to cartap hydrochloride. It seems that the enhanced antioxidant defense mechanism and diminished lipid peroxidation, resulting from *Ocimum sanctum* treatment, was able to protect the liver from oxidative damage caused by cartap hydrochloride, as evidenced by decreased hepatic enzyme activities. This may be attributed to the presence of linolenic acid in the *Ocimum sanctum*, which can suppress the cyclooxygenase and lipoxygenase pathways of arachidonic acid synthesis, resulting in anti-inflammatory action (Upadhyay 2017). Furthermore, the presence of beta carotene in the *Ocimum sanctum* aids in the prevention of cellular damage. (Upadhyay 2017). A similar type modulatory property of tulsi was found against arsenic toxicity in fish (Bhattacharya, 2017).

CONCLUSION

The present investigation revealed that *Oreochromis mossambicus* exhibited alterations in survivability and behavioral responses at the acute level as biochemical stress responses at sublethal level upon addition of pesticide. Thus, it is exposed from the work that cartap hydrochloride is prodigiously toxic to aquatic organisms. Therefore, the present findings on the toxicity of cartap hydrochloride to *Oreochromis mossambicus* may be used as a potential tool for creating awareness among people regarding the excessive use of agrochemicals. Furthermore, our studies also demonstrated that the addition of tulsi to the diet potentially abridged the toxic responses in fish induced by the addition of pesticide. Therefore, special attention should also be given to manufacturing the feed of the fish by including medicinal plant extracts in the feed to reduce the stress responses in fish induced by exposure to several contaminants in water. Further studies are required to elucidate the toxic effect of cartap hydrochloride on *Oreochromis mossambicus* and its modulation using *Ocimum sanctum* supplemented diet at the molecular and ultrastructural level.

Funding: The research did not receive any specific grant from funding agencies in the public, commercial or nonprofit sectors.

Conflict of interest: The authors have no conflict of interest.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Zoology, The University of Burdwan and DST PURSE PHASE II for providing infrastructural facilities to carry out the work.

Data Availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Sharmistha Medda: Writing original draft, Validation, Methodology, **Ritwick Bhattacharya:** Software, validation, Formal Analysis, Data curation, Sarmila Pal: Investigation, Resources, Editing; Nimai Chandra Saha: Conceptualization, Writing Review and Editing, Visualization, Supervision.

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On the Dominant Behavior of Zooplankton in Different types of Domestic Sewage Oxidation Ponds

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ABSTRACT

Plankton are small microscopic organisms, comprising diatoms and algae, which form the basis of primary production, whereas zooplankton are mainly crustaceans, rotifers and larval stages of larger animals which constitute the higher aquatic food web. Both are bio-indicators of a water body, constituting the main food for the carnivorous and omnivorous fishes in the aquatic ecosystem. In the present work, a detailed analysis of zooplankton diversity along with variations in species, and seasonal abundance with average and total population density was carried out in different types of domestic sewage oxidation ponds. Similar analysis for a comparison was done in a freshwater control pond situated in Bhopal India. In this study out of 8 ponds, 4 were selected for zooplankton analysis two being primary and two being secondary sewage oxidation ponds and one freshwater control pond was selected for comparative analysis. The results indicated that the number of zooplankton in all ponds ranged from 162 to 14776 org/L, seasonally it was observed that the sewage oxidation ponds had the maximum population of zooplankton in winter, followed by rainy and summer seasons. Regarding the population of zooplankton in the individual oxidation ponds, it was found that sewage secondary pond IIIB had the maximum zooplankton followed by IIIA. Interesting observations on the cascading effects of physicochemical parameters on plankton dynamics, including the variations in the diel population of various species of zooplankton have been recorded. The phenomenon of plankton grazing in different types of domestic sewage ponds is being reported for the first time.

KEY WORDS: COMPARATIVE ANALYSIS, TROPICAL SEWAGE PONDS, ZOOPLANKTON

INTRODUCTION

Plankton are small aquatic plants or minute animals which float and drift on the surface or are found at the bottom of water bodies. Phytoplankton forms the basis of primary production and zooplankton mainly protozoa, crustaceans and rotifers are excellent natural food for fishes in such nutrient-rich aquatic ecosystems, (Park and Shin, 2007, Ramachandra, 2009a, Ramachandra, 2009b, Goswami and Mankodi, 2012, Nanasahab et al., 2012, Suresh et al., 2013, Yusuf, 2020).

Plankton are also present in the sewage ponds, due to their photosynthetic activity, release oxygen into the water which is then made available to increase the aerobic decomposition of the organic wastes by bacteria. As zooplankton is a good bio-indicator of water quality, and being more sensitive to organic pollutants, many studies have been conducted on the zooplankton diversity in freshwater lakes and

ponds, however, there are very few studies on the seasonal abundance, population diversity of various species of zooplankton in sewage ponds (Jha, et al., 1997, Goswami & Mankodi, 2012, Yusuf, 2020, Grabicova, 2020, Tulsankar et al., 2021).

In the present work, a detailed analysis of zooplankton diversity, variations in species, and abundance with average and total population density was done in different types of tropical domestic sewage oxidation ponds along with a freshwater control pond situated in Bhopal India. Important physicochemical parameters which correlate with the growth of zooplankton were also studied seasonally, to know their cascading effects if any, on the zooplankton abundance and composition in both, wastewater oxidation ponds and the freshwater pond.

