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BIODIVERSITY ASSESSMENT: TOOL FOR CONSERVATION VOLUME II

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PREFACE

Biodiversity is the lifeblood of our planet, a dazzling tapestry of species, ecosystems, and genetic variations that have evolved over millions of years. It is a testament to the immense creativity and resilience of nature, providing us with invaluable resources, ecological services, and a source of inspiration and wonder. However, our planet's biodiversity is facing an unprecedented crisis.

Human activities, including habitat destruction, climate change, pollution, and overexploitation of natural resources, have resulted in a staggering loss of biodiversity. Species are going extinct at an alarming rate, ecosystems are unraveling, and the delicate balance of nature is being disrupted. We stand on the precipice of a sixth mass extinction event, one caused not by natural forces but by our own actions.

The urgency to protect and conserve biodiversity has never been greater. In order to tackle this monumental challenge, we must first understand the extent of our loss and assess the state of biodiversity across different landscapes and ecosystems. This is where the Biodiversity Assessment becomes an indispensable tool.

The Biodiversity Assessment: Tool for Conservation is a comprehensive guide that aims to equip researchers, conservation practitioners, and policymakers with the knowledge and tools necessary to assess and monitor biodiversity effectively. It brings together the collective wisdom of experts from various disciplines, who have dedicated their lives to the study and preservation of biodiversity.

This book covers a wide range of topics, including the principles and methodologies of biodiversity assessment, the use of cutting-edge technologies and data analysis techniques, the integration of traditional ecological knowledge with scientific research, and the development of conservation strategies based on sound assessment findings. It also explores the ethical and social dimensions of biodiversity conservation, highlighting the importance of engaging local communities and fostering a sense of stewardship for the natural world.

Ultimately, the goal of this book is to inspire and empower individuals to become agents of change in the fight to safeguard Earth's biodiversity. It is a call to action, reminding us that we have the power and responsibility to protect the natural world that nurtures us. By using the tools and knowledge provided in this book, we can make informed decisions, advocate for policy changes, and contribute to the preservation of biodiversity for future generations.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

Editors

TABLE OF CONTENT

SELECTIVE BREEDING OF GUPPY (*POECILIIA RETICULATA*): ENHANCING TRAITS FOR EXOTIC VARIETIES

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Abstract:

Selective breeding is a process by which specific traits of a species are enhanced over generations. Guppies, a popular aquarium fish, have been selectively bred to create a variety of exotic colors and patterns. This paper aims to provide an overview of the selective breeding process of guppies, including the criteria used for selecting parent fish, the breeding techniques employed, and the resulting exotic varieties. The paper also discusses the potential benefits and drawbacks of selective breeding in guppies and its impact on their health and welfare. Overall, the study highlights the potential of selective breeding as a tool to enhance the aesthetic appeal of guppies, while also raising important ethical considerations for breeders and enthusiasts.

Keywords: Selective breeding, Guppies, Exotic varieties, Traits enhancement.

Introduction:

Selective breeding of guppies (*Poeciliia reticulata*) has a long history, dating back to the early 20th century. According to a Houde and Endler (1990), fish enthusiasts in Europe and the United States began selectively breeding guppies in the 1920s to create new color and pattern variations. These early breeders observed that certain guppies had brighter or more distinct colors, and they selectively bred these fish to create offspring with similar characteristics. Over time, the selective breeding of guppies became more sophisticated, with breeders using various methods to isolate and enhance desirable traits. A review article published in the journal Aquaculture International notes that breeders have used inbreeding, outcrossing, and hybridization to produce new strains of guppies with specific characteristics (Basavaraja *et al.*, , 2017). This process has led to the creation of many different strains of guppies, each with its own unique color and pattern combinations. In addition to their aesthetic appeal, guppies have also been studied extensively for their reproductive and behavioral biology. Selective breeding has been used to study the genetic basis of certain traits, such as color and pattern variation, as well as the role of behavior in mate selection and courtship (Bartley *et al.*, , 2001). Researchers have also used guppies to study the impact of environmental factors on traits and the evolution of

complex traits. The selective breeding of guppies has therefore contributed to our understanding of genetics, evolution, and behavior, and continues to be an important tool for research and aquaculture.

Taxonomic hierarchy of Guppy:

- **Kingdom:** Animalia (animals)
- **Phylum:** Chordata (vertebrates and their relatives)
- **Subphylum:** Vertebrata (vertebrates)
- **Class:** Actinopterygii (ray-finned fishes)
- **Order:** Cyprinodontiformes (toothcarps and killifishes)
- **Family:** Poeciliidae (livebearers)
- **Genus:** Poecilia
- **Species:** *Poecilia reticulata*

Identification characters

Guppies, a popular freshwater fish species, exhibit sexual dimorphism, which means that males and females have different physical characteristics. Male guppies are known for their flamboyant and vibrant coloration, with splashes, spots, or stripes that can be found in a wide range of colors. On the other hand, female guppies have a more subdued appearance, typically exhibiting a greyish coloration that allows them to blend into their environment and protect them from predators.

In terms of size, guppies range from 1.5 to 3.5 cm in length for males and 3 to 6 cm for females. Despite their small size, guppies are highly adaptable and can thrive in a variety of aquatic environments. Their small size also makes them an ideal choice for smaller aquariums or community tanks with other non-aggressive species. With their striking appearance and peaceful temperament, it is no wonder that guppies are a favorite among aquarium enthusiasts.

General information about Guppy

Guppy small, freshwater fish is a true beauty, flaunting a slender, elongated body with a pointed head and a fan-shaped tail that features intricate patterns and designs. The guppy is a popular choice for aquariums, not just because of its stunning appearance but also because of its peaceful temperament and adaptability. It is no wonder that this graceful fish has captured the hearts of fish enthusiasts around the world. Table 1 presents general information about the guppy.

Table 1: General characteristics of Guppy

Conservation status	Least concern
Distributions	Central, North, and South America, Asia
Threat	Water pollution
Distinctive feature	Bright coloured body and fins.
Gestation period	3-4 weeks
Water Quality parameters	pH: 5.0-7.0
	Hardness: 250 to 300 ppm
	Temperature: 24 to 26 degree Celsius
Habitats	Streams, shallow pools
Feeding Behaviour	Omnivore
No of species	276
Body Colours	Yellow, red, black, orange, white, grey, and multi coloured (Khoo *et al.,* , 2007)
Skin type	Scaled fish
Weight	Less than 1g
Length	0.6-2.4 inches
Other names	Million fish, Rainbow fish

Source: A-Z Animals: Guppy, (2022)

Different types of Guppies

There are several types of guppies, each with its own unique characteristics and traits. The following tables, Table 2 and Table 3, present some of the most popular types of guppies.

Table 2: Common types of Guppies

Common types	Common fancy guppy, Endler guppy, Swamp guppy

Table 3: Other popular varieties of Guppies

Based on Tail type	Veil tail, Triangular tail, Fan tail, Scarf tail, Double sword tail, Top sword tail, Bottom sword tail, Lyra tail, Cofer tail, Spear tail, Round tail, Pin tail, Halfmoon tail
Based on eye colour	Real red eye, Real red eye albino
Based on body pattern	Tuxedo guppies, Cobra guppies, Snake skin guppies
Based on tail pattern	Glass guppies, Leopard guppies, Mosaic guppies, Lace guppies

Based on body colour	Albino guppy, White guppy, Black guppy, Blue guppy, Neon blue guppy, Japanese blue guppy, Green guppy, Red guppy, Yellow guppy, Purple guppy, Bronze guppy, Golden guppy, Half black blue guppy, Half black green guppy, Half black red guppy, Half black yellow guppy, Half black purple guppy, Half black pasted guppy, Bicoloured guppy, Solid coloured guppy, Multi-coloured guppy, Metal guppy, Koi guppy, Panda guppy, Jarweelazuli guppy, Platinum guppy, Mascow guppy, Dragon guppy
Based on pectoral fin	Dumboear guppy
Other type	Mutt guppy

Source: https://fishlab.com/

Global market of guppies

The global trade of live ornamental fish, including the beloved guppy, is a thriving industry that generates significant revenue for countries across the globe. According to Food and Agriculture Organization of the United Nations (FAO), Singapore was the leading exporter of live ornamental fish in 2019, with a staggering value of $100.4 million USD. The country's success in this industry can be attributed to its strategic location and well-developed infrastructure, which allows for efficient transportation of live fish to markets around the world. Following closely behind Singapore were Thailand and Malaysia, with values of $83.9 million USD and $73.4 million USD, respectively, indicating the significance of the Southeast Asian region in the global trade of ornamental fish.

On the other hand, the United States was the largest importer of live ornamental fish in 2019, with a value of $372.2 million USD, demonstrating the country's strong demand for guppies and other exotic fish. Japan followed with a value of $172.6 million USD, while the European Union (EU) collectively imported $155.5 million USD worth of live ornamental fish, highlighting the industry's significance in this region as well. Canada and Australia were also major importers, with values of $40.1 million USD and $35.6 million USD, respectively. The global trade of guppies and other ornamental fish is a complex and dynamic industry that contributes significantly to the economies of exporting and importing countries alike (Fish Stat; FAO, 2021).

Biology of guppy

Food and feeding habit:

Guppies are known for their viviparous nature, which means they give birth to live young rather than laying eggs. As a result, they have a high metabolic rate and require a significant amount of energy to support their breeding and active lifestyle. A well-balanced diet is crucial

for their health and vitality. To meet their nutritional needs, guppies should be fed at least three times daily with a variety of foods, including dry, frozen, fresh, and live foods. Being omnivorous organisms, guppies consume a wide range of foods, including algae, organic debris, diatoms, and mosquito larval parts, and protozoans, zooplankton, and fish parts. Among these, algae is the most commonly consumed and significant feed component for guppies. Algae provide essential nutrients such as protein, lipids, and vitamins, which are vital for growth, reproduction, and overall health. Therefore, providing a source of algae in their diet is essential for maintaining the health and vitality of guppies (Fernando and Phang, 1985).

Sexual dimorphism:

Guppies are known for their unique reproductive process, which involves giving birth to live young instead of laying eggs like other fish. This characteristic sets them apart from many other species in the fish kingdom. The free-swimming young are born in a fully developed state, with the ability to swim and feed immediately after birth. This ovoviparous reproductive strategy allows guppies to produce relatively large numbers of offspring, which can help ensure the survival of their species.

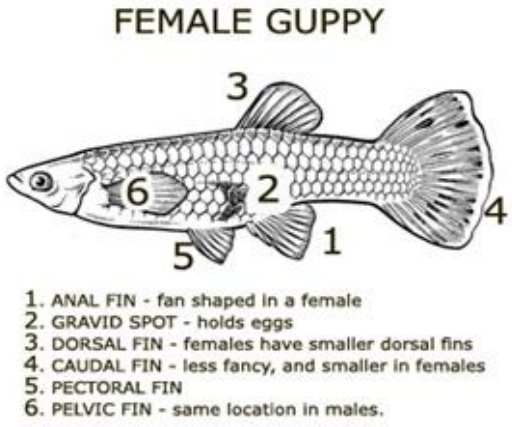

Figure 1. Sexual Dimorphism of Female Guppy

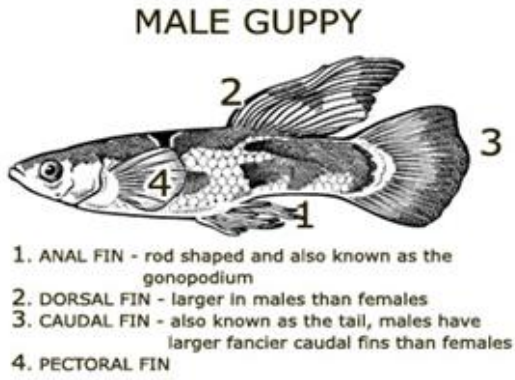

Figure 2. Sexual Dimorphism of Male Guppy

Fig 1 and Fig 2 shows the female and male guppies' physical characteristics. The males are often more vibrantly coloured, with striking splashes, spots, or stripes on their tails, while females typically have a smaller body size and less colourful appearance, with an anal gravity spot that is dark. One of the most distinctive features of the male guppy is the modified anal fin, known as the gonopodium, which is used during internal fertilisation to aid in the transfer of sperm to the female.

After fertilisation, the eggs begin to grow inside the mother's ovary, and then quickly hatch and give birth to the young. The newborn fry measure about 7-10 mm in length and are fully equipped to begin their life in the aquatic environment. This unique reproductive strategy

and the striking physical characteristics of the guppy have made it a popular species for hobbyists and researchers alike.

Sexual maturity

The gestation period of guppies lasts around 25 to 35 days, with an average of 28 days. During this time, developmental stages of the embryos can be observed through a compound microscope. It has been observed that at birth, the tail portion of the fry emerges first. Each brood can consist of anywhere between 12 to 60 fry. Newborn guppy fry have transparent or blackish skin, slender bodies, and fully developed jaws on their mouths. Despite being born as free-swimming young, they are fully capable of swimming, feeding, and avoiding danger. The fry grow rapidly and become sexually mature between 8 to 10 weeks. By 6 months, they are fully grown and display their characteristic vibrant colors and patterns.

Selective breeding of guppy

Selective breeding is a meticulous process of breeding that aims to improve the breeding value of a population by selecting and mating only the finest fish, in anticipation of producing offspring with superior traits. The success of this process depends on the ability of the selected breed to pass on their traits to their progeny, resulting in faster growth rates, increased yields, and more valuable subsequent generations. Through selective breeding, guppies can display the most desired body colours, ultimately increasing their market value, while also growing more efficiently, which would lower the cost of feed. However, this process requires careful planning and financial investment in fishing equipment. Selective breeding demands more time and effort compared to natural or spontaneous breeding, necessitating close monitoring of the pups, feeding them a healthy diet, and investing in equipment to maintain ideal conditions to home fish for several generations. Maintaining a 2:1 ratio of females to males in the tank is crucial, as males tend to stress out females by chasing them around in the water (Kodric-Brown, 1985). Thus, careful planning and management are critical for the successful breeding of guppies with desired physical traits such as specific colours or tail shapes.

Today, there are many different strains of guppies available in the market, each with its own unique coloration and pattern. Some of the most popular strains include the Cobra guppy, the Moscow guppy, the Red Delta guppy, and the Blue Neon guppy. Selective breeding has also led to the development of specific traits such as larger tails, longer fins, and brighter colors, making these fish highly desirable for both hobbyists and commercial breeders. However, there are also concerns about the impact of selective breeding on the overall health and well-being of guppies. Selective breeding can lead to the loss of genetic diversity, which can make the population more susceptible to disease and other problems (Van Oosterhout *et al.*, , 2007). Additionally, some breeders may focus too heavily on creating specific physical traits, which can lead to fish with poor overall health and reduced lifespan. To address these concerns, some

breeders are now practicing "natural selection breeding," which involves selecting fish that have survived and thrived under natural conditions. This can help maintain genetic diversity and produce healthier fish that are better suited to their environment.

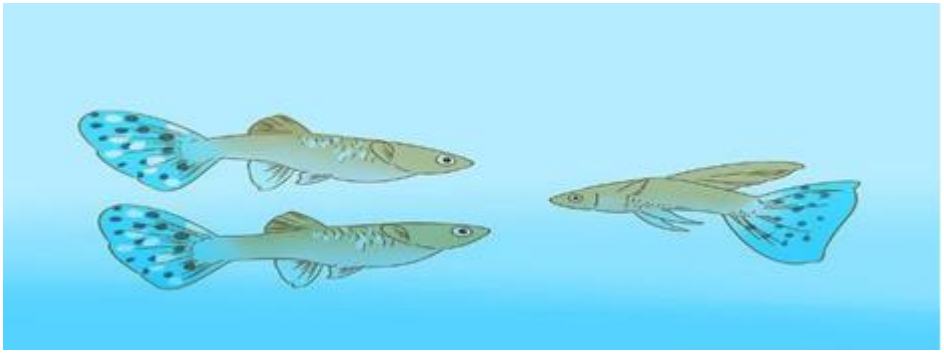

Figure 3. Selective Breeding of guppy (2 Female: 1Male)

Types of selective breeding in Guppy

1. Inbreeding

Selective breeding for specific physical traits in guppies can involve inbreeding, which is the breeding of related fish to improve fin color selection, fin confirmation, and color patterns. This technique typically involves selecting the best male guppy and breeding him resulting in offspring with similar patterns and hues (Bashey, 2008). Inbreeding can also be used to produce a specific strain in large quantities, and is likely the method used to breed the fancy guppies that are commonly sold in pet stores. Although inbreeding can produce desirable physical traits in guppies, it can also increase the risk of genetic defects and reduce the overall genetic diversity of the population. Therefore, it is important to carefully plan and monitor inbreeding programs to minimize the negative impacts. Fish breeders must also ensure that the fish are kept in optimal conditions and are provided with a healthy diet to support their growth and development. In addition to inbreeding, other methods of selective breeding can be used to produce desirable traits in guppies, such as outcrossing and line breeding. Outcrossing involves breeding two unrelated individuals with desirable traits, while line breeding involves breeding individuals from the same family lineage but with some degree of genetic diversity. These methods, along with careful planning and monitoring, can help produce high-quality guppies with desirable physical traits.

2. Line breeding

Line breeding is a selective breeding technique that focuses on breeding closely related individuals with the goal of producing an offspring that displays the desired traits of the breeder. The primary objective of line breeding is to create an improved iteration of the desired traits with each generation. This technique is considered more reliable than breeding unrelated individuals,

as it produces a unique line that preserves the desired traits. In line breeding, there are some crucial factors that breeders must keep in mind to achieve successful outcomes. It is recommended to breed female guppies with their fathers to preserve the desired traits. On the other hand, it is not advised to breed male guppies with their mothers to prevent genetic abnormalities. The careful application of line breeding is commonly used in breeding fancy guppies that exhibit unique and desirable traits such as striking coloration, fin patterns, and body shape. This technique enables breeders to produce guppy strains with consistent physical characteristics, which are highly sought after by aquarists and collectors.

3. Back crossing

In the process of breeding guppies, it is not uncommon to encounter unexpected traits that appear in the lineage, despite careful selection and breeding practices. When this occurs, it may be necessary to introduce genetic material from earlier generations in a process known as backcrossing. The aim of backcrossing is to restore or remove certain traits in the offspring by reintroducing genes from an earlier generation. However, it is essential to exercise caution and select the appropriate ancestors to avoid introducing undesirable traits. This process can take time, and multiple attempts may be necessary before achieving the desired outcome, much like other selective breeding techniques.

4. Out crossing

Outcrossing is a useful breeding method that involves mating unrelated individuals from different generations (Bashey, 2008). This technique can help maintain the health of the species and reduce the probability of defects or mistakes that can arise from inbreeding, which is a common issue with line breeding. By using outcrossing, breeders can introduce new and desirable characteristics into the line without having to worry about potential flaws, such as a curved spine or other undesirable traits that may arise from too much inbreeding. It is important to note that every few generations, it is advisable to introduce a new outcross in order to maintain the genetic health of the fish. By doing so, breeders can ensure that the guppies remain healthy and continue to exhibit desirable traits, such as interesting coloration or unique tail shapes, while avoiding negative consequences that may result from excessive inbreeding.

Selective breeding vs. Line breeding:

In the realm of fish breeding, two breeding techniques that are commonly used are selective breeding and line breeding. The process of selective breeding involves identifying and choosing fish that have desirable traits and breeding them with the intention of increasing the occurrence of those traits in the offspring. By continually selecting and breeding fish with the desired characteristics over many generations, strains can be developed that have particularly high levels of the desired traits.

On the other hand, line breeding is a specific type of selective breeding that involves mating two related guppies in order to enhance specific traits. This method is often employed in guppy breeding, whereby a female offspring with favourable traits is paired with her father to reinforce those traits in the breeding group. While guppy sons can also be mated with their mothers, the resulting offspring are generally less desirable than those produced by mating guppy daughters with their fathers. Consequently, breeders who opt for this method must maintain meticulous records to avoid complications in the future.

The application of line breeding in guppy breeding is highly favoured by breeders, as it significantly improves the chances of producing offspring that possess the desired traits. By choosing and breeding related fish that exhibit the preferred characteristics, breeders can steadily advance and refine the desired traits in the subsequent generations of guppies.

Useful tips for selective breeding of guppy:

In order to ensure the proper care and breeding of guppies, it is imperative that each aquarium tank is set up correctly. This entails installing a trap (net), introducing aquatic plants, and affixing the appropriate labeling system that corresponds to a number or letter. Given the cannibalistic tendencies of guppies, it is best to separate offspring and brooders. It is important to monitor the interactions between fish, particularly relationships such as daughter-father, siblings, and half-siblings, as certain combinations may result in unfavorable offspring when breeding occurs (such as male pups reproducing with their mother). When selecting healthy brooders, one must consider the type of offspring desired. Gender differentiation is also important to distinguish between male and female tanks, and it is recommended to familiarize oneself with the distinctive traits such as colors, patterns, and tail size that characterize the strain being developed. Finally, the breeding program should be closely monitored, including the breeding methods utilized, the outcomes obtained, and the distribution and breeding dates. By following these guidelines, optimal breeding outcomes can be achieved. Grammatically, the sentence structure and word choice are appropriate and coherent.

Conclusion:

Guppies are highly valued and sought-after ornamental fish due to their wide variety of colors, body shapes, and fin arrangements, which have been selectively bred over time.

However, breeding guppies can be a challenging task for aquarists due to the scarcity of quality traits and lack of technical expertise. To successfully breed high-quality guppies, aquarists must possess a deep understanding of the different breeding practices and focus on selecting traits that meet desired standards. Additionally, advancements in genetic research can aid in the creation of new and exotic guppy varieties that meet the demands of guppy fanciers, ensuring their continued interest in this beloved species. Overall, safeguarding the interests of guppy fanciers requires a comprehensive approach that considers both traditional breeding practices and emerging technologies.

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CHALLENGES AND OPPORTUNITIES IN INDIAN FISHERIES SECTOR

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Introduction:

The export production in fisheries was worth USD 6,678.69 in the year 2019-2020 with 7.18% growth in USD/Kg. However, there is strong possibilities to increase the production capacity by increasing and utilizing proper quality control measures and infrastructures. At present India is the third largest producer in capture fisheries with 5.4 MT, second largest producer in aquaculture with 6.2 MT and fifth largest exporter of fish and fishery products in the world (USD 6.6 Billion) along with this India accounts for 4.1% share in the world seafood exports. And the industry employs more than 28 million people in India.

Marine resources constitute a coastline of 8,118 kms, Exclusive Economic Zone (EEZ) of 2.02 million sq.mt and continental shelf area of 0.53 sq.mt. The inland resources include 0.27 million kms of rivers and canals, 2.45 million hacters of ponds, 3.15 million hactars of tanks and 1.2 million hectars of floodplain lakes.

In 2020-21, the total marine and inland fish production stood at 14.73 million metric tonnes (MMT), which includes 11.25 MMT and 3.48 MMT from inland and marine sectors, respectively. Fisheries sector plays a crucial role in the national economy and is one of the key contributors to the Inland fisheries exchange earnings. In 2020-21, about 66% of the marine fisheries and 51% of the inland fisheries potential were harnessed. The overall production of marine production has been growing consistently for the last ten years. Total fish production grew from 8.67 MMT in 2011-12 to 14.73 MMT in 2020-21.

There are eight major fish producing states: Andhra Pradesh, Gujrat, Karnataka, Kerala, Maharashtra, Odisha, Tamil Nadu, and West Bengal. Andhra Pradesh is the largest producer of marine products with 2019-20 production at 4.2 MMT. The share of Gujarat, Karnataka, Odisha and Maharashtra during the same period was 6%, 4.5%, 5.8% and 4, respectively. In addition, India is among the top five exporting countries and mainly exports frozen shrimps, fish, cuttlefish, squids and dried, live and chilled items.

Among them, frozen shrimps are the largest exported product contributing to more than 50% of the total and about 75% of the total export value. In addition, frozen fish, cuttlefish and squid contributed 6.7%, 3.73% and 4.6% of the total fish products export value, respectively.

The formulation of policy for export in fisheries facilitating and promoting exports of fisheries and allied as well as processed products. The Marine Products Export Development Authority (MPEDA), which is a statutory body under the Ministry of Commerce and Industry, Government of India, entrusted with the overall development, promotion and export of fish and fishery products from the country.

Major export Indian markets

India exports fish and fish products primarily to China, USA, Japan, The EU, the Middle East and South-East Asia. USA was the largest importer of seafood, accounting for 41.2% of the total export value and imported 291948 MT of fish food in 2020-21. The exports to USA increased 0.48% in rupee terms over the previous year, frozen shrimp is the main product exported to USA with a share of 95.63% in USD terms. Second and third importers were China and Europe, with a share of 15.8% and 13.8%, respectively in terms of value. China imported 2,18,343 MT of fish food (USD 939.17 million). The EU imported 152770 MT of fish food (USD 821.8 million) during 2020-21. Apart from this, the other importers are Canada, Russia, Bangladesh, Tunisia and Dominican Republic.

Export data

About 12,89,61 MT of Seafood has been exported from India with worth US Doller 6.68 Billion in the year of 2019-20. The major importer of Indian fish food is the USA followed by China furthermore, the seafood exports of India have improved by 0.16% in terms of rupee.

Exports during 2019-20 compared to 2018-19

Export Details	**2019-20**	**2018-19**	**Growth in %**
Quantity in Tons	12,89,651	13,92,559	-7.39
Values in Crores	46,662.85	46,589.37	0.16
USD in Million	6,678.69	6,728.50	-0.74
Unit Value (USD/Kg)	5.18	4.83	7.18

As the world shrimp market witnessed an increased supply of farmed shrimp of Asian origin, the markets such as USA, South East Asia and Japan witnessed higher shrimp inventories. This situation has brought down the export prices as the demand lagged. The importers were also prompted to offer discounts at retail level to push forward the inventories.

The increased production of shrimp in South East Asian countries has also affected Indian shrimp exports to South East Asian region. The routing of shrimps to China through Vietnam has got decimated, and instead the export of seafood, especially shrimps, directly from India to China has increased remarkably. India's exports to EU is affected by the spate of past rejections due to antibiotic residues and the consequent delisting of certain establishments.

Challenges for fisheries sector in India

The fisheries industry in India is confronted with a range of economic, institutional and environmental concerns. According to IUCN (International Union for Conservation of Nature) study, number of floodplain fish species are in danger of extinction and the pressure of fishing is so heavy in the floodplains that less than 2% of produced fish survives the end of each year. Recurrent floods and natural disasters are believed to be main underlying to sea-level rise and is ranked first among countries to be affected by the adverse effects of climate changes. Their geographical position makes it highly prone to natural disasters as well. Climate change have devastating impacts on fishery-based livelihoods and on domestic food supply. Vulnerability of fishery-based livelihoods may substantially increase in the coming decades due to climate change, and in the absence of adaptation, increased frequency and intensity of cyclones and floods would result in greater damage to fishing materials and loss of fish

Government initiatives

Pradhan Mantri Matsya Sampda Yojna (PMMSY)

Pradhan MantriMatsyaSampdaYojna was introduced in fisheries to increase the productivity, production capacity, area under cultivation and exports. It was launched by Prime Minister Shri Narendra Modi in the year of 2020 with an earmarked investment of Rs. 20,050 crores (USD 2.53 billion). The shares are distribute into three main parts, the share of central govt. was Rs. 9,407 crores (USD 1.12 billion), share of state was 4,880 crores (USD 617 million), and share of beneficiaries was Rs. 5,763 crores (USD 729 million). The main objective of PMSSY is to increase the fish production to 22 million MT by 2024-25, enhance aquaculture productivity to 5 tonnes per hectare and increase the contribution of the fisheries sector to 9% y 2024-25.

Fisheries and Aquaculture Infrastructure Development Fund (FIDF)

To meet the infrastructure requirement of the fisheries sector, a fund was formed of total Rs. 7,522.48 crores (USD 951 million) by department of fisheries, ministry of fisheries, animal husbandry and dairying in 2018-19, which is known by fisheries and aquaculture infrastructure development fund (FIDF). The objective of the fund is to provide concessional finance to eligible entities like state govt. /union territories and state entities for developing the identified

fisheries infrastructure through loaning entities namely- national cooperatives development corporation (NCDC), national bank for agriculture and rural development (NABARD) and all scheduled banks.

Government body

The Marine Products Export Development Authority (MPEDA)

The MPEDA was formed for fisheries industry in India in 1972. The focus of MPEDA is on market promotion, capturing fisheries, research and development, quality control, infrastructure processing and value fishing vessels, storage premises, processing plants and conveyances.

Department of Fisheries (DoF)

The department of fisheries was formed by dividing the fisheries division from the now known as the department of animal husbandry, dairying and fisheries. And the roles are developing the fish industry, promoting fisheries and the welfare of fishermen, statistics, regulations and carrying out surveys.

Major trade promotion activities

- Establishment of online catch and ICCAT certification system to facilitate export.
- Conduction of national residue control monitoring programme (NRCP) with respect to aquaculture for export to EU.
- Initiation of online validation of S 2031 certificates for shrimps to facilitate to USA.
- Country of origin certificate to facilitate exports.
- Registration and monitoring of various entities involved in the export of fisheries product from the country.
- Setting up processing plants, storages and handling centres using financial assistance.
- Participation in international seafood trade fairs for branding of Indian seafood products in major seafood markets such as USA, EU, Japan, China and Middle East.
- Organization of trade delegations to Japan, South Korea, China etc. and increasing market access and enhance market services.
- Aquaculture diversification through Modern technologies

Conclusion:

This chapter provides an overview of the fisheries sector and it challenges and opportunities in India. It is evident that the performance of fisheries sector is crucial from a national macroeconomic and food as well as nutrition security perspective. Therefore, it propose that a more efficient and sustainable management of the aquaculture resources will contribute

greatly to health and economy of the country. Policy makers must spare no effort to ensure the functioning of this sector in full swing by enhancing investment and research infrastructure, more strict environmental policies and introducing better storage and marketing facilities. More importantly, the situation of fishers must be taken into account and special task force should build to assess their vulnerability and strategies to tackle them. In order to meet the soaring demand for food for the burgeoning population, there is a need for increased rice and fish production in India. But attention should also be given to the negative environmental externalities such as land and water biodiversity and water and air pollution which is inextricably linked with the success of agricultural sector.

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ADDITIVES USED IN FISH PROCESSING

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Definition:

Additives are substances not normally consumed as a food by itself and as such not nutritive but are added to food to improve its nutritive quality, freshness and sensory quality or as a processing aid in the manufacture of food. The Food Protection Committee of the Food and Nutritional Board (NFCFNB) defines additive as "a substance or mixture of substances other than the basic foodstuff, which is present in a food as a result of any aspect of production, processing, storage or packaging". The Food and Drug Administration (FDA) defines food additive as "A substance that does not become a component of food, but is used in preparing an ingredient of the food to give a different texture, flavor or another characteristic in the food". A material used in the production of containers and packages is subject to this definition. If it is reasonably a component of food, it affects directly or indirectly the characteristics of the food packed in the container. The characteristics of the food do not include any physical effects, as containers and packages protect contents, preserve shape and prevent moisture loss. If there is no migration of a packaging component from the package to the food, then it does not become a component of the food and thus is not a food additive.

History of food additives

Before the Civil War, people ate the food that they raised and processed themselves. Food additives are very limited mostly to home-grown colorings and substances needed for the preservation of vegetables and fruit juices using salt, spices and smoke. Food additives were used in the good old days too. The ancient Egyptians used food coloring made from vegetables and insects, while the Romans preserved fruits in honey. Salting of foods was very common in the middle Ages.

Our system of food supply changed after the Civil War, many rural people flocked to the cities to work in factories and they needed food grown and processed by others. Cheap methods of food preservation became important and food purity was not taken as a major constraint.

Chemicals were used to keep products good just by hiding the smell. So, dangerous adulteration became very common. The problem of food additives then became acute. Copper sulfate, a powerful emetic also known as "blue vitriol" was added to canned vegetables to keep them green and fresh. Salicylic acid, borax and formaldehyde were also used generously and carelessly.

Food and Drug Protection came into operation under the US Federal Government under the leadership of Dr. Harvey Washington Wiley, the Chief Chemist for the US Department of Agriculture. He announced that the American people were being steadily poisoned by the dangerous chemicals that were being added to food. He formed "Dr. Wiley's Poison Squad" to learn about the reactions in the human body due to the ingestion of these chemicals. His efforts and the publication of another book 'The Jungle' by Upton Sinclair helped the Congress to pass the Food and Drug Act in 1906 as well as the Meat Inspection Act of 1906.

In 1927, Food, Drug and Insecticide Administration was formed and later renamed the Food and Drug Administration (FDA). In 1938, the Federal Food, Drug and Cosmetic Act was signed to broaden the scope of the Food and Drug Act of 1906. The problem of chemicals in foods became unmanageable. There were thousands of new compounds used in crop production, food processing and packaging. Some of the chemicals were so acutely toxic. It later became clear that 'chemicals in foods' have to be regulated in at least two separate categories – 'pesticides', which were used on raw agricultural commodities and 'food additives', which were substances added to improve food products or to facilitate processing or packaging. The Pesticide Chemicals Act became law in 1954, the Food Additives Amendment was approved in 1958 and the Color Additives Amendment in 1960. All laws did not ban the use of food additives but ensured their safety.

In many homes, additives are still employed when food is made. e.g., baking soda (sodium bicarbonate) is added to bread dough, pectin and sugar are added to make a jam, and vinegar (acetic acid) is added in many recipes.

Categories of food additives

The number of additives consumed every year is relatively small; hence, additives pose a minor health risk to consumers. There are over 2000 additives, many of which are common chemicals, and all are continually scrutinized for any adverse effects on human health.

Additives are divided into two categories:

1. Intentional or direct additives
2. Incidental or indirect additives

1. Intentional additives

These are the additives of known composition that have been purposely added to foods to achieve specific effects during production or processing or to impart or retain desired characteristics.

2. Incidental additives

These are chemicals which have no planned function in food, but become part of it during some phases of processing, packaging or storage.

Need for food additives

1. **To maintain and improve nutritive value**

   Foods are fortified with vitamins and minerals to maintain and improve nutritive value.

   eg. Vitamin D in milk, Vitamin A in margarine, Iodine in table salt, Vitamin A and Iron in bread and cereals.

2. **To maintain freshness**

   Preservatives and antioxidants are added to retard spoilage, to preserve natural color and flavor and to keep fats/oils turning rancid. Nitrites and nitrates protect cured meat, fish and poultry from contamination from botulinum toxin. Ascorbic acid keeps uncooked peach turning brown. BHA and BHT prevent color, flavor and texture changes in foods exposed in air.

3. **To help in processing and preservation**

   Emulsifiers are added to give body and texture to foods. Thickeners are used to evenly distribute particles of one liquid to another. Leavening agents affect cooking and baking. Acidulants control acidity and alkalinity. Humectants are used to retain moisture and prevent caking and lumping.