MATERIAL AND METHODS

Analysis of zooplankton was done in the domestic sewage oxidation ponds situated at Shahpura sewage ponds located

Article Information:*Corresponding Author: drshariqali@yahoo.com

Received 03/09/2022 Accepted after revision 10-11-2022

Published: September 2022 Pp- 542-546

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Available at: <https://bbrc.in/> DOI: <http://dx.doi.org/10.21786/bbrc/15.4.9>

10 km South-East of Bhopal City (25-17 L). There are 8 sewage oxidation ponds constructed in two series of primary and secondary as per specifications of the of National Environmental Engineering Research Institute (NEERI) Nagpur. Out of eight ponds, four were selected in the present study. (Two being primary, designated as IA & IIIA and two as secondary as IB & IIIB). One freshwater control pond was selected for comparative study. Each pond had an area of 0.4 hectares, designed to treat biologically 3 million gallons of sewage per day. Sewage from adjacent areas is collected in a sump and is pumped to the oxidation ponds where it is detained for a period of 15-20 days for microbial transformation and biological stabilization. The raw sewage enters the primary pond through 3 inlets and after the detention period the biologically treated effluent goes out from the secondary pond through outlet. The treated effluent is either let out into a small canal or into the fields for vegetable cultivation in an area extending more than 50 hectares. The morphometric features of the ponds are: Length = 100.65 mts /Breadth = 50.32 mts Average depth = 1.20 mts.

As large-scale fish mortality and unfavorable conditions occurred in the primary ponds 1A & 1B, fish culture experiments could not be carried out in these ponds and only two distant oxidation ponds, IIIA & IIIB along with a

control freshwater pond with fish culture were analyzed in the present study. In our previous studies, various aspects of fish culture along with the role of nutrients in primary and secondary oxidation and control ponds have been reported, (Ali et al., 2020, 2021).

There was large-scale post-stocking mortality of fishes within 24 hours in the initial (primary) ponds i.e. 1A & 1B due to untreated heavy loads of sewage coming to these ponds, whereas only 10-15% fish mortality was observed in the secondary oxidation ponds III A & III B, which received biologically treated sewage. Hence in the present study, collection and identification and species enumeration of zooplankton (different species) were done only in two oxidation ponds (IIIA & IIIB) as per standard methods and compared with their composition in the control freshwater pond.

Physicochemical parameters like light penetration, water temperature, pH and dissolved oxygen were also studied in these oxidation and control ponds to know the quality of the water and their effect on the survival and growth rates of planktonic organisms. The physico-chemical parameters were estimated as per the procedure described in Standard Methods, 1995 (APHA & AWWA). The identification of zooplankton was carried out by using standard methods described by Palmar (1980), Wetzel (1983).

Table 1. Showing the average seasonal ranges of different physico- chemical parameters of different domestic sewage oxidation and control ponds (Data from Ali et al 2021).

Seasons	Ponds	Light penetration (cm)	Temperature (°C)	pH	Dissolved oxygen (mg/l)
Winter	IIIA	15.40-25.80	18.30-23.80	9.10-10.00	5.30-13.30
	IIIB	15.60-26.30	18.10-23.90	9.40-10.20	5.10-10.40
	CP	35.60-41.30	18.50-24.10	7.80-8.40	5.40-7.20
Summer	IIIA	12.60-14.80	29.00-34.70	8.70-9.90	3.00-17.30
	IIIB	9.20-11.60	32.00-36.20	8.60-9.90	3.80-10.50
	CP	39.30-77.00	32.34-36.50	8.20-9.00	4.50-7.20
Rainy	IIIA	11.00-12.90	24.30-28.10	9.20-10.20	3.60-12.80
	IIIB	11.00-13.10	23.60-27.70	9.60-10.00	4.30-16.90
	CP	26.60-30.30	24.50-28.30	7.80-8.60	5.20-8.40

(IIIA, IIIB – Sewage oxidation ponds: CP - Fresh Water control ponds)

RESULTS AND DISCUSSION

Important physicochemical parameters such as light penetration, water temperature, pH and dissolved oxygen were analyzed seasonal-wise in primary, secondary and freshwater control ponds and are depicted in Table No:1.

Light penetration and the poor transparency, high presence of algae and solids and other undesirable materials are the reasons for low light penetration in the primary pond. Light penetration was found to be high in winter, followed by rainy & summer seasons in our studies, (Table No 1). These parameters are known to cause adverse effects on

various species of zooplankton and their assessment. An important physiochemical variable in aquatic environment is DO, which plays a vital role in survival and growth of organisms such as zooplankton. DO levels are known to reach minimum levels in highly fertile ponds prior to sunrise.

During the course of study, it was observed that there were twenty-one species of zooplankton present in the primary and secondary oxidation ponds under investigation. These species of zooplankton comprised *Copepods*, *Rotifers* & *Brachionus*, out of which *Keratella*, *Cyclops*, *Daphnia*, *Brachionus*, *Eubranchiopus*, *Naupleus* larva regularly occurred in both type of oxidation ponds. The number of zooplankton in all ponds ranged from 162 to 14776 org/L, and seasonally it was observed that the oxidation ponds had the maximum population of zooplankton in winter followed by rainy and summer. Regarding the population of zooplankton in the individual oxidation ponds it was found

that secondary pond IIIB had the maximum zooplankton followed IIIA. (Table No. 2) *Cyclops* (486 to 10965 org/L), *Keratella* (324 to 8748 org/L) *Brachionus* (1000 to 5000 org/L), *Naupleus* larva and *Daphnia* (162 to 106) were the dominant species recorded in these ponds.

On the other hand, the total number of zooplankton species in the fresh water control pond was only ten and the dominant ones were *Cyclops*, *Keratella*, *Daphnia* and *Naupleus* larva. The total population of zooplankton in the fresh water pond ranged between 162 to 3240 org/1. Thus, it is evident that the number of zooplankton in the fresh water control pond were many times less than the sewage secondary oxidation ponds. The average population density of zooplankton in the oxidation ponds was four times greater than that of the fresh water control pond. The seasonal dominance of the zooplankton in the fresh water control pond was seen more during winter followed by summer and rainy seasons (Table No. 2, 3).

Table 2. Showing the seasonal presence of various species of zooplankton in different domestic sewage oxidation ponds along with a freshwater control pond (numbers/litre) IIIA&IIIB- Sewage oxidation ponds CP- Fresh water control ponds

Season	Ponds	Cyclops	Keratella	Daphnia	Naupleous larva	Brachionus	Eubran chiopus	Total	Average
Winter	IIIA	2106	2430	810	972	2106	4212	12636	2106
	IIIB	2268	4212	0	0	1944	1296	9720	1620
	CP	810	972	0	324	648	0	2754	459
Summer	IIIA	648	810	810	648	1458	0	4374	729
	IIIB	1458	648	648	810	810	0	4374	729
	CP	810	972	486	324	648	0	3240	540
Rainy	IIIA	972	972	324	810	1296	0	4374	729
	IIIB	1134	810	162	324	1134	648	4212	702
	CP	810	486	162	162	648	0	2268	378

Another interesting observation made regarding zooplankton of fresh water pond was total disappearance of some of the species in the pond during the course of study. It was observed that *Daphnia* and *Eubranchiopus* maintained their absence in summer and rainy also whereas *Daphnia* occurred during summer and rainy in the fresh water pond though its population decreased to half in rainy from summer (Table No. 2). During the diel study of zooplankton, it was observed that the following species of *Cyclops*, *Keratella*, *Daphnia*, *Naupleus* larva and *Brachionus*, were most of the time found during night hours, preferring 10 PM to 2 AM period in the types of different oxidation ponds, their population was greater by about two times in night than day (Table No. 2). The variations in the diel behaviour of the zooplankton population during different seasons in various oxidation ponds are shown in (Table 3).

In the present investigation more than twenty one species of zooplankton have been found to be present in different types of oxidation ponds, ie primary and secondary, ranging from 162 to 14,726 cell in thousands / L, where dominant species belonged to the groups of *Rotifera*, *Copepods* and *Cladocerans* (Table No. 3). The most frequently recorded species were of *Cyclops*, *Keratella*, *Brachionus*, *Naupleus* larva and *Daphnia*. The zooplankton found in the oxidation ponds had considerable influence of the season. Maximum zooplankton were found in winter followed by rainy and summer, and the ponds which had maximum population were of following order- IB > IA > IIIB > IIIA. An interesting feature observed in the seasonal influence on the population density of zooplankton with that of phytoplankton density. Maximum phytoplankton had minimum zooplankton, whereas minimum phytoplankton

had maximum zooplankton. The control pond zooplankton species comprised only the following two groups Rotifera and Copepods, which had about ten species in dominance.

An interesting feature observed in the present study was the seasonal influence on the population density of zooplankton on plankton, being vice versa in population density in the

oxidation ponds which were highly nutritious. The control pond zooplankton species comprised only the following two groups Rotifera and Copepods, which had about ten species in dominance. The seasonal order of zooplankton dominance in freshwater was maximum in winter followed by rainy and summer. It has been observed that oxidation ponds generally do not have excessive growth of zooplankton (Havel and Shurin, (2004, Rajagopal et al., 2010, Goswami and Mankodi, 2012, Pearson & Duggan, 2018).

Table 3. Showing the presence of total zooplankton in secondary sewage oxidation pond and fresh water control pond (in thousands /litre)

Season	Month	Total Zooplankton		Cladocerans		Density	
		Copepods		OP	CP	OP	CP
		OP	CP				
Rainy	June	44	10	20	7	11	0
	July	32	10	21	7	16	2
	Aug	30	11	26	7	18	4
	Sept	29	11	30	8	20	4
Winter	Oct	89	21	62	15	46	8
	Nov	92	22	64	15	48	10
	Dec	100	24	60	18	50	11
	Jan	103	24	62	21	54	11
Summer	Feb	69	11	46	10	35	5
	Mar	71	14	49	12	39	6
	Apr	73	14	51	12	40	6
	May	64	15	47	12	42	6

OP- Oxidation Pond (sewage pond), CP- Control fresh water pond

The low population of zooplankton as reported by these workers has also been observed in the present investigation. However in the oxidation ponds, we have observed significantly low population of zooplankton, which greatly enabled a bio-equilibrium of prey and predators, exhibiting the phenomenon of plankton grazing in sewage pond which is being reported for the first time. As the algae form the trophic base for the secondary production it is obvious that the grazing and predator relationship would influence their population density and as such an inverse relationship of zooplankton with that of phytoplankton density, particularly that of grazing in fresh water bodies, as observed presently is in full corroboration with the earlier findings of (Michael, 1968, Singh et al., 2021, Ramaekers et al., 2022).