4. **To make food more appealing**

   Coloring agents, natural and synthetic flavours, flavor enhancers and sweeteners are used to make food look and taste better.

Functions of food additives

1. Improve the palatability of food
2. Improve the appearance of processed food
3. Improve the quality or stability of food
4. Improve or extend the storage life of food
5. Improve the safety of food
6. Minimize the wastage of food

Classification of food additives

1. **Nutrients**:

   Vitamins, minerals, proteins, etc are added to maintain or improve nutritive value of food

2. **Preservatives**:

   Preservatives include antimicrobials, antioxidants and anti-browning agents.

3. **Texturizers**:

   They are used to stabilize, thicken, retain moisture and prevent caking. They mainly aid in processing or preparation of food.

4. **Colors**:

   They impart desired, appetizing or characteristic color to the food.

5. **Flavours**:

   Spices and natural/ synthetic flavours are used to complement, magnify or modify the taste and/or aroma of food.

Classes of food additives

Food additives are grouped into 23 functional classes according to their technological functions and given in the Table 1.

Table 1. Functional classes of Food Additives

1. Acid	9. Emulsifier	17. Humectant
2. Acidity regulator	10. Emulsifying agent	18. Preservative
3. Anti-caking agent	11. Firming agent	19. Propellant
4. Anti-foaming agent	12. Flavour enhancer	20. Raising agent
5. Antioxidant	13. Flour treatment agent	21. Stabilizer
6. Bulking agent	14. Foaming agent	22. Sweetener
7. Colour	15. Gelling agent	23. Thickener
8. Colour retention agent	16. Glazing agent	

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IMPORTANCE OF BIRD COMMUNITY FOR THE ENVIRONMENT AND HUMAN LIFE

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Biodiversity, or biological diversity, is defined as the diversity of all life forms (i.e. microorganisms, plants and animals) in the Earth's biosphere. Biodiversity includes three levels of organization - ecosystems, species and genetics. It considers species diversity between ecosystems, diversity within a species population, and diversity of genetic information and functional differences within species (MEA 2005). Biodiversity is essential for the healthy functioning of ecosystems. It serves as a primary source of ecosystem services that are central to the livelihoods and well-being of all people. It plays a key role in providing economic, ecological, scientific, cultural and recreational services. Various ecosystems have greater financial value because they are various raw materials needed in medicine, food, construction, or other industries that can boost national or local economies (Naeem *et al.*, , 1999).

Similarly, biodiversity enables the efficient recycling of energy and nutrients between different species and within ecosystems. These chemical and biological material flows contribute to ecological services such as greenhouse gas regulation, water purification, soil formation, plant pollination, pollution, nitrogen fixation, etc. (Lachman *et al.*, , 2007; Naeem *et al.*, , 1999).

Assessment of biodiversity determines the richness, equality and heterogeneity of living organisms in an area. Richness is measured by the number of unique species. Evenness (or relative abundance) determines the equality of species by counting the organisms of each species. And heterogeneity is the difference between species in an ecosystem. Assessing the status of extinction risks and species population trends is essential to assess the effectiveness of conservation policies and biodiversity management strategies to protect, conserve and manage endangered habitats and wildlife. Therefore, assessment is a prerequisite for the implementation of conservation plans and actions that promote sustainability (IUCN, 2014).

The overall importance of biodiversity

Humans appreciate biodiversity for a number of reasons that make them continually appreciate it. In fact, most biologists believe that biodiversity has intrinsic value, meaning that every species has value and a right to exist, whether or not it is perceived as providing value to humans. Following are some of the importance of biodiversity to humans.

Financial value

Humans rely on biodiversity to obtain raw materials for consumption and industry. As the economies of most developing countries are more dependent on natural resources, industries related to biodiversity, such as agriculture and forestry, account for the majority of their total production.

Ecological life support

Biodiversity ensures that ecosystems function properly, providing people with oxygen, clean air, water, plant pollination, insect control, sewage treatment, and many other ecosystem services. Entertaining most of the leisure activities are based on the special nature of the diversity of nature. Activities such as bird watching, camping, hiking and fishing would not exist if biodiversity did not exist. Therefore, the assessment of ecosystems that attract tourism is very important.

Conservation of cultures

Most cultures are closely related to biodiversity through identity, spirituality and appreciation of the aesthetics of nature.

Scientific value

Until recently, research and development techniques for medical, agricultural and industrial applications of biodiversity varied widely. Individual samples of animals, plants or microbes can now be kept in culture and tested for possible use in all these sectors. For example, the discovery of Taq polymerase, a thermostable enzyme developed by a thermophilic bacterium, to amplify specific DNA target sequences from small amounts of DNA using a technique known as polymerase chain reaction (PCR).

How important are birds for environmental protection and human life?

Birds play countless roles in healthy ecosystems, so preserving bird diversity helps the environment and everyone.

Birds add beauty

Birds provide intangible aesthetic pleasure and enrich our lives with their presence. This intangible value comes from knowing that our world is still big and healthy enough to support a variety of bird species.

Birds spread seeds

This seed dispersal may be the most important ecological function of birds, since many plant species depend almost entirely on bird reproduction.

Birds pollinate plants

When you think of pollinators, bees and butterflies come to mind, but bird pollinators like hummingbirds make a big contribution. Their role as pollinators directly benefits us.

Birds feed on pests

Birds eat tons of insects, up to 400-500 million tons of insects per year. They are a natural way to control pests in gardens, farms and parks. Studies on the damage caused by birds to various crops show that the amounts are less than 1 percent of the production.

Scavengers are nature's cleanup crew

The highly acidic secretions of the vulture's stomach kill all but the most resistant spores, reducing the number of pathogenic bacteria that feed on the corpses and thus disease. These birds provide a public health service by arriving days before other less efficient scavengers such as wild dogs or rats can take the remains, preventing the development and spread of deadly diseases such as rabies and tuberculosis. In India, the decline of vultures led to an increase in rabies and contributed to the 1994 outbreak of bubonic plague.

Birds indicate environmental hazards

Because birds are sensitive to changes and easy to count, they are an important tool for measuring environmental health. Birds integrate and accumulate environmental stresses over time because they are usually high in the food chain and have relatively long lifespans. This makes birds our early warning system.

Birds promote nature conservation

When people discover the wonder of birds, their interest often leads to support for conservation.

Birds preserve and change entire landscapes

Habitats such as forests, swamps and grasslands support people across the planet, store carbon, keep the climate stable, oxygenate the air and convert pollutants into nutrients. But without birds, many of these ecosystems would not exist. Birds maintain a delicate balance between plants and herbivores, predators and prey, and are an integral part of food chains and food webs.

Birds fertilize

Birds, especially seabirds, play an important role in nutrient cycling and fertilization of marine ecosystems such as coral reefs. Their droppings are high in nitrogen, phosphate and potassium, three essential nutrients for plant growth.

Birds inspire science

From flight technology to the invention of zippers, humans have been inspired by birds for centuries. A notable example: Darwin's studies of the finches of the Galapagos Islands proved decisive in shaping the ideas of evolution through natural selection.

Birds feed us

Birds and their eggs have been at least an occasional source of food for humans since the dawn of time and will continue to be in most societies. Chickens were domesticated in Asia at least 3,000 years ago.

Feathers keep us warm and cozy

Bird feathers are used all over the world to fill pillows, mattresses, sleeping bags, jackets and blankets. They are a natural and renewable source of insulation that can have a lower environmental impact than synthetic oil-based microfibers.

Bird watching connects us with nature

Birds are a shared interest and a gateway to a greater natural connection.

Bird tourism

Bird tourism can sustainably support communities and promote greater conservation. Birds are a good reason to travel to other places and cultures and to keep in touch with other places and cultures.

Birds and Ecosystem

Birds are naturally important members of many ecosystems. They are an integral part of food chains and food webs. For example, in a forest ecosystem, some birds get food mainly from plants. Others mainly eat small animals such as insects or earthworms. Birds and bird eggs are in turn food for animals such as foxes, raccoons and snakes. Nutritional relationships between all the animals in an ecosystem help prevent one species from getting too big. Birds play an important role in maintaining this natural balance. In addition to being important parts of food webs, birds also play other roles in ecosystems.

Birds are important in the formation of new soil

Since there are so many birds, it makes sense that a large number of them die each year. Their bodies are rich in nutrients and organic materials. The soil absorbs nutrients with the help of bacteria. The bodies decompose in the soil and then become part of the soil. Birds are excellent carriers of organic material and this enriches the soil beneath us.

The importance of birds in the ecosystem

Birds are important to the ecosystem because they create many nests, tree holes and even burrows during their lifetime. When they die, these structures can be used to protect other animals.

Conclusion:

Birds are naturally beautiful creatures. Birds are important to the ecosystem in many ways; they pollinate flowers and spread seeds. They are also important to people in many ways; they are a source of food and fertilizer. From the tiniest hummingbird to the heaviest bird, birds are easily some of the most interesting animals in the world. They are intelligent and simply fascinating to watch. They make wonderful pets. With all the amazing facts about birds, it is clear that it is simply tragic to see them die out.

How to protect Avian biodiversity

Below are the ways to protect biodiversity:

Legislation

The government has the power to regulate what happens to the ecosystems within their boundaries. Legislation that protects natural ecosystems by prohibiting development, natural extraction of natural resources or other forms of human activity significantly affect the conservation of biological diversity.

Reduction of invasive species

Invasive species are often introduced into new areas every day, either intentionally or accidentally. To reduce the number of invasive species that are accidentally transported, aircraft, ships and cargo must be carefully inspected before they are unloaded in a new country. Also, a person may not introduce new types of animals or plants into the area without first contacting ecologists who know the area.

Habitat restoration

If human activities have negatively affected the environment, efforts can be made to restore it to its natural state. In this case, the appropriate measure is the restoration of the naturally occurring or dominant plants and animals in the area.

Stop climate change

The consequences of climate change are dire for all living things, animals and microorganisms on the planet. Climate change is caused by the widespread use of fossil fuels and other human activities that change the ecosystem.

What can we do to help birds?

With all these advantages, don't we have an ethical duty to ensure that our children inherit as much as we do now? Despite their functional values, should we not let a bird species disappear like destroying a masterpiece of art?

Make outdoor spaces bird-friendly

One of the best ways to help native bird species is to make outdoor spaces bird-friendly. In winter, it can provide additional nutrition in the form of seeds and nuts, and in summer it can ensure the availability of drinking and bathing water for the birds.

Help protect habitats

Habitat loss is one of the most important reasons for the decline of bird populations. Important habitats such as forests and wetlands must be protected.

Support charities

It is important to support charities that work to protect birds, such as the World Bird Organization. We should encourage governments to protect wildlife, such as birds, through policies and regulations before important species disappear.

Participate in local bird counts

Another great way to help these creatures is by participating in local bird counts. This will help scientists collect more data to estimate the number of birds in populations. This is only possible with the help of the public who can post notes about their local bird sightings.

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ODONATAN DIVERSITY IN AROUND THE CAMPUS AREA OF PT. RAVISHANKAR SHUKLA UNIVERSITY RAIPUR (C.G.) INDIA

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Abstract:

The bulk of Madhya Pradesh and Chhattisgarh lies on the tableland of central India and gifted with diverse habitats for Odonate diversity. A check list of 106 species of odonata belonging to 53 genera representing to 12 families of which 14 species were new record. The highest number of Odonate were recorded belonging to the family Libellulidae (39 species), followed by Coenagrionidae (29 species), Gomphidae (10 species), Protoneuridae (5 species) and Lestidae (6 species), Calopterygidae and Aeshnidae (4 species of each), Chlorocyphidae (3 species), Platycnemididae, Macromiidae (2 species) and Euphaediae and Cordyliidae (one species). The study provides the Odonatan diversity in around the campus area of Pt. Ravishankar Shukla University Raipur (C.G.) India.

Keywords: Odonata, Biodiversity, Chhattisgarh States, India.

Introduction:

The order Odonata (dragonflies and damselflies), compising three suborders Anisoptera, Anisozyoptera and Zygoptera are one of ancient group of Insects. Within India, 463 species belonging to 140 genera have been recorded representing 8% of the world known species (Subramanian, 2009). The larvae and adults are predatory and very important biocontrol agents for insect (Khaliq, 2002). Moreover, studies across the world have shown that they are good indicators of ecosystem health and ideal surrogate taxa for identifying freshwater biodiversity hotspots for conservation (Hart *et al.*, , 2014).

The Odonates have strong association with water because of their aquatic larvae. Dragonflies have been extensively used as indicators of environmental quality in aquatic ecological units. Dragonflies are key organisms of the food web as predators both as larvae and as imagoes. They usually have definite habitat preference and territorial behavior. Odonates are ecologically impotant as both predators and prey. In India, Odonata status gives valuable insight about ecosystem health. They are among the dominant invertebrates predators in any ecosystem. Being predators both at larval and adult stages, they play significant role in the food chain of the

ecosystem. Their aquatic larvae constitute a natural biological control over mosquito larvae and help to control several epidemic diseases like malaria, dengue, filaria etc.

The present study was carried out in four different sites in an around Pt. Ravishankar University Raipur campus. The objectives undertaken in the present study were: To survey and identify the dragonfly species found in four study areas, to evaluate the species diversity, evenness, richness and abundance of the species in both the study areas and to identify bio indicator dragonfly species in the four ecosystems.

Materials and Methods:

Study sites:

The present study was done in Pt. Ravishankar Shukla University, Raipur campus, district Raipur State of Chattisgrah.Site 1 Boyes hostel campus, Site 2 Guest house campus, Site 3 University main get, Site 4 Dam near H.R.D.C.guest house.The study area is located between latitude 21.2469 N and longitude 81.5974 E. The university campus has total area of 150 acres.

Data collection:

The study was conducted for a period of fourteen days from four July 2018 to Seventeen July 2018. All surveys and photograph were taken in morning and evenings, by Canon, Digital 7 camera, lens 55-250.for identification keys provided by Fraser, Subrimanian, Prasad and Varshney, Manoj V Nair.

Results and Discussions:

In the present study 15 species of dragonflies and damselflies were recorded from four survey sites (Table 1). It has been found that 08 species of Coenagrionidae family is mostly present in abundance followed by family Libellulidae comprising 06 species. The detailed species names and the site from where they have been observed are given in Table 1.

It has been found that Coenagrionidiae comprises 53.33 % of total species identified from survey sites followed by 40 % of Libellulidae and 6.66 % of Gomphidae. In the present investigation, a study of dragonflies of Pt. Ravishankar Shukla campus was carried out to ascertain the number of species present in the beautiful landscape flanked by forest areas and water bodies and a checklist preparation was initiated. The survey showed remarkable species diversity of dragonflies dominated by Coenogrionidiae comprising 08 species followed by 06 species of Libellulidae and 01 species Gomphidae families. This investigation showed some addition to the number of dragonfly species already described from Raipur district of Chhattisgarh.

Crocothemis servilia, Brachythemis contaminate, Diplocodes trivialis, Trithemis pallidinevis, Orthetrum Sabina, Ditch Jewel, Ctinogamphus rapax, Bradinopyga geminate,

Ischnure aurora were found from all study areas and these were the most dominant species in the campus. Their dominance may be attributed to the presence of large green trees and small pond around Pt. Ravishankar Shukla campus. It is reported that the dragonflies and damflies are ideal model insects for the investigation of the impact of the environmental warming and climate change due to its temperatures remains moderate throughout the year, except from March to June, which can be extremely hot. Study on diversity patterns of this creature thus can give an idea about changing climatic condition and its effect on surrounding vegetation. This checklist, a first of its kind, showed remarkable dragonfly diversity and distribution in the beautiful land scape of university and it assumes a great taxonomic significance as it is already mentioned that this checklist shows new additions to the previously known number of species from Raipur district of Chhattisgarh.

Observations:

S. No.	Genus and Species of Odonata	Family	Site 1	Site 2	Site 3	Site 4
1.	*Crocothemis servilia*	Libellulidae	Y	Y	N	Y
2.	*Brachythemis contaminata*	Libellulidae	Y	Y	Y	Y
3.	*Diplocodes trivilis*	Libellulidae	Y	Y	Y	Y
4.	*Trithemis pallidinervis*	Libellulidae	Y	Y	Y	Y
5.	*Orthetrum sabina*	Libellulidae	Y	Y	Y	Y
6.	*Bradinopyga geminata*	Libellulidae	Y	Y	Y	Y
7.	*Ictinogamphus rapax*	Gomphidae	N	N	N	Y
8.	*Pseudagrion rubriceps*	Coenagrionidae	N	N	N	Y
9.	*Ischnura senegalansis*	Coenagrionidae	N	N	N	Y
10.	*Psudogrion coromandelian*	Coenagrionidae	Y	Y	Y	Y
11.	*Psudogrion decorum*	Coenagrionidae	N	N	N	Y
12.	*Pseudogrion microcephalum*	Coenagrionidae	N	N	N	Y
13.	*Agrionemis pygmea*	Coenagrionidae	N	N	Y	Y
14.	*Agrionemis femina*	Coenagrionidae	N	N	Y	Y
15.	*Ischnura aurora*	Coenagrionidae	Y	Y	Y	Y

Conclusion:

From the present study in the four study sites Pt. Ravishankar Shukla University, Raipur, Chhattisgarh 15 species of dragonflies and damselflies were found, belonging to a three family Libellulidae, Gomphidae and Coengrionidae Odonates are good indicators of the quality of environment. Their distribution and abundance depicts the changing environment. These species are at the least concern category according to IUCN red list, but due to loss of habitat and various anthropogenic activities they could be in danger in near future. So, far, very less study has been done on these beautiful creatures, especially in central India. Effort to study the diversity and abundance of these species in these should be made, to keep these species away from any to its existence in the near future.