The most pronounced effect seen was that the zooplankton had on phytoplankton, in an aquatic environment was grazing. In the present investigation, the reduction of the algal standing crop with the resultant increase of zooplankton indicates the grazing of phytoplankton by zooplankton. There have been conflicting reports regarding the effects

of grazing of plankton in nutrient-enriched environments. Some workers found that heavy grazing depressed primary production (Kvale et al., 2021, Zheng, 2022). Whereas few studies detected little or no effect of herbivory on primary productivity (Chenillat et al., 2021).

Other results showed a positive correlation between the density of grazers and chlorophyll concentration (Goswami and Mankodi, 2012) or primary productivity (Pearson & Duggan, 2018). These results suggest that there is a unimodal response of primary productivity to zooplankton grazing. The data of the present finding support the view that grazing by zooplankton decreases the algal population thereby affecting the primary productivity and chlorophyll concentration.

CONCLUSION

It is concluded that about 21 species of zooplankton have been found in varying abundance in both the primary the secondary waste-stabilization or oxidation ponds as

compared to the freshwater control pond. The reason for high and variable species of the zooplankton is attributed due to high and conducive nutrient levels which can offer excellent opportunities for natural survival and faster growth of the food chain including the poly carps in domestic tropical secondary oxidation ponds. Due to optimum levels of vital parameters such as light, pH, oxygen and excessive nutrients, along with conducive conditions present in the domestic sewage waters, secondary sewage oxidation ponds can offer excellent fish culture opportunities as a low cost economically viable concept of waste water aquaculture.

Conflict of Interest: The authors declare no conflict of interests.

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16s rRNA Amplicon Sequencing Approach for Community and Predictive Functional Diversity of Therapeutically Valuable Formulation of Cow-derivatives

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ABSTRACT

Panchagavya is the blend of five ingredients obtained from cows and used in traditional Hindu rituals. Panchagavya preparation is a microbial mediated process that possibly involves microbial succession. The present study was conducted to decode the microbial community that exists in the preparation of three hours of old Panchagavya. DNA was isolated from Panchagavya using HiPurA™ Stool DNA Purification Kit followed by quality checking by Agarose electrophoresis and Qubit fluorometer. The V3 and V4 region of 16s rRNA based amplicon sequencing was performed using the Illumine MiSeq platform. Taxonomic profile encoded by using One Codex, kraken and MG-RAST. Functional traits detected through the abundance of specific genes using Tax4Fun. Taxonomic result suggests the total 2000 species were identified. The most abundant was *Streptomyces griseocarneus* (2.65%) followed by *Clostridiales bacterium* (2.26%), *Bacteroidales bacterium* (1.38%), and *Verrucomicrobia bacterium* (1.13%). Community based analysis revealed the microbial diversity and presence of anaerobic, unclassified, and uncultivable microbes in metagenomes, which may be associated with the pharmacological properties of Panchagavya. Functional analysis predicts around 351 metabolic pathways for metabolism of carbohydrates, synthesis of secondary metabolites and degradation of xenobiotic compounds. The detection of various secondary metabolites genes associated with pharmacological molecules correlated with its traditional clinical applications. The present study revealed the advantages of cultivation approach for exploring untapped and unique bacterial diversity, and also utilities for various biotechnological and environmental applications.

KEY WORDS: AMPLICON SEQUENCING, COMMUNITY METAGENOMICS, COW, FUNCTIONAL METAGENOMICS, PANCHAGAVYA.

INTRODUCTION

Panchagavya is an organic product derived from five products of the cow. The three are direct constituents i.e. dung, urine and milk and the two derived products are curd and ghee. According to the old Ayurveda literature, the five individual constituents of Panchagavya possess medicinal properties and can be used singly or in combination with some other

herbs. This kind of treatment is called Panchgavya therapy or cowpathy (Muthukapalli et al., 2022).

The potential applications of Panchagavya are as antimicrobials, immune boosters, antidiabetics, anticancer, anticonvulsant, aphrodisiac, blood purifiers, and anti-HIV agents. Panchagavya is also used in Ayurveda for the treatment of several disorders such as hyperlipidemia, leucoderma, arthritis, acidity, renal disorders, asthma, dietary disorders, gastrointestinal disorders and asthma as an antistressor. It also acts as a general tonic or immunomodulator to enhance the immunity (Dhama et al., 2022, Athavale et al., 2012 & Kuldeep et al., 2013).

Article Information:*Corresponding Author: hnguraj@gmail.com

Received 03/10/2022 Accepted after revision 20-12-2022

Published: December 2022 Pp- 547-551

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Available at: <https://bbrc.in/> DOI: <http://dx.doi.org/10.21786/bbrc/15.4.10>

Panchagavya is essential in organic agriculture for disease control, plant growth, flowering, germination, and canopy development. It also plays a significant part in compost decomposition, improves soil nutrient status by promoting better mineralization, and aids in the creation of beneficial microbes in the root rhizosphere, which can be helpful in the supply of beneficial macro and micro nutrients to plants (Kumar et al., 2022).

Panchagavya contains several macronutrients such as nitrogen, phosphorus, potassium and micronutrients like Zn, Fe, Cu, and Mn which are required for the normal growth and development of plants. In the Indian subcontinent, the application of the Panchagavya is well-known and used in an agricultural sector to protect the crops and growth promotion of the crops (Gugalia et al., 2021, Kumaravelu et al., 2009, Dhama et al., 2005, Chauha, R.S., 2002, Krupanidhi et al., 2008, Matthews and Jenks, 2013, Kumar et al., 2022).

By culture dependent study few bacterial species isolated from the Panchagavya were *Lactobacillus*, *Saccharomyces*, *Streptomyces*, and *Rhodopseudomonas* (Leo et al., 2013), *Azospirillum*, *Azotobacter*, *phosphobacteria* (Dhama et al., 2013), *Pseudomonas*, *Azotobacter*, *Actinomyces*, *Rhizobium* (Ram et al., 2013), *Acetobacter*, *Bacillus*, *Micrococcus*, *Leuconostoc*, *Enterococcus*, *Microbacterium*, *Pseudoxanthomonas*, *Corynebacterium*, *Escherichia*, *Paenibacillus*, *Shigella*, *Rhodobacter*, *Lactococcus*, etc. (Anandham et al., 2015). Nowadays, the Prophylactic potential of a Panchagavya formulation was tested against certain pathogenic bacteria (Patel et al., 2018). So, based on such diverse properties and the presence of different bacterial species; there is a need to decode the microbiome of the Panchagavya formulation. To the best of our knowledge; at present, there is not a single study of the metagenomics analysis of Panchagavya. This is the first microbial profiling of the Panchagavya by culture independent methods using a metagenomics approach.

MATERIAL AND METHODS

Panchagavya was prepared at Madhuvan Dairy Farm, Haldarva, Bharuch, India. Fresh cow dung, urine, milk, curd, and ghee were mixed in equal proportion thoroughly in a sterile glass beaker. This mixture was allowed to stand for three hours and subjected to filtration through a muslin cloth aseptically.

DNA was isolated from the filtrate using HiPurA™ Stool DNA Purification Kit (MB544) according to the manufacturer's instruction. DNA concentration was measured using the Qubit Fluorometer. The sequencing library generated from V3 and V4 amplicons from the sample were sequenced using an Illumina MiSeq sequencing platform. Diversity and abundance were analysed using available standard bioinformatics software. The taxonomic assignment of unassembled metagenomic sequences was performed using FASTQC followed by Pear, One Codex

(Minot et al., 2015) and predictive functional metagenomics performed by SILVAngs, MicrobiomeAnalyst (Dhariwal et al., 2017), Tax4fun (Abhauer et al., 2015), KO and KEGG mapper (Kanehisa & Sato 2020).

Figure 1: Krona based chart at family level. (Core of chart indicated phyla and peripheral edge indicates the family's abundance in percentage)

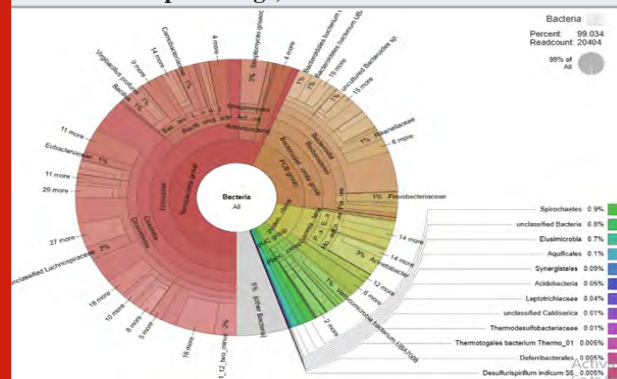
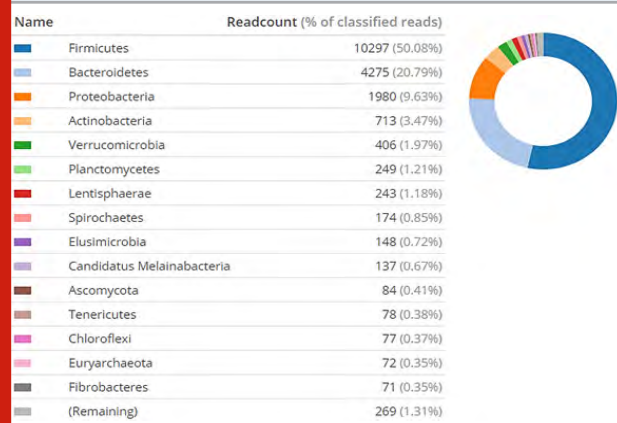


Figure 2: Community at Phyla level based on the percentage of reads in metagenome sample (Pie chart includes the most 15 dominant phyla presented in clockwise direction)



RESULTS

The present study is based on the 16s rRNA amplicon NGS metagenomic analysis of Panchagavya. A total number of 27301 bacterial 16S V3-V4 high-quality sequences with an average read length of 71 to 490, were obtained. Profiling the taxonomic composition of the community also can be accomplished by the analysis of the distribution of k-mers (e.g., using Kraken or One Codex). Metagenomic analysis using One Codex was performed by uploading the 2D FASTQ data to the One Codex platform at <https://app.onecodex.com>. This cloud-based k-mer method was selected, because it is reportedly more accurate than either the MG-RAST or the Kraken tools and it provides for community access to the data and analytical results. Sequence clustering resulted in the identification of 2000 different bacterial species, 87 diverse microbial phyla and 510 families together with an unclassified category were depicted in the metagenome (Figure 1).