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A PRELIMINARY REPORT ON TRADITIONAL USE OF SELECTED PLANTS OF THE SOLANACEAE FAMILY AT LATHOR VILLAGE, H.S. ROAD, BALANGIR DISTRICT, WESTERN ODISHA

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Abstract:

The present research work was designed together indigenous knowledge of medicinal plant species from Solanaceae family, which are being utilized by the local inhabitants of the area of Lathor village, H.S. Road, Balangir district, Odisha, India. A total of 10 medicinal plants species belonging to Five genera were used by the natives for the treatment of different diseases. There is a maximum of six species belongs to genus *Solanum* and rest Four genus consist of one species each. Out of the ten reported medicinal plants eight species (80%) are shrub, and two species (20%). These reported medicinal plants are generally used for the treatment of lipoma, asthma, cough, skin diseases, diabetes, hypertension, bleeding in gums, ulcer, increase body stamina, etc. Plants like *Capsicum frutescens* L., *Solanum lycopersicum* L., *S. lycopersicum* var. *cerasiforme* (Dunal) A. Gray, Kon Bilahi, *S. melongena* L., *S. tuberosum* L. are cultivated in kitchen garden and used as vegetables by the native of the study sides. During the investigation it was found that the person practicing this art does not easily reveal knowledge to others present investigation carried out during the month of January 2022 to December 2022.

Keywords: Solanaceae, Medicinal plants, Traditional use, Tribal.

Introduction:

Although modern scientific medicine has made significant advances in recent years, traditional medicine continues to be the primary method of treating diseases for the vast majority of people in developing countries, including India. Even among those who have access to western medicine, the number of people who use alternative medicine or complementary is rapidly increasing around the world. The growing understanding of metabolic processes and the effects of plants on human physiology has broadened the scope of medicinal plants' potential use in medicine (Dey *et al.*, , 2012). The oldest evidence we know of human beings using plants for medicinal purposes has been unable to be identified. Whether accidentally or persistently, it

seems probable that man has been experimenting with nature for some time. For the most part, regular people were responsible for accumulating most of the collected information about valuable plants. However, when one is aware of the medical applications that thousands of wild plants around us have developed, the plant takes on a new significance, a new value that surpasses its aesthetic worth, its cooling shade, or its pleasant aroma (Hussain *et al.,* , 2009). Nature has always been a first-rate medication shop, thanks to its great variety of plants that have been shown to offer medicinal properties that are effective. Traditional civilizations have amassed a wealth of information about herbal medicines via trial and error through hundreds of years of experience. Even more importantly, the most significant remedies were handed down orally from one generation to the next (Hussain *et al.,* , 2010). Natural alternatives to synthetic chemicals, such as medicinal plants and plant-derived medications, have long been practised in civilizations all over the globe, and they are becoming more popular in contemporary society as natural alternatives to synthetic chemicals.

The Solanaceae, also called nightshades, comprise more than 3000 species many of which evolved in the Andean/Amazonian regions of South America in habitats that vary dramatically and include rain forests that receive more than 3 meters of rainfall annually to deserts with virtually no rainfall and high mountains with regular snowfall and subfreezing temperatures (Jagatheeswari, 2014). The centre of diversity of the Solanaceae is near the equator and thus species were undisturbed by the ice ages and have had time to accumulate adaptive genetic variation for extreme ecological niches. The Solanaceae are also the third most important plant taxon economically and the most valuable in terms of vegetable crops, and are the most variable of crops species in terms of agricultural utility, as it includes the tuber-bearing potato, a number of fruit-bearing vegetables (Tomato, Eggplant, Peppers), ornamental plants (Petunias, Nicotiana), plants with edible leaves (*Solanum aethiopicum, S. macrocarpon*) and medicinal plants (eg. *Datura, Capsicum*). The Solanaceae include a number of commonly collected or cultivated species. Perhaps the most economically important genus of the family is *Solanum*, which contains the potato (*Solanum tuberosum*, in fact, another common name of the family is the "potato family"), the tomato (*Solanum lycopersicum*), and the aubergine or eggplant (*Solanum melongena*). Another important genus, *Capsicum*, produces both chili peppers and bell peppers (Jagatheeswari, 2014). Traditional knowledge of the medicinal plants and their use by tribal people need to be conserved for their cultural tradition along with the biodiversity.

Lathor village of Balangir district is one of the tribal dominant village in western Odisha. People of this village depend on plant resources for their domestic and primary health care needs. They collect all the useful plant and plants part from the forest, grasslands, wetlands, agricultural

fields etc. and use it by long-established way (Behera and Sahu, 2023). This paper is the initial step for the documentation and conservation of medicinal knowledge of different plant species comes under Solanaceae family which helps the society to restore to health.

Materials and Methods:

Plants collection and identification

The study area was visited frequently and close interaction were made with the senior tribal people practicing herbal medicines. During field work, interviews were conducted with local knowledgeable villagers, the herbal healer called 'Vaidyas' (local physicians in Indian System of Medicine), old woman and medicinal plant vendors. Photographs of different plants and plant parts were taken; plants were also identified by using local flora book (Haines 1921-25, Saxena and Brahmam 1994-96). Further the local names were crossed checked by using our earlier reports (Sahu *et al.*, , 2010; Sahu *et al.*, , 2013; Sahu *et al.*, , 2021). In this study it is also observe that the traditional medicinal values and knowledge are facing challenges because the young generations don't want to adopt the traditional knowledge, values and migrate towards the city. Due to this reason the knowledge on medicinal plant cannot pass to the next generation. Their fore it is very much important to conserve it in the form of documentation which further helps in research works.

Enumeration

In this present report, a total of Ten medicinal plant species from Five genera belong to Solanaceae family have been reported from the study site. Ethno-medicinal inventory was developed consisting of botanical name, followed by their local name, Medicinal and other traditional Uses including the part used, and mode of preparation in tabulated format. The statistical analysis was carried out using MS-EXCEL.

Result and Discussion:

A total of 10 medicinal plant species from Five genera have been found and reported in this present study (Table 1, Figure 1). There is a maximum of six species *Solanum indicum* L., *S. lycopersicum* L., *S. lycopersicum* var. *cerasiforme* (Dunal) A. Gray, Kon Bilahi, *S. melongena* L., *S. nigrum* L., and *S. tuberosum* L. are belongs to genus *Solanum* and rest Four genus consist of one species each. From the ten reported medicinal plants eight species (80%) are shrub, and two species (20%). These reported medicinal plants are mostly used for the treatment of lipoma, asthma, cough, skin diseases, diabetes, hypertension, bleeding in gums, ulcer, increase body stamina, etc. Plants like *Capsicum frutescens* L., *Solanum lycopersicum* L., *S. lycopersicum* var. *cerasiforme* (Dunal) A. Gray, Kon Bilahi, *S. melongena* L., *S. tuberosum* L. are cultivated in kitchen garden and used as vegetables by the native of the study sides.

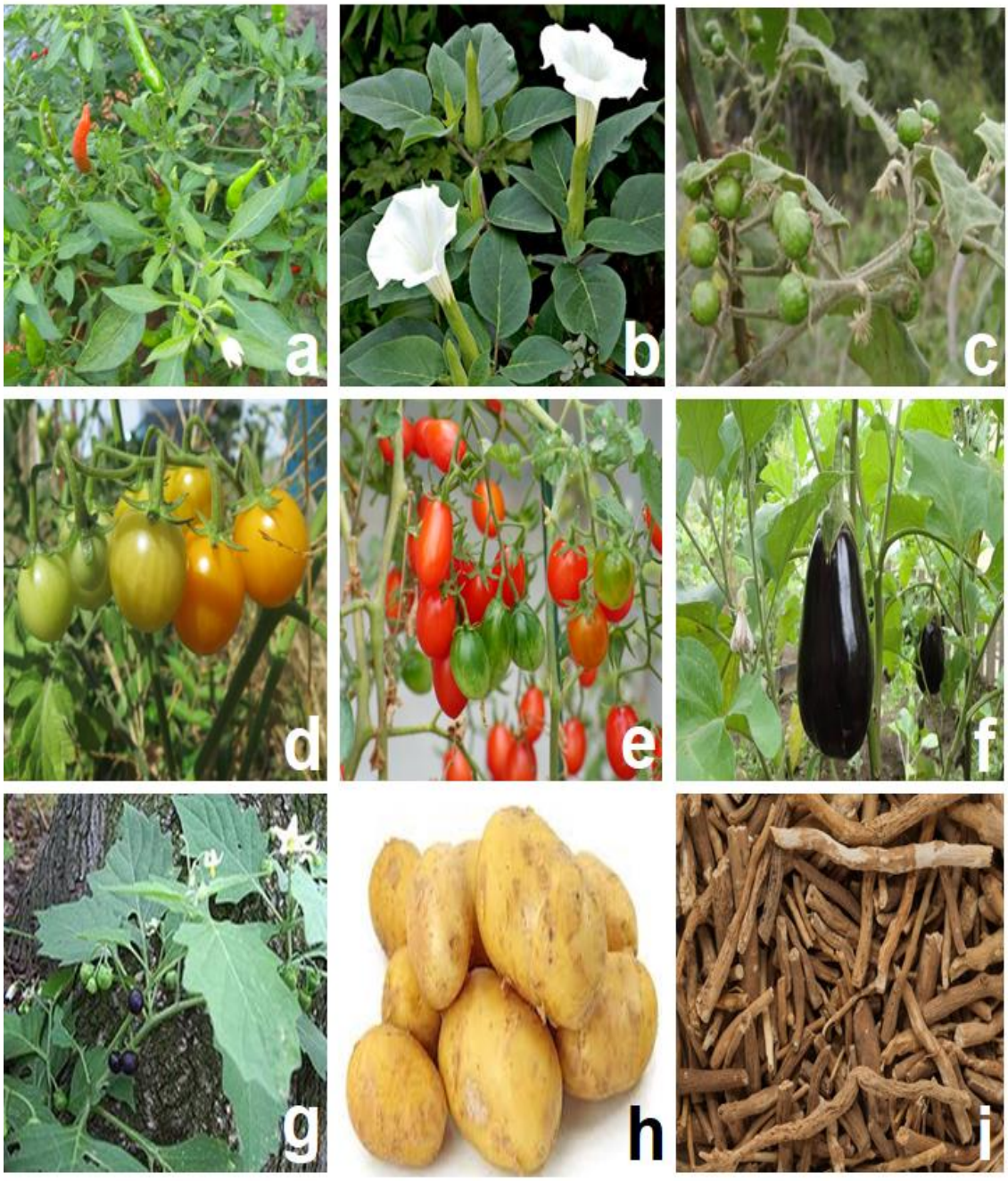

Figure 1: Photograph of *Capsicum frutescens* L. (a), *Datura fastuosa* L. (b), *Solanum indicum* L. (c), *Solanum lycopersicum* L. (d), *Solanum lycopersicum* var. cerasiforme (Dunal) A. Gray, Kon Bilahi (e), *Solanum melongena* L. (f), *Solanum nigrum* L. (g), *Solanum tuberosum* L. (h), and root of *Withania somnifera* (L.) Dunal (i).

Table 1: Traditional use of some medicinal plants of Solanaceae family used by the native of Lathor Village, H.S. Road, Balangir District, Western Odisha

Botanical Name	Common Name	Uses
Capsicum frutescens L.	Chilly (Mircha)	➢ Chilly is used as spices to increase digestive power. ➢ Green chilly is helps to increase R.B.C in blood. This can also help to control blood sugar level.
Datura fastuosa L.	Dhatura (Dudhra)	➢ Leaf is used to treat ear problem like swelling and pain. Leaf is also use to treat lipoma, asthma. ➢ Fruit helps to cure asthma ➢ Seed oil is use to increase sex power and stamina, this oil is also helping to remove swelling and skin problems.
Nicotiana tabaccum Linn.	Tobacco (Bhang)	➢ Tobacco leaf and root help to heal all old sores, cancerous ulcers, ring worms and scabies. ➢ Leaf is use in cigar to get temporary pleasure. The leaf is also use as antiseptic to stop bleeding; this also use get relief from headache.
Solanum indicum L.	Bush Tomato (Bhejri)	➢ The juice extracted from the leaves is used with fresh ginger juice to alleviate nausea and vomiting. ➢ The fruit is used to treat leukoderma, pruritus, and bronchitis. ➢ Powder of dried fruit is used in cigarette and the smoke is kept inside the mouth for about 10 minutes to relieve dental caries.
Solanum lycopersicum L.	Tomato (Patalghata)	➢ Fruit helps to control diabetic, reduce belly fat, and reduce skin dullness. ➢ Take 1tometo with 2 black paper to increase eye site.

Solanum lycopersicum var. cerasi forme (Dunal) A. Gray, Kon Bilahi	Cherry tomato	➢ Fruit help to treat scurvy, bleeding gums and mouth ulcer. ➢ Fruit is use to increase eyesight, and to treat oily skin problem.
Solanum melongena L.	Brinjal (Biagan)	➢ Fruit is help to treat diabetes, lose body fat and weight. ➢ Root help to get relief from asthma ➢ By consumption of food it controls hypertension and stress.
Solanum nigrum L.	Black nightshade (Nunnunia)	➢ The leaves are used as poultice for rheumatic and gouty joints. ➢ Berries are used to possess tonic, diuretic and cathartic properties.
Solanum tuberosum L.	Potato (Alu)	➢ Potato is use as food to increase digestion; potato past is use for glowing skin. ➢ Leaf extract is used to treat in cough, exima, itchy.
Withania somnifera (L.) Dunal	Winter cherry (Ashwagandha)	➢ Take 3 leaves for 3 days to lose body weight. ➢ Root is use to help to get relief from swelling and in arthritis. ➢ Root tea is use to control cough ➢ Root, stem, leaf, extract use to increase body stamina.

It is observed that a large number of populations lives in villages and they are directly or indirectly depending on the plants for the fulfillment of their health care and sociocultural need. It is also observed that the villagers mostly prefer the traditional healer and herbal healer known as "Vaidyas" for diagnosis of health issue and use the prescribe medicine by the Vaidyas to get recover from the health issue (Mishra, 2006). This is happening due to the high cost price of allopathic medicine as well as the side effect of these allopathic medicines. Long established medicines are the applying source of health care, apart from this the study indicate that most of the plants have multiple socio-cultural usage by the local tribal. The study also reviles that the different plant parts like root, stem, leaf, flower, fruit, seed, etc. of these reported plant species

are primarily use for medicine. Sahu *et al.*, , (2010) reported the medicinal use of *Capsicum frutescens, Datura fastuosa, Solanum melongena, S. xanthocarpum, Withania somnifera* from Solanaceae by the native of Bargarh district. Devi *et al.*, , (2012) reported the ethnomedicinal use of about Eight plant species from Solanaceae family by the native of Hamirpur district (H.P). Awan and Murtaza (2013) reported about the ethnobotanical use of 15 plant species belonging to family Solanaceae by the local inhabitants of the area of Muzaffarabad Division, Azad Jammu and Kashmir, Pakistan. Sahu *et al.*, , (2020) reported *Solanum melongena L., Capsicum frutescens* L., and *Lycopersicon esculentum* Mill., by the rural peoples of Bargarh district as summer seasonal plants. Sahu *et al.*, , (2021) reported the use of *Withania somnifera* (*L.*) *Dunal for* Relieving Urogenital Ailments by the native of Bargarh district.

Acknowledgement:

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VETERINARY DRUGS FOR FISH

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Introduction:

Aquaculture is a multibillion industry with a global production of 122.6 million tonnes in 2020, with a total value of USD 281.5 billion. Aquatic animals accounted for 87.5 million tonnes and algae comprised 35.1 million tonnes. Asia continued to dominate world aquaculture, producing 91.6 % of total. Fishes and shrimps are susceptible to several diseases by bacteria, viruses or fungi. They are also prey to many parasites like protozoan, trematodes and helminths. New diseases like COVID regularly appear and cause threats. Aquaculture bacterial and parasitic infections are often treated with veterinary drugs. Veterinary drugs are substances or compounds, including medicinal products, Vaccines, bio-preparations, microorganisms and chemicals permitted to use by animals for prevention, treatment, diagnosis, rehabilitation and improvement of growing and reproductive functions of animals. In addition, Agents that cannot be considered under antibiotics include antihelmintica (active against parasites), antimycotica (against moulds and fungi) and hormones (used in sex reversal). Hence, all these substances are grouped under "veterinary drugs".

Few veterinary drugs are allowed for use in aquaculture. Withdrawal times (*i.e*., the time between the end of treatment and the clearance of the drug from the organism) are an essential issue. Continuous use of antibiotics has led to the emergence of resistance among bacteria, which forced the farmers to increase the dosage or shift to another drug. Some drugs are thus prohibited by many countries e.g., chloramphenicol and nitrofurans.

Origin of veterinary drug residues

Some veterinary drugs have extremely long depletion times. Malachite green and its metabolite, leuco-malachite green, deplete within 100 days from 7 to 15 µg/kg in eel. Low levels of antibiotics are observed in commercial feed. Crabs are contaminated during processing by the commercial hand cream containing chloramphenicol. In general, veterinary drugs undergo fast and extensive metabolism in the animal. Some drugs *e.g*. nitrofurans, produce small breakdown

products which are used as indicators of the parent substance *e.g*. semicarbazide is the indicator metabolite of nitrofurazone.