Firmicutes (50.08%) and Bacteroidetes (20.79%) were abundant along with Proteobacteria (9.63%), Actinobacteria (3.47%), Verrucomicrobia (1.97%) and Planctomycetes (1.21%) (Figure 2).

Total of 516 families was detected (figure 1) along with following *Ruminococcaceae* (8.46%), *Lachnospiraceae* (7.34%), *Bacteroidaceae* (3.57%), *Enterobacteriaceae* (3.27%) and *Moraxellaceae* (3.12%) were major dominant (Figure 3).

Figure 3: Microbial communities at species level based on the percentage of reads in metagenome sample (Pie chart includes the most 15 dominant species presented in clockwise direction)

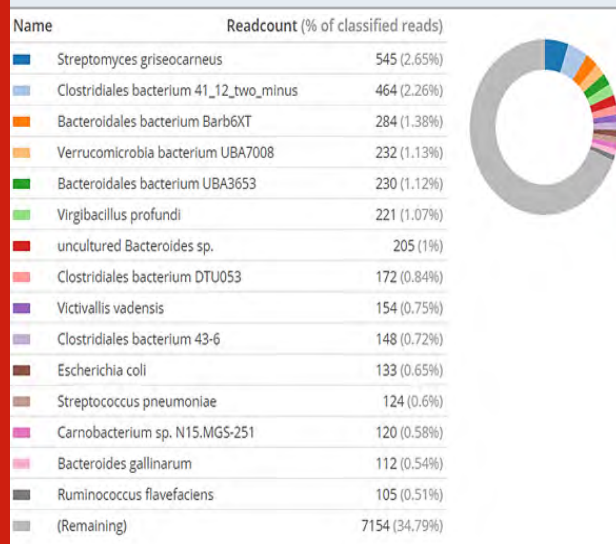
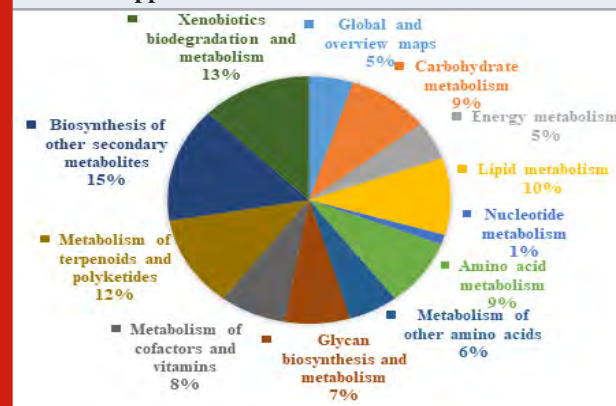


Figure 4: Secondary metabolites pathways analysis using KEGG Mapper



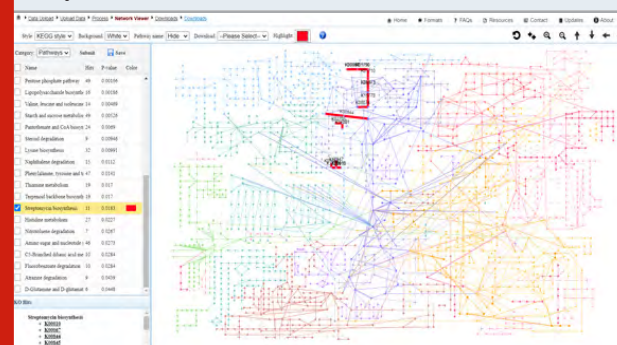
Based on the microbiome analyser, community analysis data was subjected to the functional analysis to decode the functional potential of the microbiome. In the present study pathways modules for the metabolism of cofactor and vitamins, biosynthesis of secondary metabolites, xenobiotic degradation, drug resistance, lipid metabolism, and energy metabolism were detected which correlates its pharmaceutical and agricultural application (Figure

4). Furthermore, we have analysed the 10 categories of Reconstruction Pathway (Table 1). The highest number of the pathway was found in Environmental information processing. To confirm the pharmacological and medicinal attributes of the Panchagavya, we have further analysed the Biosynthesis of other secondary metabolites pathways and were find out the 12 important biosynthetic pathways which give the antimicrobial substances. Furthermore the biosynthesis of Streptomycin was highlighted in the global metabolic network (Figure 5).