Analysis of veterinary drugs

Two types of tests were conducted to analyse drugs in the aquatic animal body, namely the biological test system and the instrumental method. The details of both the study have given below-

1. Biological test system

The inhibition of microorganism growth by an antibacterial drug is utilized for their detection. These tests are not very selective. Good sensitivity is reported for penicillin. Nitrofurans or sulphonamides produce weak or even no inhibition. Enzyme-linked immunosorbent assay (ELISA) permits fast and highly selective screening and semi-quantification of various veterinary drugs. This method is well-suited for a large number of processed samples. The high selectivity is due to the recognition of an epitope of the veterinary drug. ELISA is still a 'one drug, one test' approach.

2. Instrumental method

Liquid chromatography (LC) is more suited for analyzing veterinary drugs. Gas chromatography (GC) coupled with mass spectrometry (MS) is still useful for hormone analysis. Samples are extracted and cleaned up prior to separation and quantification. Based on the group of a veterinary drug, polar or non-polar, highly buffered or strongly acid solvents are used for the extraction. Some alternatives for liquid extraction: are matrix solid-phase dispersion extraction (MSPD) and accelerated solvent extraction (ASE). MSPD is laborious, while ASE is limited to thermostable analysis. Modern methods of veterinary drug clean-up are solid phase extraction (SPE) *e.g*., C-18 cartridges. RP-SPE removes proteins and peptides that otherwise precipitate on the analytical column, reduces separation performance and causes signal suppression in MS. Anion or cation exchange cartridges are highly selective, producing clean extracts, but are unsuitable for some veterinary drugs. Hence, less selective RP-SPE cartridges are preferred. LC and UV detection are sued for the assay of a few drugs but provide insufficient selectivity and sensitivity. LC with a fluorescence detector permits high selectivity and sensitivity, but only a few veterinary drugs possess an inherent fluorescence *e.g.* chinolones. Most of these drugs are unsatisfactory and require pre or post-column derivatization before detection and. LC coupled with MS opened new frontiers for analysing veterinary drug residues. Interfaces like electrospray (ESI), atmospheric pressure chemical ionization (APCI) and photoionization (PI) are used. Most veterinary drug analysis is done by LC coupled to tandem mass spectrometry (LC-MS-MS).

Mass selection of a precursor ion, induced fragmentation, and selective monitoring of one or more derived product ions gives good selectivity and sensitivity.

Route of drug administration

1. **Water medication**

The most typical method by which drugs have traditionally been administrated to fish is by medication of water the fish inhabit. In addition to its simplicity, water medication has the advantage of being adaptable to mass medication of large numbers of fish.

2. **Immersion or dipping**

This term means preparing a small volume of medicated water in a separate container from that holding the fish. The fish, usually held in a net, are immersed in it for a short period and then returned to their normal environment. Dipping has a disadvantage compared to water medication in that the fish are exposed to the stresses of chasing, handling and netting. Dipping has a particular advantage in using certain antibacterial drugs in aquaria where filters are used to effect bacterial oxidation of ammonia to nitrites and nitrates.

3. **Hyperosmotic infiltration**

Hyperosmotic infiltration is a development of immersion designed to accelerate the absorption of macromolecules or even of particles such antigenic bacteria. The procedure as originally devised consisted of two separate immersions. The first was in a pharmacologically inert solution, hypertonic to fish plasma; 10% urea and 5.23%sodium chloride, both being1650 mOsm/l, have been used. This immersion was for 3 minutes and was followed immediately by the solution to be absorbed.

4. **Flushing**

Where fish are kept in running water that is not recirculated; for example, in a raceway, immersion can be achieved by flushing or, as the process is sometimes called, a California flush. Flushing is more wasteful and environmentally polluting than dipping; obtaining a homogenous distribution of the medication in water may be difficult. This procedure is commonly adapted in hatcheries to control fungal infection.

5. **Bath treatment**

In bathing, the bottom of the net cage is raised, typically to 2 meters, thus limiting the volume of water to be medicated. This reduces the weight of the drug, reducing both the cost and degree of environmental contamination. Bath treatment is wasteful and environmentally contaminating, and additionally is labour-intensive.

6. In-feed medication

In-feed medication or the provision of medicated feed is a much less wasteful method of administration than water medication. In-feed medication is standard practice for many diseases but is prophylactic, not therapeutic.

7. Pelleted medicated feed

The ideal way to medicate feed is to add the medicinal product to the feed mix before pelleting.

8. Surface-coating pelleted feed

This process is suitable for the medication of small batches of feed and can be used for heat-liability drugs. It is, therefore, the standard means of medicating feed on fish farms.

9. Spray-medication of pelleted feed

Sex hormones are important examples of a class of drugs that are, for practical purposes, insoluble in water and which are used in tiny doses.

10. Leaching

Leaching of drugs into the water occurs with all forms of in-feed medication but is a particular problem with surface-coated feed. The extent of leaching varies according to the solubility of the active ingredient in water and the time the feed is in the water. A further factor affecting the leaching rate from medicated pellets is the size of pellets and hence the ratio of surface area to weight. The smaller the pellets, the faster the leaching will be.

11. Micro-encapsulation of drug

One micro-capsule structure that has been well-researched has a calcium alginate core into which the drug is mixed and a chitosan-alginate shell. Whether a micro-capsule is retained in the stomach is an essential question of its size.

12. Gavage

Gavage is an oral administration used extensively in experimental work because the dose can be known accurately. It is rarely used in routine fish management as it is labour intensive and stressful to the fish. Gavage is a helpful technique where a few fish, such as brooder or valuable ornamentals, must be dosed orally.

13. Injection

a. Manual injection

A prerequisite for injection is that the fish should be anaesthetized; without this precaution, injury is likely to be caused to the fish and possibly the operator.

b. Intramuscular injection

Intramuscular injections are given into the epaxial musculature, normally approximately mid-way between the mid-dorsal and lateral lines.

c. Intraperitoneal injection

Intraperitoneal injections are made into the mid-ventral line, just cranial to the vent. This is a widely used injection route, but it can sometimes cause peritoneal adhesions. In consequence, intraperitoneal injection should be avoided; the dose is likely to be made into a viscus or the lumen of the gut. Injection into the dorso-median sinus. The administration route recommended for salmonids is the dorso-median sinus (DMS). The needle insertion point is in the mid-dorsal line at the angle at the caudal margin of the cranial dorsal fin. This route is unsuitable for most fish other than salmonids because of their different anatomy.

d. Automatic Injectors

Multiple-dose syringes such as those available for injecting drugs into mammals, rarely deliver sufficiently accurate doses of the volumes used in fish. However, automatic injectors used for vaccinating poultry can inject fish.

e. Machine injection

Machines are available for the rapid injection of large numbers of fish. In practice, this means vaccination by the intraperitoneal route. Anaesthesia is not needed with the machine, but it was found safer for the operator than manual procedures with anaesthesia.

14. Implantation

Where there is a need for prolonged medication with a drug that, for either economic or biochemical reasons, can only be administered by injection for economic or biochemical reason, it is sometimes formulated as a pellet or capsule for Implantation. As with other injections, implantations may be intramuscular or intraperitoneal.

15. Tropical application

Tropical application of drugs on fish is rare, where it is usually done to treat skin ulcers on valuable ornamental fish.

Veterinary drugs used in aquaculture

I. Bactericidal drugs (antibiotics)

1. Sulphonamides

Sulphonamides are exclusively produced in a synthetical manner at low price for wide use. They are antibacterial and antiprotozoal. They show activity against a broad spectrum of gram negative and positive bacteria. Their use for more than 50 years, had led to widespread resistance against many animal pathogens. They are administered in combination with

potentiators such as ormeroprim and trimethoprim that enlarges their therapeutic range. They are quite stable molecules to wide pH and temperature. There is no significant concentration of metabolites, except the only one in urine, N-acetyl metabolites. ELISA and microbiological tests are available for detection. LC-MS-MS in the positive ESI permits quantification of sulphonamides together with potentiators.

2. Tetracyclines

Tetracycline is an antibiotic produced by *Streptomyces*. Their semi-synthetic derivatives are chlorotetracycline or deoxycholine. They are broad spectrum drugs with activity against gram negative and positive bacteria. They are widely used in aquaculture and animal husbandry because of their low price. Development of resistance is a common problem and hence, they are less stable than sulphonamides. They form epimers and complexes with metals, which complicate quantification. Specific microbiological tests are available for detection. Tetracyclines are extracted by acid aqueous buffers containing oxalic acid and EDTA and identified by fluorescence detection after post column derivatization with improved specificity and sensitivity. LC-MS-MS (positive ESI) is most often employed.

3. Chinolones

Chinolone is a group of synthetically produced bactericidal drugs, active against a broad spectrum of gram negative bacteria. They are new groups of drugs reserved for human treatment. Residues of enrofloxacin and its metabolite, ciprofloxacin are found in shrimps and fish. Chinolones are stable molecules and most of them show intensive fluorescence, and hence, LC detection is often employed. LC-MS-MS (positive ESI) is preferred for confirmation.

4. β lactams

The β-lactams include both the pencillins and the cephalosporins

5. Penicillin

Penicillin is the first discovered antibiotic produced by the mould, *Penicilliumnotatum* and *P. chrysogenum*. It is active against gram positive and negative bacteria. Their long use had led to extensive resistance. A number of pathogens produce β-lactamase that inhibits the action of penicillin. A number of semi-synthetic penicillin derivatives are now available. They are amino pencillins (amoxicillin, ampicillin) and more moderncephalosporins that are not affected by β-lactamase. These drugs are reserved for human treatment. They are very labile molecules and are easily degraded by enzymatic or chemical hydrolysis. Microbial assay are available for detection. Extraction with polar solvents and deproteinization by addition of acetonitrile or tungstate are required prior to detection following derivatization by LC or LC-MS-MS.

6. Nitrofurans

Nitrofurans are purely synthetic class of antibiotics that show a wide spectrum of activities against many microorganisms. Their use is banned or strongly limited due to their possible carcinogenic and mutagenic potential. Furanace (nifurpirinol) designed as a chemotherapeutic for fish is now not permitted for use in fish. Nitrofurans fed to animal are quickly metabolized and escape analytical detection, if parent drug is searched. Only the discovery of metabolites that are covalently bound to tissue proteins permitted their detection. The detectable metabolites are considered as "markers". They do not have the nitrofuran structure typical to the parent drug. When the food is treated with hypochlorite, nitrofurazone produces semicarbazide, the marker molecule. Semicarbazide is also a degradation product of azodicarbonamide, used as flour improving agent and as blowing agents in the gaskets of certain food jars. Semicarbazide is also reported to be an endogenous compound in Finnish crayfish. Samples are hydrolyzed, derivatized by use of HCl and nitrobenzaldehyde, processed with SPE and analysed by LC-MS-MS detection at 1µg/kg level. Quantification is preferably done by using isotope labelled standards available for four different nitrofurans.

7. Chloramphenicol

Chloramphenicol is a very effective antibiotic for human and animal treatments. They have very serious, life threatening side effects. This occurs seldom, so there is apparently no safe concentration of the drug. This has led to the ban of this drug for human and veterinary use. Florfenicol, a derivative of chloramphenicol was developed and used in aquaculture. Chloramphenicol is a small polar molecule and has low minimum required performance limits (MRPL) of 0.3 µg/kg. Commercial ELISA tests are widely used for screening. Confirmation was done after derivatization by GC-MS. LC-MS-MS (negative ESI) had replaced GC-MS since it does not require derivatization and produce excellent sensitivities.

8. Macrolides and Lincosamides

They are group of semi-synthetic antibiotics that are active against gram positive bacteria. They are used against microorganisms that have developed resistance against penicillins. Macrolides are often derivatized and detected by LC-FD. LC-MS-MS gives best results in terms of selectivity and sensitivity.

9. Aminoglycosides

This group of antibiotics includes bactericidal compounds produced by *Streptomyces* and *Micromonospora* spp. Apramycin, bambermycin A, B1, B2 and C; dihydrostreptomycin, kanamycin A, B and C, neomycin A, B and C, and tobramycin are derived from *Streptomyces*spp, in which the name ends in the suffix, 'mycin'. Gentamicin C1, C1a and C2 are

derived from *Micromonospora*spp, in which the antibiotic name ends in the suffix 'micin'. They are all composed on one or more amino sugar units in the form of a glycosamine and/or a disaccharide containing D-ribose, which are connected in glycosidic linkage to a central a glycon moiety.

They are polar compounds used against aerobic gram negative bacteria. They show narrow therapeutic range due to their toxicity against the animal. Amikacin and gentamycinare used for ornamental fish and aquaculture. Aminoglycosides require low pH (harsh) extraction. Cation exchange SPE is used for clean-up and their detection is by LC-FD after derivatization. LC-MS-MS permits detection of more than one or two aminoglycosides.

10. Nitroimidazoles

Nitroimidazoles such as nitrofurans are banned drugs, which are suspected to be carcinogens and mutagens. These drugs viz. metroimidazole and ipronidazole are still used in aquaculture for prophylactic and therapeutic treatment of diseases. Nitroimidazoles undergo extensive metabolism and detected as hydroxylated metabolites. LC-MS-MS permits the quantification and conformation of nitroimidazole residues.

II. Antimycotica

Fungal and protozoal infections of fish can be treated by triphenylmethane dyes. *E.g.* crystal violet and malachite green. These compounds are often used in aquaculture and not registered in most countries.

a. Malachite green

Malachite green residues are very persistent and detected months after their application. Malachite green is generally detected in their leuco form. LC-UV detection utilizes chromphoric changes when oxidizing leuco malachite green to malachite green. Low detection limits are best achieved by LC-MS-MS.

b. Crystal violet

Crystal violet (CV), also referred as gentian violet, is a compound of the triphenyl methane dyes group. They are effective in the treatment of fungal and protozoa infections. It is easily absorbed into aquatic animal tissue from water exposure and metabolized to the reduced leuco forms - leucocrystal violet (LCV). The leuco form has a longer half life time in aquatic tissue than its parent compound. Crystal violet may be carcinogenic and also promote in vivo mutageneses in humans. In many countries, crystal violet is not approved for use in aquaculture. Originally, crystal violet and leucocrystal violet did not have maximum residue limits (MRL) nor minimum required performance limits (MRPL) as defined in Commission Decision 2002/657/EC. The EU has established the new criterion which does not allow shrimp and fish to

contain more than 0.5 ppb crystal violet. But the new regulation has blocked over 50% of Bangladesh shrimp export to EU which used the old maximum residue limit of crystal violet at the level of 2.0 ppb. Methodology for the determination of CV and LCV has been limited. The lack of a chromophore group in the metabolized form causes difficulty to analyze these compounds. The oxidization of LCV back into CV is the way to overcome the problem. The convertibility reaction is taken before measuring the results as total CV by using HPLC/UV or fluorescence detector or LC-MS/MS. To confirm the results at ppb level, LC-MS/MS is the best technique to be utilized according to Commission Decision 2002/657 EC.

III. Tranquilizers

Tranquilizers are used in aquaculture to sedate fishes and reduce mortality during transport and handling. The use ofbenzacaine and tricaine are reported in aquaculture. The residue target organ for tranquilizers is the kidney, but this organ is not eaten. In muscle tissue, degradation occurs within hours. Detection is only possible, if the drug is applied directly prior to catching or freezing.

IV. Antiparasitica

Avermectins act against parasites like sea lice. Members of this group, emamectin and ivermectin were used to treat salmon and trout against such parasites. The marker residue for emamectin benzoate is "emamectin B_1a" and it is detected by LC-FD.

V. Antihelmintic drugs

Benzimidazolese.galbendazole are active against a broad spectrum of intestinal helminths. These drugs are developed to treat mammalians, however activity against fish pathogens. Albendazole is extensively metabolized in fish forming albendazole sulfoxide, albendazole sulfone and albendazole 2 aminosulfone. LC-FC is used for the determination of albendazoles.

VI. Hormones

Hormones are used in aquaculture for sex reversal of newly hatched fry. Tilapia males are known to grow faster and larger than femalee.g. methyl testosterone. Since, such treatment is applied on juvenile fishes, residues are not likely to detect when it comes to market. In some cases, hormones are used as growth promoters. Analysis of hormones is mainly by GC-MS. Now, LC-MS-MS with APCI interfaces is reported.

Conclusion:

Veterinary drugs are an essential component of aquatic animal and modern food production, but their residues can persist in animal-derived foods and present potential food safety risks. To minimize these risks, national authorities establish strict controls for the

authorization, labelling, and use of veterinary drugs in food-producing animals, and they conduct surveillance programs to detect unsafe drug residues in animal-derived foods.

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BIOCONTROL EFFICACY OF SILVER NANOPARTICLES SYNTHESIZED FROM FRUIT EPICARP OF *GLYCOSMIS PENTAPHYLLA* AGAINST SOME CROP PATHOGENS

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Abstract:

The aim of the study is to Characterize and assessment of antimicrobial effect of silver nanoparticles (AgNPs) synthesized from fruit epicarp of *Glycosmis pentaphylla* against some crop and human pathogens. The present study first time confirms the ability of fruit epicarp extract of *Glycosmis pentaphylla* for the biosynthesis of silver nanoparticles (AgNPs) grown under in vitro condition. The green synthesis of AgNPs from EtOH extracts were performed through standard protocols. The synthesized AgNPs were confirmed by colour changes (green → brown) within <10 minutes and characterized by UV-visible spectral, SEM and TGA analysis. Antimicrobial activity of the silver nanoparticles was performed by well diffusion method against five crop pathogenic fungus and four human pathogenic bacteria. The highest antifungal activity of silver nanoparticles was found against *Colletotrichum lindemuthianum* and *Alternaria alternata.* The antibacterial activity was measured through zone of inhibition against *B. subtilis* (18 mm), *S. typhimurium* (17.33 mm), *S. mutants* (17 mm) and *E. coli* (17 mm). The AgNPs performed the antimicrobial potentiality against five crop pathogens and four human pathogens by means of minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC). In addition, it displayed the significant synergistic antibacterial effect when AgNPs were combined with streptomycin and Ciprofloxacin at the ratio of 1:1. This eco-friendly, biocompatible and sustainable phytofabrication approach of bioactive AgNP synthesis is a progressive step towards various applications to control the crop and human pathogens in near future.

Keywords: *Glycosmis pentaphylla*, fruit epicarp, silver nanoparticles, antimicrobial activity, synergistic

Introduction:

The present status of worldwide agricultural production has reduced over the past few years due to crop pathogens. The harmful effects on fruits and vegetables of these pathogens are

decreasing the quality of the crops along with economical loss in globally. The consumption rate of fruits and vegetables were increased up to 40% during the past few years. In every year, the amount of lost is approximately 20% of all fruits and vegetables (Droby S., 2006). The fresh fruits and vegetables exposed to contamination by microorganisms, especially plant fungi due to direct contact with soil, dust, water during harvesting or post harvesting (Eni *et al.,* , 2010). Now a day's the traditional antibacterial treatment against growing resistance of human pathogenic bacterial strains become a big issue (Kaweeteerawat *et al.,* , 2017). The microbial infection is a serious problem in agriculture and healthcare sector in worldwide. Therefore, it is needed to develop new antimicrobial agents including different characteristics, such as eco-friendly, low toxicity, antimicrobial potency, and great compatibility. In this situation, nanoparticles (NPs) have accepted as alternative to chemical pesticides worldwide, due to their electrostatic attraction between positively charged NPs and negatively charged microbial cells, and a large surface to volume ratio, resulting in improved physicochemical properties and enhanced antimicrobial activities of the NPs (Jogaiah *et al.,* , 2019; Kavyashree *et al.,* , 2015). The antibacterial and antifungal proparties of NPs have recently been widely reported (Mallmann *et al.,* , 2015, Salem *et al.,* , 2015, Elgorban *et al.,* , 2016).

The application of metal composite specially at nano scale to control the drug resistance bacterial infection (Mei *et al.,* , 2014; Kumar *et al.,* , 2015; Pavoski *et al.,* , 2019). The increased the potentiality of antibiotics could increase when applied antibiotics and metal NPs in combined condition (Wang *et al.,* , 2017). Hence, NP-antibiotic conjugate could lower the amount of both dosages, which reduces noxiousness and increases antimicrobial properties. The various research areas like drug discovery, biomedical sciences, cosmetics, luminescence, and renewable energy technologies focus on novel properties of NPs which make the extremely versatile (Rauwel *et al.,* , 2015). Among the different types of metallic nanoparticles, silver nanoparticles are very attracting and interesting for several applications because of their unique and remarkable properties, enhanced permeability, retention effect and antimicrobial activity (Mariana *et al.,* , 2019, Gupta *et al.,* , 2017; Loo *et al.,* , 2018). Therefore, the market value of AgNP increased day by day due to these properties which enable the promotion of AgNP applications as an antimicrobial agent in different arrays of products, such as soaps, plastics, food and textiles (Franci *et al.,* , 2015). The binding and absorption rate of drug on patient cells increased due to existence of protein caps on nanoparticles which both stabilization and binding to bacterial cell surface. The mode of action of AgNPs showed that it decreased the rate of bacterial cell permeability, cellular respiration, DNA and protein function inside the cell (Ragaa *et al.,* , 2019). Many researchers focus on numerous evidences of synthesis of different

AgNPs and their antimicrobial activity but MIC, MBC and Synergistic effect against human pathogenic bacteria are rare.

The modern age of nanotechnology, there is going on competition to identify "green" pathways to synthesize metallic nanoparticles using biological resources mainly the plant resources. There are still several scopes for improvement in green pathway for a single step rapid synthesis of AgNPs at room temperature by different modifications. In earlier report Ag-Np were synthesized by the reduction of aqueous Ag^+ ions using unexploited weed resources (*Ipomia, Enhydra,* and *Ludwigia*) and sunflower, *H. annus* (Roy and Barik, 2010; Roy and chowdhury., 2015). There is still several scope for improvement in bio-based methods for single step rapid synthesis of metallic Nps at room temperature by modulating their size.

The fruit epicarp of *Glycosmis pentaphylla* has many medicinal and reducing property which have been used for synthesized Ag-NPs by the reduction of $AgNO_3$ by using aqueous extract of the fruit epicarp extract at room temperature with in 72hr. However, the synthesis of AgNPs using such plant constituents has not yet been fully studied along with their antimicrobial activity.The nanoparticles synthesized from various plant parts used to control different plant and animal disease causing microorganisms. There are numerous evidences of synthesis of different AgNPs but the fruit epicarp antifungal activity of AgNPs with detail study (MIC & MFC) against human pathogenic bacteria is rare.

In the present work we have analyzed the antifungal activity against some crop pathogens, antibacterial activity against different gram-positive bacteria and gram-negative bacteria with MIC and MBC study. Individual and synergistic antibacterial activities of AgNPs with two conventional antibiotics (Streptomycin and Ciprofloxacin) were investigated to evaluate their biomedical applications in minimizing antibiotic dose over exploitation.

Materials and Methods:

1. Experimental microorganisms

The referred strains of fungus and bacteria obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Reference fungal strains included *Colletotrichum gloeosporiodes, Colletotrichum lidemuthianum, Fusaruim moniliforme, Fusarium oxysporum, and Alternaria alternata.* The bacterial strains included two Gram-positive (*Bacillus subtilis*, *Streptococcus mutans)* and two Gram negative bacteria (*E. coli* and *Salmonella typhimurium).*

2. Plant sample collection and extract preparation:

The ripening fruits of *Glycosmis pentaphylla* were collected from college ground of Sreegopal Banerjee College, Bagati, mogra, Hooghly. The fleshy epicarp separated from fruit

and dry in hot air woven at 40°C. The dry fruits epicarps were crushed in dust form and extracted in different solvents to analysis its phytochemicals. The fruit epicarp extract was prepared by taking 50 g of dry dust fruit epicarps in a 500 ml conical flask with 100 ml of 30% ethanol (EtOH) for 24h at 30°C room temperature. The crude extracts were filtered through Whatman`s No.1 filter paper and stored at 4°C for the synthesis of AgNPs.

3. Phytochemical analysis of the crude extract

The fruit epicarp extract were dipped in different solvents for extraction of different secondary chemicals. The biochemical analysis were done for various chemicals estimated, such as total phenolics (Bray and Thorpe., 1953), flavonoids (Zhishen *et al.*, , 1999), tannin (Trease and Evans., 1983), saponins (Trease and Evans., 1983) and alkaloids (Harborne., 1973), phytate (Reddy and Love., 1999) and oxalate (Day and Undrwood., 1986). Determination of each biochemical analysis was repeated for three times and expressed in percent dry weight basis.

4. Synthesis of Silver nanoparticles (AgNPs)

The aqueous solution of 1 mM silver nitrate ($AgNO_3$) [analytical grade (AR), purchased from E. Mark (India)] was prepared and used for the synthesis of AgNPs. The fruit epicarp extract (5 ml) was added into 50 ml of aqueous solution of 1 mM $AgNO_3$ for reduction of Ag+ to Ag^0. The reaction mixture was incubated (15 minutes) at room temperature till the turn up of green to brown colour. The particles were isolated by centrifugation (6000 rpm up to 15 minutes), repeated washing and drying at 75°C for further characterization.

5. Characterization of synthesized AgNPs using UV-visible spectrophotometer

The reduction of Ag^+ to Ag^0 was monitored by measuring the UV-Vis spectrum of each reaction mixture at different time intervals (10, 20, 30, 40, 50, 60, 120, 180, 240, 300 minutes) within the range of 370-500 nm in the UV-Vis spectrophotometer (Shimadzu UV-VIS Spectrophotometer, Japan) because the absorption spectrum of aqueous $AgNO_3$ and green synthesized AgNPs solution exhibited λ_{max} at about 220 nm and 430 nm, respectively.

6. Characterization of synthesized AgNPs using Scanning electron microscopy

Morphological characterization of AgNPs was done by Scanning electron microscopy. For SEM analysis, the EtOH used as a blank reference. The isolated dried and powdered AgNPs were used for SEM study. A thin film of each sample was prepared separately on a small glass cover slip (3x3 mm), and set on a copper stab for electron microscopy using Hitachi made Scanning Electron Microscope (SEM) (Model: S530 with IB2 ion cotter, Japan).

7. Characterization of synthesized AgNPs using Thermal gravimetric analysis

TGA analysis of the synthesized AgNPs was also observed with increasing temperature range of 160˚–550˚C as in Roy and Barik, 2010.

8. *In vitro* antifungal activity of green synthesized AgNPs

The antifungal activity of bio–synthesized AgNPs was tested against various crop pathogens by the standard agar well diffusion method. To examine the antifungal activity of biosynthesized AgNPs, Potato Dextrose agar plates were sterilized and allowed to solidify. After solidification, 30 µl of each fungal spore's suspension containing 1×10^6 CFU/ml was inoculated on the Petri plates by a sterile glass rod and 8 mm cup was cut with the help of sterile cork borer in each inoculated plates. The well filled with 10µl antifungal AgNPs solution and incubated at 28°C for 5 days. Controls of silver-free plates were incubated under the same conditions.

9. Determination of Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC)

The minimum inhibitory concentration (MIC) of the test AgNPs samples was done on crop pathogenic fungal strains. The stock AgNPs solution was serially diluted using sterile 30% ethanol to obtained 50 µg/ml, 25 µg/ ml, 16.66 µg/ml, 12.5 µg/ml,10 µg/ml, 8.33 µg/ml, 7.14 µg/ml, 6.2 µg/ml, 5.55 µg/ml, 5.26 µg/ml concentration and 30% ethanol was serve as control. The spore suspension of test fungus was prepared by scrapping the spores from 7-day-old PDA slant culture. 10µl spore suspension was picked up from slant through micropipette, checks the CFU and poured into each fresh Potato dextrose agar plates. 10 µl of the test samples from each concentration were loaded into the 5 mm diameter well in five test fungus plates and incubated at 28°C for 48 hrs.

The MIC end-point criterion was defined as the lowest AgNPs concentration showing no visible growth after 48-hrs incubation. MIC values were calculated by comparing the germination of spores in PDA plates containing different concentration of AgNPs. The lowest concentration considered as MIC that resulted in inhibition of spores germination compared to the germination in control well.

The MFC means the lowest compound concentration at which no visible growth was observed. Evaluation sets for MFC were prepared as same as MIC experimental sets. To determined MFC values of AgNPs used higher concentration value than MIC value.

10. *Invitro* antibacterial activity of green synthesized AgNPs

10.1. Antibacterial activity by the Agar-well Diffusion Method

Assessment of antibacterial activity of AgNPs sample against two Gram-positive bacteria (*Bacillus subtilis*, *Streptococcus mutans*) and two Gram negative bacteria (*E. coli* and *Salmonella typhimurium*) was measured by the agar well diffusion method. 5 mm wells were cut in each fresh inoculated bacterial plates and 10 µl of different concentration of the test sample was loaded into the 5 mm diameter well seeded with test bacteria and incubated for 24 h. at 37 °C.

The potency was compared by measuring zone diameter of growth inhibition with standard antibiotics, Streptomycin (10 µg/ml).

10.2. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) of the test samples was done following by the standard protocol of on sensitive bacterial strains. Different concentrations of the AgNPs sample (50µg/ml, 25µg/ml, 16.66µg/ml, 12.5µg/ml, 10µg/ml, 8.33µg/ml, 7.14µg/ml, 6.2µg/ml, 5.55µg/ml, 5µg/ml) was prepared by diluting the stock solution using sterile double distilled water. 10 µl of the test sample of different concentrations was loaded into the well of pre inoculated nutrient agar plate of target bacteria, incubated for 24 h at 37 °C and observed for zone of growth inhibition. The MBC was determined by checking the viability of the bacterial cells after treating with 2 × concentration of MIC of AgNPs and dilution plating on Nutrient agar plate. In brief, the actively grown bacterial strains (log phase growth) were treated with test samples (different AgNPs samples) at higher concentration of MIC and incubated for 1 h. The treated culture was then plated on nutrient agar plate at a dilution of 10−2 to 10−4 in triplicates and incubated in similar condition for observing for any viable colony formation. MBC is noted as the concentration where no viable cells were noticed.

11. Synergistic Activity with Antibiotics

To performed this experiment, 6 mm diameter sterile Whatman No.-1 filter paper discs were soaked with the AgNPs sample at MIC values (5.5 µg/ ml) and filter sterilized antibiotic solutions at MIC values (i.e. Streptomycin, 0.5 µg/ml and Ciprofloxacin, 0.5 µg/ ml) and placed at the centre of the each culture plate seeded with target bacterium and incubated at 37 °C for 24 h and were observed growth inhibition. The synergistic potential was determined by comparing the magnitude of antibacterial activity of AgNPs and antibiotics with the antibiotics alone using the following formula: FI-Fold Increase (FI) = [(b − a)/a] × 100; where, ‘b’ stands for ‘inhibition zone diameter (mm) for antibiotics + AgNPs’; ‘a’ stands for ‘inhibition zone diameter (mm) for antibiotics alone’.

12. Statistical analysis:

All the data of phytochemical regime and antimicrobial activity were analyzed using one way ANOVA, Tukey HSD and Pearson correlation (Zar, 1999). All the statistical analysis was performed using the statistical program SPSS v. 13.0 (SPSS, 2004).

Results and Discussion:

1. Phytochemical regime:

The biochemical analyses of fruit epicarp extract represents variation in secondary (Phenols, flavonoids, tannin, saponins, alkaloids, phytate and oxalate) chemicals which is very much similar with the previous report of Roy and Barik, 2010. The phytochemical regimes of the plant is presented in figure 1. All secondary chemicals were higher than other plant parts.

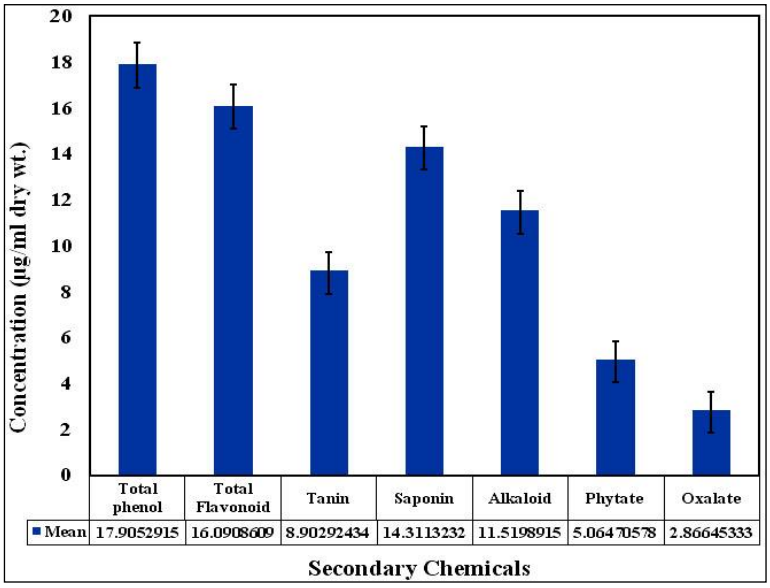

Figure 1: Phytochemical variations of *Glycosmis pentaphylla* Fruit Epicarp extract (Mean ±5 obsevations)

2. UV-VIS Spectroscopy characterstics of AgNPs:

During green synthesis of AgNPs through the fruit epicarp extract changes in colour from green to brown was observed like previous researchers (figure 2). The brown colour due to the reduction of Ag^+ confirms the formation of AgNPs and was characterized by UV-Vis Spectroscopy as in Roy and Barik, 2010. The reduction of pure silver ions (Ag^+) was estimated by UV-Vis spectral analysis in frequency range of 270 to 500 nm at room temperature and which was represented the peak at around 420-430 nm for long time interval (10-300 minutes) specific for the synthesis of AgNPs with longer stability (figure 3). The band at 420-430 nm can be attributed to the property surface plasmon resonance (SPR) due to oscillation of electrons (Mie scattering) for strong interaction of light with the AgNPs. In both cases EtOH act as blank. The λ_{max} of AgNPs was observed at around 430 nm whereas in EtOH extract it was at around 380 nm, respectively within the time span of 10-300 minutes. The UV-visible spectra showed absorption bands in 350 to 550 nm region which confirms the formation of AgNPs (Sastry *et al.*, 1997; Sastry *et al.*, 1998).