Table 1. List of reconstruction pathway found in the metagenome sample (number of pathways indicates total different pathways operated in microbiota of sample under each categories)

Sr. No.	Reconstruction Pathway	No. of Pathway
1	Carbohydrate metabolism	41
2	Energy metabolism	62
3	Lipid metabolism	32
4	Nucleotide metabolism	08
5	Amino acid metabolism	53
6	Glycan metabolism	24
7	Metabolism of cofactors and vitamins	31
8	Biosynthesis of terpenoids and polyketides	45
9	Xenobiotics biodegradation	21
10	Biosynthesis of other secondary metabolites	28

Figure 5: A depiction showing functional enrichment analysis and visualization within the global metabolic network. (Highlighted red lines shows the Streptomycin biosynthesis)



DISCUSSION

Panchagavya plays major role in crop production, especially in organic agriculture, maintaining genetic biodiversity, helps as a growth promoter, root growth enhancer, increasing water holding capacity, increase photosynthetic activity in

plants, human diseases and improving the body's immunity, the metabolic profiles (hormones, proteins, nutrients, etc.) and microbial profiles have not been explored completely (Somasundaram et al., 2007; Kumar et al., 2022).

Moreover, the profile and dynamics of the entire microbial community associated with the Panchagavya are unavailable due to limitations in culturing several genera/species using conventional microbiological techniques. Not many efforts have been made to explore the complete biology involved in agriculture, indicating its potential for increasing crop output by the Panchagavya. Only by combining different omics approaches can the fundamental aspects of a complex system be understood (Segate et al., 2013).

Therefore, we have used different omics approaches to decode the microbial community (metagenomics) and metabolite profile (metabolomics) in the Panchagavya. These approaches can reveal the microbial composition and their abundance, the functional annotation of genes and important protein compounds, hormones, and so forth, in the Panchagavya formulation. Several bacteria that used as starter cultures for dairy products were found to be predominant in Panchagavya. Among them *Lactococcus fermenticola*, *Lactococcus fujiensis* JCM 16395, and *Lactococcus fujiensis*, is widely used as starter cultures for various cheeses (Lahtinen et al., 2011). Our findings are in contrast to a recent report that the use of *Bacillus cereus*, *Bacillus subtilis*, *Lactobacillus camellia*, *Lactobacillus ozone*, and *Paenibacillus* as a probiotic dietary supplements is expanding rapidly with increasing number of studies demonstrating immune stimulation, antimicrobial activities and competitive exclusion (Kamilya et al., 2022; Cutting, 2011; Sorokulova et al., 2008).

CONCLUSION

Using metagenomic sequencing, the Panchagavya's related microbiota can be profiled. An extensive range of bacterial taxa from the domain bacteria were found in the Panchagavya, according to the research. In Panchagavya metagenomes, the Firmicutes, Bacteroidetes, and Proteobacteria phyla are widely present. Its health benefits are suggested by the presence of a few probiotic species and antibiotic-producing species. The research sheds light on the existence of several bacterial species, whose immune-boosting characteristics and therapeutic potential may be the basis for the Panchagavya's health-tonic properties. According to pathway research, the secondary metabolites that the bacteria create may be the cause of the Panchagavya's antibacterial abilities. The Panchagavya is a low-cost instrument for enhancing gut immunity and has a positive impact on health because the enormous microbial variety and the detection of rare species and uncultivable species play a crucial role in maintaining an intestinal barrier and metabolising nutrients.

Data availability statement: Metagenome sequences data are deposited in EBI-Metagenomics under the accession

number PRJEB31987.

ACKNOWLEDGMENTS

We are extremely grateful to Madhuvan Dairy Farm, Haldarva, Bharuch, India for providing the samples for this study and for sharing knowledge on panchagavya.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Incidence of Climate on the Number of Admissions and Deaths Due to Cerebrovascular Diseases Through Mathematical Modeling In Sagua La Grande, Villa Clara, Cuba

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ABSTRACT

The number of deceased cases and admissions for annual cerebrovascular diseases were modeled in Sagua la Grande, Villa Clara province, Cuba, between 1993 and 2017. A statistical description of these two variables was made, with an average admission of 148.7 cases, with a standard deviation of 24.9. The deceased had an average of 35.7 with a standard deviation of 13.2, the results were obtained using the Regressive Objective Regression (ROR) methodology. The impact of the minimum temperature and atmospheric pressure of the Sagua La Grande station was studied, reaching the conclusion that as the minimum temperature increases, the income and deaths increase, so this impact is related to the climate change, when the minimum temperature increases by 1 °C, deaths increase by 9 cases, while when the maximum temperature increases by 1 °C, deaths increase by 11.6 cases. In the case of revenue, as the atmospheric pressure increases by 2 hPa, the amount of revenue decreases by 1 case.

KEY WORDS: CUBA; CVD; DEATHS; CLIMATE IMPACT; INCOME; MODELING; ROR REGRESSION.

INTRODUCTION

Cerebrovascular disease is a hierarchically broad term, I a syndrome that includes a group of heterogeneous diseases with a common point: an alteration in the vasculature of the central nervous system, leading to an imbalance between oxygen supply and oxygen requirements, the consequence of which is a focal dysfunction of brain tissue (Garcia et al., 2019).

The frequency of cerebrovascular disease increases with age and predominates in patients of male sex and black

complexion. According to data from 11 studies conducted in Europe, Russia, Australia and the United States, the worldwide incidence of CVD was estimated at 300 to 500 x 105 inhabitants per year, between 45 and 84 years of age. Every 10 years, the incidence increases significantly above 35 years of age and triples to 3000 x 105 inhabitants in individuals over 85 years of age. The incidence of cases/year in the USA is 531 to 730 000, 127 000 in Germany, 112 000 in Italy, 101 000 in the United Kingdom, 89 000 in Spain, 78 000 in France, 60 000 in Poland and 55 000 cases in Japan. In Brazil, a rise in the crude CVD mortality rate has been observed in the last three decades; a trend of shifting to younger ages was detected (Priscilla and Ikefutiligia, 2018; Sera et al., 2018).