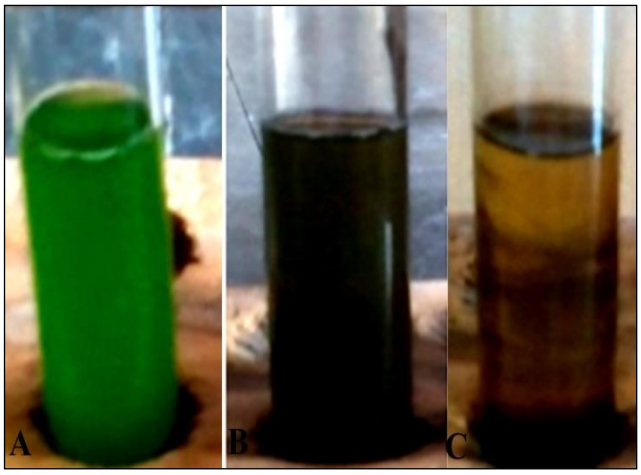

Figure 2: The colour change from green (A) to brown (B and C) during the reaction of Ag^{+} into AgNPs due to the phytochemicals present in fruit epicarp extract of *Glycosmis pentaphylla*

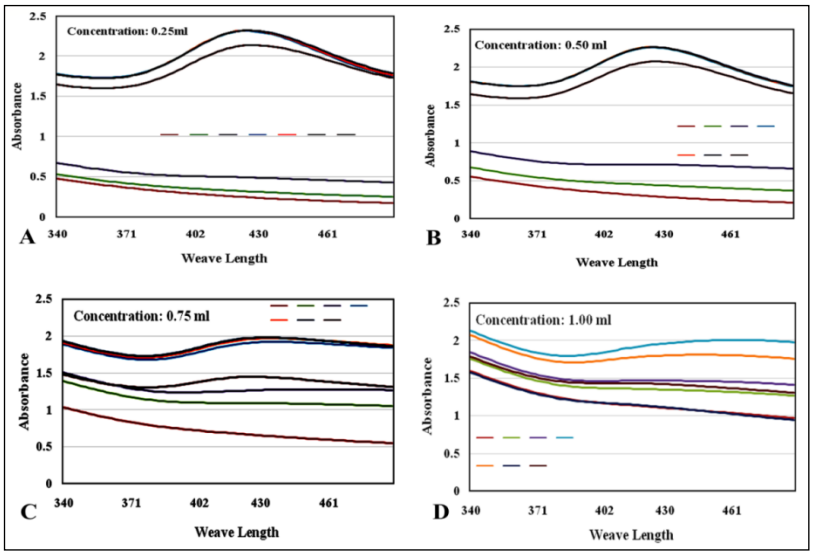

Figure 3:- UV-Vis absorption spectra recorded at different time intervals (10 min, 30 min, 1h, 24h, 48h, 72h) of AgNPs synthesized from fruit epicarp extract *Glycosmis pentaphylla* (Mean of 3 observations)

3. SEM and TGA characterstics of AgNPs

Microscopic surface features including morphology and particle size of synthesized AgNPs was assessed by SEM analysis. The SEM image provided roughly spherical topography of AgNPs was about 75 ± 5 nm in size (figure 3). SEM image also confirms that the synthesized nanoparticles are well separated with no aggregation (Fig. 4 a and b).TGA data of the synthesized AgNPs showed steady weight loss due to desorption of its bioorganic compounds with increasing temperature range of 160˚–550˚C as in Roy and Barik, 2010.

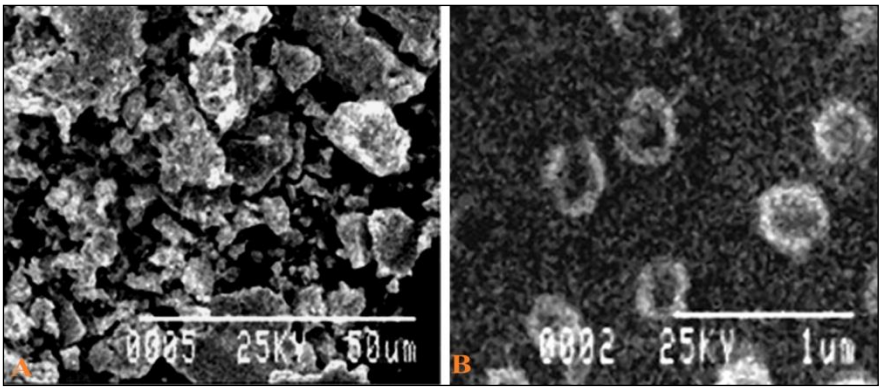

Figure 4: The SEM images of AgNPs synthesized from fruit epicarp extract *Glycosmis pentaphylla* at 25.0 kV × 1 k

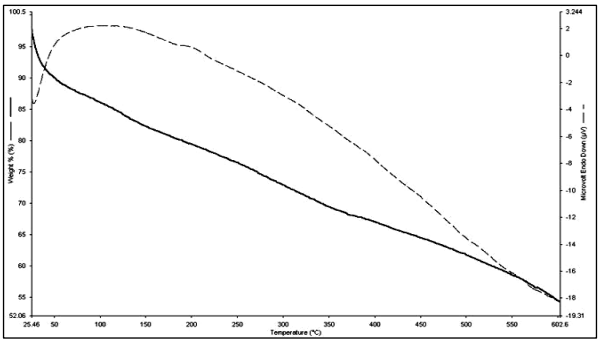

Figure 5: TGA of the synthesized AgNPs showed steady weight loss within the temperature range of 160˚–550˚C

4. *In vitro* antifungal potentiality of of AgNPs

The antimicrobial activity of AgNPs against various crop pathogenic fungi were investigated as shown in Figure 6. The biothensized AgNPs inhibited the growth of *Fusarium oxysporum, Fusarium moliniliforme Alternaria alternata, Colletitrichum lindemuthianum* and *Colletotrichum gleosporiodes*. Thus, AgNPs could be considered as excellent broad-spectrum antifungal agents for sustainable crop production and also could potentially be used widely in clinical applications against human pathogenic fungi.

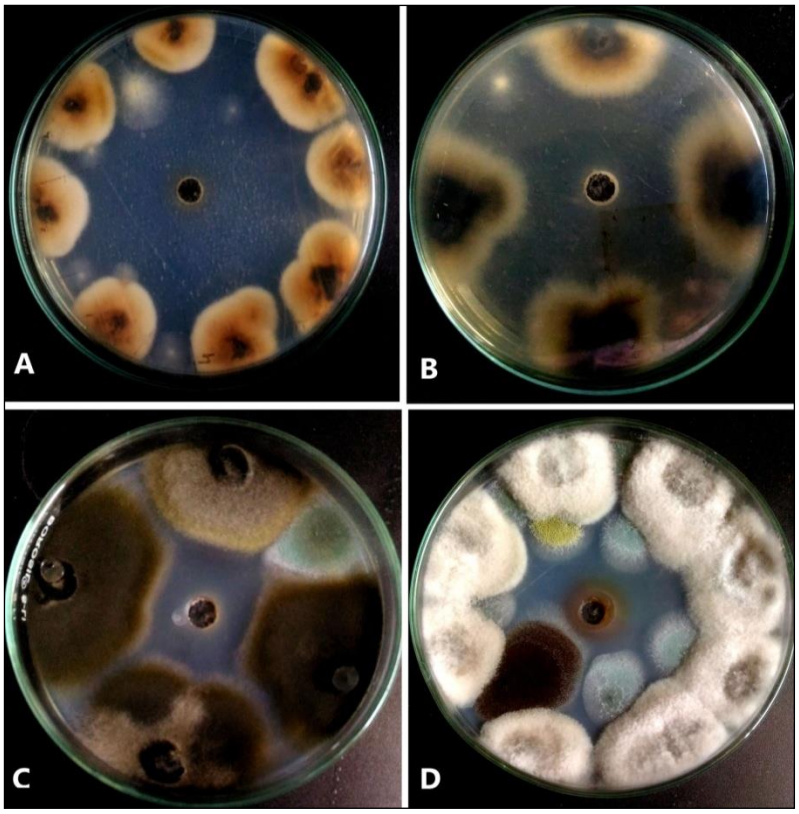

Figure 6: Antifungal activity of AgNPs in PDA media by agar well diffusion method; (A) *Colletitrichum lindemuthianum;* (B) *Alternaria alternata;* *(C) Colletotrichum gleosporiodes; (D)Fusarium moliniliforme*

5. Determination of MIC and MFC of AgNPs

The minimum inhibitory concentration and minimum fungicidal concentration of AgNPs for 5 different fungal strains as shown in Table – 1. The results suggest that the plant synthesized AgNPs are capable of inhibiting crop fungi like *Fusarium oxysporum, Fusarium moliniliforme Alternaria alternata, Colletitrichum lindemuthianum* and *Colletotrichum gleosporiodes.* The highest MIC values shown in *Alternaria alternata, Fusarium oxysporum, Fusarium moliniliforme* which were 6.2 µg/ml and the highest MFC values were 7.14 µg/ml.

According to Zubaidi *et al.*, , 2019, biosynthesized silver nanoparticles had antimicrobial effects against *A. flavus, F.oxysporum,* and *P. digitatum* on PDA in vitro. Inhibition (97.3%) was obtained against *A. flavous* treated with a 10 µg/ml concentration of silver nanoparticles and minimal level of inhibition was found against *P. digitatum* and *F. oxysporium* with 2 µg/ml concentrations of AgNPs. This could be possible the adhere AgNPs to fungal hyphae and deactivating plant pathogenic fungi. DNA loses its ability to replicate upon treatment with Ag+ resulting in inactivated expression of ribosomal subunit proteins, as well as certain other cellular

proteins and enzymes essential to ATP production (Feng QL *et al.,* , 2000; Yamanaka M *et al.,* , 2005).

Table 1: MIC and MFC of AgNPs sample at different concentrations against the five fungal crop pathogens

Name of the crop pathogens	**Concentration of AgNPs (µg/ml)**									
	50 µg/ml	**25 µg/ml**	**16.66 µg/ml**	**12.5 µg/ml**	**10 µg/ml**	**8.33 µg/ml**	**7.14 µg/ml**	**6.2 µg/ml**	**5.55 µg/ml**	**5.26 µg/ml**
Alternaria alternata	–	–	–	–	–	–	**MBC**	**MIC**	–	–
Colletotrichum gloeosporioides	–	–	–	–	–	–	–	**MBC**	**MIC**	–
Colletotrichum lindemuthianum	–	–	–	–	–	–	–	**MBC**	**MIC**	–
Fusarium moniliforme	–	–	–	–	–	–	**MBC**	**MIC**	–	–
Fusarium oxysporum	–	–	–	–	–	–	**MBC**	**MIC**	–	–

6. Antimicrobial activity of AgNPs Sample

The application of AgNPs against human pathogenic bacteria showed the significant growth as shown in Figure 7. The antibacterial activity was measured through zone of inhibition against *B. subtilis* (18 mm), *S. typhimurium* (17.33 mm), *S. mutants* (17 mm) and *E. coli* (17 mm) which was shown in figure 8. It was observed that the AgNPs was more potent antibacterial compound than other. The comparison of single application of AgNPs, Strptomycin, Gentamycin and Cifrofloxcin against the Gram-positive and Gram-negative bacterial strains which was shown in figure-9. The results showed that antibiotics and AgNPs have more or less parallel potency by means of formation of inhibition zone (mm) by the application of same volume (10µl) and same concentration (6µg/ml).

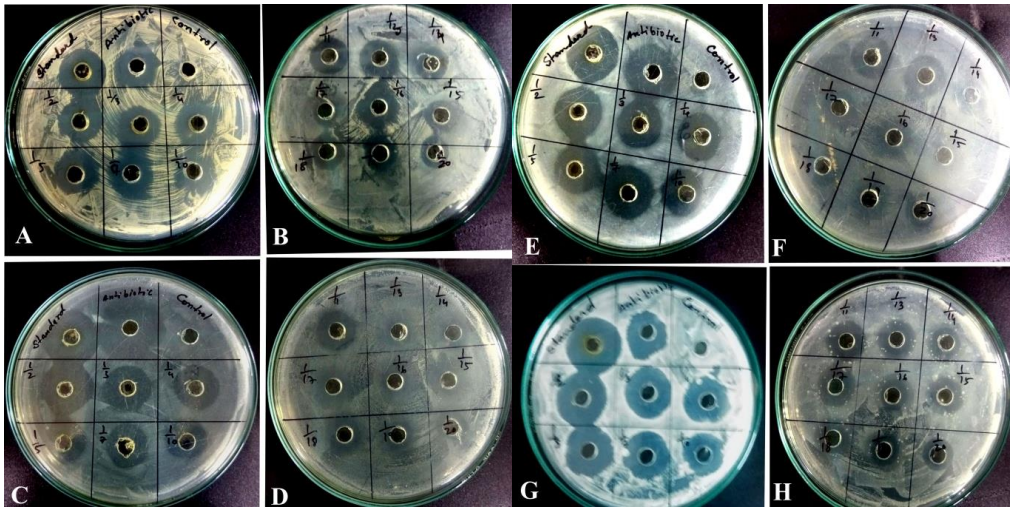

Figure 7: Agar well diffusion assay of the AgNPs. (A-B) Against *B. subtilis*; (C-D) against *S. mutans*; (E-F) against *E. coli*; (G-H) against *S. typhimurium*

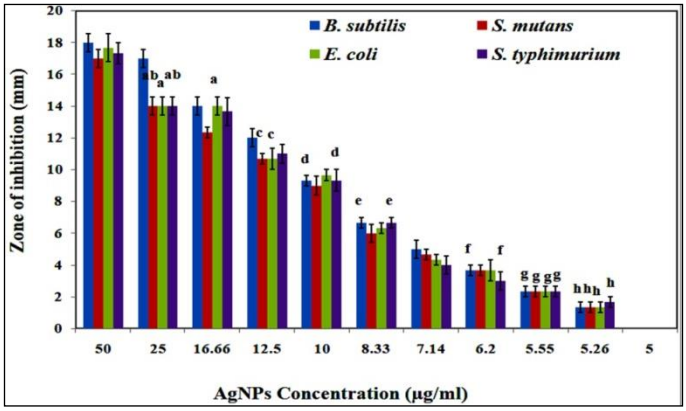

Figure-8: Antibacterial assessment of the AgNPs. Here, the data were the average diameter of inhibition zone of triplicate trials. The data are displayed as mean ± standard error. Bar with the same letters indicate no significant differences according to Tukey (HSD) test (P <0.05)

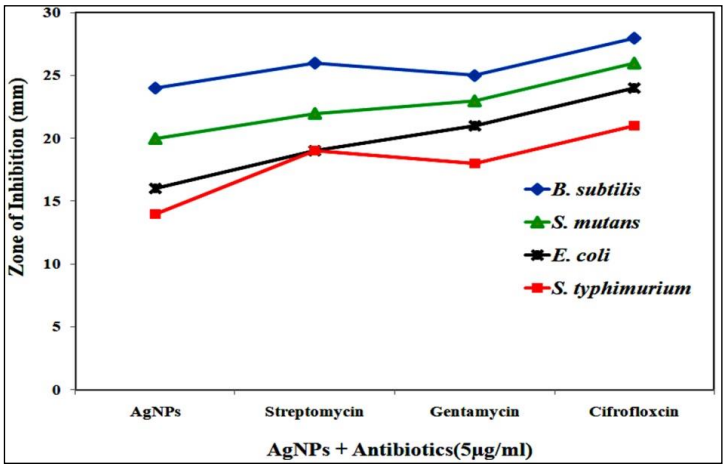

Figure 8: Potency of antimicrobial activity of AgNPs, Strptomycin, Gentamycin and Cifrofloxcin against the Gram-positive and Gram-negative bacterial strains

8.1 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The results of Table 3 showed that the MIC values varied from 6~5 µg/ml. The MIC values for *B subtilis* and *E. coli* was 5 µg/ml and 5.26 µg/ml MIC values for *S. mutants* and *S. typhimurium* respectively. Similarly the MBC value for *B subtilis* and *E. coli* was 5.26 µg/ml and for *S. mutants* and *S. typhimurium* it was 5.55 µg/ml.

From the above observations, it is very much clear that AgNPs has the highest antibacterial effect which causes highest interaction with bacterial cell wall. Similar observation on antibacterial activity was also observed by the previous studies (L. Cui *et al.*, , 2013). We found no particular trend of antibacterial effect for four pathogenic bacteria. This clearly indicates that the antibacterial effect not only manifested by the penetration of AgNP in the bacterial cell wall because if it was the only reason then it was always produced greater effect for gram negative bacteria than gram positive one as gram positive bacteria has associated with thicker peptidoglycan layer (M. Griffith *et al.*, 2015). Phytofabricated AgNPs with antimicrobial properties have also been investigated against different microbes which actually depend on size, shape, environmental conditions (pH, ionic strength) and capping agent (Kim *et al.*, , 2012;). Recently, efficient antimicrobial activity of green AgNPs was observed against multi drug resistant (MDR) and highly pathogenic bacteria (*P. aeruginosa*, *S. aureus*, *S. typhi*, *S. epidermidis* and *E. coli*) (Singh *et al.*, 2014;) and fungai (*C. gloeosporioides*) (Chowdappa *et al.*, 2014).

Our study shows that *S. mutans* (gram positive) and *S. typhimurium* (gram negative) were most sensitive which indicate mode of action was not only affected by cell wall thickness of the bacteria. Ag+ release from AgNP is the another reason for antibacterial activity. As the smaller AgNPs has the higher surface area associated with faster release of Ag+ (G.A. Sotiriou *et al.*, , 2010) and exerts higher toxicity. High affinity of Ag+ towards protein thiol groups of respiratory enzymes inactivated enzymes even died out (S. Liau *et al.*, , 1997). AgNPs also plays important role in biocompatibility as it controls the interaction of AgNP with living organism.