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Received 15/09/2022 Accepted after revision 28-12-2022
Published: December 2022 Pp- 552-554
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Available at: <https://bbrc.in/> DOI: <http://dx.doi.org/10.21786/bbrc/15.4.11>

Ischemic stroke is triggered by a series of biochemical processes resulting from the disruption of cerebral blood flow. It involves a complex mixture of different biomolecular events that originate and develop through the ischemic cascade, generating excitotoxicity, which together trigger irreversible cellular injury (Salas, 2020).

Stroke is caused by risk factors that, when affecting a patient, trigger the entity, but the triggering or predisposing factors are those that, when affecting vulnerable patients with several risk factors, are able to start or provoke a disease crisis. One component of the physical environment (environment) that potentially influences diseases of the circulatory system is the local meteorological variables. This influence is direct and indirect and can act positively or negatively (Gonzalez et al., 2019). One of the diseases, which according to multiple research results, is also influenced by weather and climate changes, are cerebrovascular diseases (Alcalá, 2021; Icalá, 2021).

In Spain, variations in atmospheric pressure act as a trigger factor for stroke, and it has been shown that increased atmospheric pressure increases the incidence of hemorrhagic stroke (Jiménez, 2008). With the arrival of cold weather in the southern and southeastern regions of Brazil, it may bring with it an increase in the number of deaths from cerebrovascular accidents (CVAs), mainly among the population over 65 years of age (Ikefutiligia et al., 2018; Icalá, 2021).

In Russia the number of strokes increases with temperature, daily thermal amplitude. Strokes were more sensitive to inclement weather than myocardial infarctions (Shaposhnikov et al., 2014). In Cuba, in 2020 there were 10 821 deaths with a crude rate of 90.4 X 100 000 inhabits. and an adjusted rate of 39.7 X 100 000 inhabits. In Villa Clara in this year, the total number of deaths from this cause was 668 for a crude rate of 85.9 per 100 000 inhabitants (MINSAP, 2021).

Various studies estimate that a large part of the population is vulnerable to sudden changes in temperature, humidity and atmospheric pressure. Respiratory and cardiovascular pathologies are usually the most related to atmospheric impacts (Altea, 2016; Sauchay et al., 2017; Priscilla and Ikefutiligia, 2018).

In view of the above, there is a need to investigate the effects of meteorological variables on human health, which are seen with greater incidence due to climate change and have a direct impact on cerebrovascular disease.

The objective of the research was focused on using descriptive statistics of these variables, modeling them and predicting their quantity, so that measures can be taken to reduce their influence, as well as to measure the impact of climate variables, both on deaths and admissions due to this cause.

The Objective Regressive Regression (ORR)

Methodology:

For the forecast of admissions and deaths, we modeled using the methodology of the Regressive Objective Regression ROR (Osés and Grau, 2011), for which we create in a first step, dichotomous variables DS, DI and NoC where:

NoC - Number of base cases,

DS = 1, if NoC is odd; DI = 0, if NoC is even, when DI=1, DS=0 and vice versa.

Subsequently, the module corresponding to the Regression analysis of the statistical package SPSS version 19.0 (IBM Company) was executed, specifically the ENTER method where the predicted variable and the ERROR are obtained.

Then the autocorrelations of the variable ERROR were obtained, paying attention to the maximums of the significant partial autocorrelations PACF. The new variables were then calculated taking into account the significant Lag of the PACF. Finally, these regressed variables were included in the new regression in a process of successive approximations until a white noise in the regression errors was obtained.

The data correspond to the years from 1993 to 2017 belonging to the hospital of Sagua La Grande, Villa Clara, Cuba. The climatic variables correspond to the same period from the meteorological station of Sagua La Grande (Latitude: 22°13' N, Longitude: 80°02' W).

Meteorological variables act as precipitating factors of CVD, both ischemic and hemorrhagic, where the slowing of circulation, increased viscosity, redistribution of blood flow, and increased sympathetic action are the main impacts on the cerebral circulatory system. As the temperature rises, the number of admissions increases; rising temperatures, both maximum and minimum, increase the number of fatalities. Temperature and its variations cannot be catalogued as risk factors, but as triggering factors, capable of causing a stroke in patients with risk factors and low adaptive capacity. Elderly patients are therefore the most vulnerable group.

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Bioscience Biotechnology Research Communications

P-ISSN: 0974-6455 E-ISSN: 2321-4007

CODEN (USA): BBRCBA

Indexed in Thomson Reuters ISI Now Clarivate Analytics Web of Science (ESCI)

Publishers: Society for Science and Nature, Bhopal India

Journal Unique Identifier: Cross Ref DOI: <http://dx.doi.org/10.21786>

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Bioscience Biotechnology Research Communications

An Open Access International Journal www.bbrc.in

Post Box 01, GPO, Bhopal 462001 India

P-ISSN: 0974-6455 O-ISSN: 2321-4007 CODEN USA: BBRCBA

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