Table 2: MIC and MBC of AgNPs sample at different concentrations against the gram-positive and gram-negative bacterial strains

Sl. No.	Strains	AgNPs (100 µg/ml)										
		50 µg/ml	25 µg/ml	16.66	12.5	10 µg/ml	8.33	7.14	6.25	5.55 µg/ml	5.26 µg/ml	5 µg/ml
Gram-positive	***B. subtilis***	+	+	+	+	+	+	+	+	+	MBC	MIC
	S. mutans	+	+	+	+	+	+	+	+	MBC	MIC	_
Gram-negative	***E. coli***	+	+	+	+	+	+	+	+	+	MBC	MIC
	S. typhimurium	+	+	+	+	+	+	+	+	MBC	MIC	_

Synergistic Effect of AgNPs

The combined effect of antibiotics and AgNPs against different human pathogenic bacteria showed in table 3. The highest inhibition zone was observed in dual (AgNPs + antibiotic) application than single (AgNPs). The combined effect against bacteria increased the diameter of inhibition zone that may be possible due to bonding between antibiotics and AgNPs as the antibiotics generally contain active groups like hydroxyl or amino, which bind AgNPs by chelation (K.I. Batarseh, 2004). The application of Streptomycin with AgNPs showed the highest increasing fold 38.24% against *E. coli* followed by 31.89%, 25%, 20% against *B. subtilis*, *S. mutans* and *S. typhimurium.* Similarly combine application of Ciprofloxacin showed the highest fold increase 30% against *B. subtilis* followed by 24.64%, 15.95% and 5.67 against *S. mutans* and *S. typhimurium*. When bacteria acquire antibacterial resistance, synergistic effect plays an important role as AgNPs and antibiotics kill bacteria in different mechanism (P. Li, 2005). So, our observations clearly indicate that, synthesised AgNPs is able to decrease the concentration of Streptomycin and Cifrofloxcin against *S. mutans* & *S. typhimurium* with lowering the side effects and cost effectiveness of antibiotics.

Table -3: Synergistic effect of two antibiotics with AgNPs sample against pathogens *B. subtilis, S. mutans* and *E. coli, S. typhimurium*; FI-fold increase FI% = [(b − a)/a) × 100]

Sample	Name of the Pathogens	Inhibition zone diameter (mm) for AgNPs	Inhibition zone diameter (mm) Streptomycin (AB			Inhibition zone diameter (mm) Ciprofloxacin (AB		
			Only AB (a)	AB+ AgNPs (b)	FI %	Only AB (a)	AB+ AgNPs (b)	FI %
AgNPs	*B. subtilis*	21.000 ± 0.577	24.000 ± 0.577	30.000^{af} ± 0.577	25	23.333^{g} ± 0.333	30.333^{a} ± 0.333	30
	S. mutans	20.333^{d} ± 0.333	23.000^{be} ± 0.577	30.333^{f} ± 0.333	31.89	23.000^{bg} ± 0.577	28.667^{h}± 0.882	24.64
	E. coli	20.000^{d} ± 0.577	22.667 ± 0.333	31.333 ± 0.667	38.24	23.000^{g} ± 0.577	26.667 ± 0.882	15.95
	S. typhimurium	19.000 ± 0.577	23.333^{e} ± 0.333	28.000^{c} ± 0.577	20	22.667 ± 0.333	28.333^{ch} ± 1.202	5.67

The data are displayed as mean ± standard error according to Tukey (HSD) test (P <0.05

Conclusion:

The biosynthesis of nanoparticles by plant is low cost, safe, environmental friendly and less time consuming, it provides effective satisfactory results without any hazardous chemicals involvement. In the present study, fruit epicarp of *Glycosmis pentaphylla* was exploited to biosynthesize silver nanoparticles by reducing silver nitrate. Different concentrations of extract were employed to synthesize of AgNPs structure. Furthermore, a detailed characterization was part of the physical and chemical analysis of obtained AgNPs product. The appearance of broad optical absorption peak of UV–Visible spectral analysis confirmed the synthesis of AgNPs. The SEM studies confirmed that the concentration of the fruit epicarp extract is highly efficient in controlling the shape and size of AgNPs structures. TGA was detecting the steady weight loss due to desorption of its bioorganic compounds with increasing temperature. In *invitro,* AgNPs exhibited potent antifungal effects against some crop pathogens, likely by lysis the cell wall integrity. Synthesized AgNPs also showed potential antibacterial activity comparable with standard antibiotic Streptomycin. From the comparison data of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

study, it was observed that AgNPs was more effective to kill all the four bacteria even in very low concentration (5.26 µg/ml). AgNPs play a crucial role in enhancing the antibacterial activity of Ciprofloxacin and Streptomycin with 2 to 25-fold increase against bacteria. The applications of AgNPs and antibiotics together improved its efficiency with reducing dose and also its cost. The highest potency of AgNPs with Ciprofloxacin and streptomycin against all human pathogenic bacteria immense the importance biomedical applications against the pathogen of dental, pneumonia and colon. These results not only provide a new approach for integrative control of plant pathogens but also reduce or avoid used of various drugs. With the application point of view, this AgNPs could be used as biofungicide for sustainable agriculture and biomedical using against human pathogenic bacteria in future studies.

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BASELLA RUBRA AND ITS BIOACTIVE COMPONENTS: A RETROSPECTIVE STUDY

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Introduction:

The plants belonging to Centrospermae contain specific pigments: red- violet betacyanins and yellow betaxanthins, which have a common name, betalains. The circumstance of betalains is defined to ten families of this order. The remaining families contain exclusively anthocyanins (Chethan *et al.*, 2004).

In Latin, it's known to be- Basellorubura; In english – Ceylon spinach, Malabar spinach; In sanskrit it's known to be Upodika& its Indianname is poi., It's known to beamunututu in south western Nigeria Nightshade, *Basella rubra* poi and nivithi in Sri Lanka.(Chethan *et al.*, 2004). The leaves of the plant are generally consumed as a green leafy vegetable and are a good source of nutrients and nutraceuticals (Kumar *et al.*, 2016).

The fruit imparts a unique color to products with sugar, acid, and pectin to maintain spreadability or consistency. Fruit spreads are one of the popular ways to utilize whole fruits and fruit juices through the right combinations of fruits, pectin, acid, and sugar (Qiu *et al.*, 2018).

They have several ethnobotanical plays by handling diseases which include gonorrhoea, constipation, leprosy, dysentry, ulcer and burns, intestinal complaint and Sore throat. It's reported to melio rate thus testosterone levels in males. Thus, boosting bido, and decoction of leaves is recommended as Laxative in pregnant women and children (Chethan *et al.*, 2004),

The fruits of the plant are black or dark grandiloquent color and fleshy. The fruit of the plant has been used from a long time back for the treatment of multitudinous conditions like dysentery, diarrhoea, anemia, cancer etc., and has been utilized for different kinds of healing activities. Their pigments are soluble in water, which makes them a potential source of natural dye (Chethan *et al.*, 2004).

Basella rubra has been found to be a good source of calcium, iron, vitamin A. *Basella rubra L.* is an important green leafy vegetable vine and is known for its health benefits in

traditional medicine. Light is a basic physical factor essential to the development and bioactive secondary metabolite production in invitro callus cultures (Chethan *et al.,* 2004).

The plant has explored for it's medicinal properties in ancient Indian and Chinese traditional medicine practice to treat constipation and also as a diuretic and an anti inflammatory material (Chethan *et al.,* 2004).

Bioactive compounds in *Basella rubra*

Bioactive combinations, like as polyphenols, flavonoids, proanthocyanins, anthocyanins, β- carotene, α- carotene, and xanthophylls, like as neoxanthin, violaxanthin, lutein, and zeaxanthin with antioxidant parcels are linked in *Basella rubra (Sravan Kumar et al., 2015)*. Betalains are violet- red, natural food grade colors with health benefits; still, their stability limits its use in artificial food processing (Ajay *et al.,* 2021).

Betalains are nitrogen- containing, water-answerable, manufactory- based, natural colors generally set up in 17 families of the order Caryophyllales. *Beta vulgaris*, *Opuntiaspp*.Hylocereusundatus and Amaranthaceous are reported to be rich sources of betalains. Betanin(E162) is the commercially available betalain element pulled from beetroot. It's massively used as a natural colourant in model food systems like as logjams, ice creams, galettes, yogurts, and sticky delectables. In the current food processing industry, betalains have gained the attention of scientists, and end- users, due to their salutary, nutraceutical eventuality, cell underpinning implicit, and natural exertion. More significant is the exertion of work of betalains to battle against many conditions, for illustration, meanness, aggravation, common inflammation, neurodegenerative, and cardiovascular conditions. An arising trend of the food assiduity for advancing fortified food phrasings is to replace artificial colourants with natural colors that parade health benefits.

Despite all the precious good benefactions, betalains are delicate to external conditions, for illustration, oxygen, temperature, light, pH, water action, and chemicals. A multitudinous reports have shown betalains with bettered stability, as estimated and blazoned in fruits of *Basella rubra*. One enhancement of betalain stability is the medicine of lecithin nanoliposomes (NLs). NLs are encouraging because of their capacity to epitomize hydrophobic orhydrophilic motes, and non – poisonous quality of their endogeniclecithin(phosphatidylmcholine) composition. The NLs, set with a thin- film hydration system, are a extensively employed transport strategy for antimicrobial patches, nutrients, phenolics, and marketable betanin (Hiromu *et al.,* 1991).

Basella rubra species contain mixes with antiviral parcels. Colors attained from the pericarp of *Basella rubra* species were used for colouring jellies galettes and sweets.The use of the juice in ancient China as a ornamental color(cream) and essay for sealing. The interest in natural colors has increased in recent times, since it has been set up that numerous of the synthetic products used in the food assiduity have Dangerous goods.

Red beet *(Beta vulgaris)* is, in fact, the only cultivated factory comprising large amounts of betacyanins used to shade food (delectables, potables, protein backups).

In view of their rich bioactive profiles, they play an important role in the maintenance of the redox potential of cell functions (Krishnaiah *et al.*, 2011).

Flavour components in *Basella rubra*

The phytochemical and DPPH contents of the *Basella rubra* forms varied. All forms had goodscavenging ability on free radicals; the highest was in the *B. alba* round leaf form. The DPPHrepresents scavenging ability of leaf extracts on free radicals in the body. This may have resultedfrom higher concentration of polyphenols and vitamins A and C. *Basellaalba*, round leaf form had the highest vitamin C and A contents. All forms of *Basella rubra* possess phytochemicals in varied proportion. The antioxidantsalpha-tocopherols, ascorbic acid, carotenoids, amino acids, peptides, proteins, flavonoids, andothersplay aroleasphysiological and dietary antioxidants augmenting the body's natural resistance to oxidative damage (Hiromu *et al.*, 1991).

Volatile flavor components of Malabar-Nightshade (*Basella rubra L.)* were identified by infrared spectra and chromatography –mass spectrometry. Major components identified from volatile oil were 1-methoxypropane, (Z)-3-hexen-l-ol,3-methoxyphenylacetate, acetophenone, 4-vinyl guaiacol, isophytol and phytol. The major head space components were ethyl acetate, benzene, 3-heptanone, 2-heptene, ethylbenzene, o-xylene, and limonene.

Betalains content in leaves and fruits of *Basella* plant is less than the tubers of beet. Betalains extracted from *B. Vulgaris* and the prickly pear *Opuntiaficus indica* are used in food.Betalains extracted from fruits of *Basella rubra* plant are alsoused in food formulations. It is considered as an alternative of synthetic colourants because of itspositive effects on health and also uses in the pharmaceutical and cosmetic industry.

Fruit extracts of *Basella rubra*, which are rich in bioactive phenolics, flavonoids andbetalains were investigated for their antioxidant and anticancer activities against human cervicalcarcinoma (SiHa) cells. The fruits contained total betalain contents of 0.34 g/100 g fresh weightand 1.9 g/100 g dry weight. Betanin, isobetanin and gomphrenin I were the major

pigments identified. Phenolic compounds, such as generic acid, sinapic acid, ferulic acid, coumaric acid and chlorogenic acid, and flavonoids, such as myricetin, quercetin, luteolin, apigenin and kaempferol, were identified. Both water and aqueous methanol extracts of fruits showed significant free radical scavenging potential and ferric reducing antioxidant power. Fruit extracts at 50 mg/mL showedstrong (81%) cytotoxic activity against human cervical carcinoma cells. Thus, fruit extracts have potential application for cancer treatments and as nutraceutical or dietary supplements (Sandopu Sravan Kumar *et al.*, 2014). The flavonoid content of different parts of *Basella rubra*species ranged between 4.00 and9.87mg/g.

Nutrient and chemical composition of *Basella rubra*

S.No.	Nutrients	Quantity (per 100g)
1.	Water	93ml
2.	Energy	19kcal
3.	Protein	1.8g
4.	Fat	0.3g
5.	Calcium	109mg
6.	Phosphorus	52mg
7.	Iron	1.2g
8.	Magnesium	65mg
9.	Potassium	510mg
10.	Sodium	24mg
11.	Zinc	0.43mg
12.	VitaminA	8000IU
13.	VitaminB1	0.05mg
14.	VitaminB2	0.16mg
15.	VitaminB3	0.50mg
16.	VitaminC	102mg

A bioactive compound, BR-1 was isolated from the methanolic extract of the leaves of *Basella rubra L.* The leaf extract contains proteins, fat, vitamin A, vitamin C, vitamin E, vitaminK, vitaminB9 (folicacid), riboflavin, niacin, thiamine and minerals such as calcium, magnesium, phosphorus and iron. Kaempherol is the flavonoid present in *Basella rubra* at a con of 1.4mg/100. It contains Basellasaponins amino acid such as Arginine, Leucine, Isoleucine, Lysine, Threonine and Tryptophan, Peptide, Phenolic compounds in various extracts. The nutritional and nutraceutical potentials of *B. rubra*fruit extracts on human cervical cancer cells (SiHa) were also been demonstrated. Reactive oxygen and nitrogen species (ROS/RNS) are essential for detoxifications, immunity and chemical signaling.

The nutritional content of Malabars pinach

Basella rubra is a good source of calcium, iron, and vitamins AandC.Seedcontainfattyoilssuchaspalmitic, oleicandlinolenicacid). Carotenoids also found in the leaves of *Basella rubra* with major beta-carotene, small amounts of alpha-carotene and traces of other carotenoids. This plant is good for health due to the presence of mineral, protein, oil, carbohydrate, fibre, carotenoid, organic acid, vitamins.

Therapeutic and medical uses of *Basella rubra*

Malabar spinach oil can work as very good vegetable oil. Saturated fatty acids like lauric acid, arachidic acid, behenic acid, lignocenaricacid, palmitic acid, stearic acid and myristic acid while unsaturated fatty acids like oleic acid, eicosenoic acid, palmatoleic acid, erucic acid, docosahexanaenoic acid, arachidonic acid, linoleicacid and alpha-linolenic acid are present in the oil of both red and green species of Malabar spinach. In the oil of red Malabar spinach total amount of saturated and unsaturated fatty acid is about 22.19% and 50.7% respectively while in oil of green Malabar spinach oil total amount of saturated and unsaturated fatty acid is about 21.41% and 52.36% respectively. Malabar spinach is used to make many types of dishes in many countries like Philippines, Thailand China, Mongolia, India, Sri Lanka and many African countries. In the Philippines, a vegetable dish called utan which is cooked with sardines, onions, garlic, and parsley. In MangaloreanTuluva cuisine, a coconut-based gravy called gassi is paired with the *Basella* plant, making a delicacy called Basalegassi to be eaten with rice dumplings called pundi soaked overnight in the gravy, or with red rice. Soup is made in Chinese cuisine and Vietnam cuisine, Malabar spinach is mixed with crab meat and jute (6).

Dietary components are required to best able and bio available. Betalains are known to best able at pH5, refrigerated temperature, and ascorbic acid protects them efficiently (Mohammad *et al.*).

In Chinese traditional medicine, the leaves or the aerial parts of *B. rubra* have been used forthe treatment of constipation and also as a diuretic, toxicide, and anti-inflammatory. *Basella alba* is an important medicinal plant in ethnoveterinary for treatment of retained after birth and a plasmosis (Sushila *et al.,* 2015).

Anti bacterial activity

A bioactive compound, BR- 1 was isolated from the methanolic extract of the leaves of*Basella rubra* L. (Hiromu *et al.,* 1991). Isovitexin exhibited dose- dependent cytotoxicity against human colon cancer(HT-29)cells (Hiromu *et al.,* 1991). *Basella rubra* leaves on performing cup plate diffusion method shown that aqueous, ethanol and petroleum ether extracts of the leaves exhibited antibacterial activity against *E. coli*, *Vibriocholera*, *Staphylococcus aureus* and *Staphylococcus typhi* (Annu sharma & banshidhar behara.,2022).

Antiulcer activity

The gastric ulcers in the rats were induced by giving the treatment of ethanol and pyrolous and then the treated animals were fed with aqueous extracts of *Basella rubra* leaves. It was foundthat, the treatment of *Basella rubra* aqueous leaf extract in the ratio of 10mg/kg and 20mg/kg had significant and dose dependent antiulcer activity (Annu sharma & banshidhar behara., 2022).

Antifungal activity

Two novel antifungal peptides, designated α- and β-basrubrins, respectively, isolated from seeds of the *Basella rubra Linn*. α- and β-basrubrins exhibited a molecular weight of 4.3 respectively. The translation in a rabbit reticulocyte system with an IC50 value of 400 and 100nM, respectively was inhibited by them. α- and β-basrubrin also inhibited HIV-1 reverse transcriptase by 79.4 ± 7.8% and 54.6 ± 3.6%, respectively, at a concentration of 40μM, and 10.56 ± 0.92% and 2.12 ± 0.81%, respectively, at a concentration of 40μM.Both α- and β-basrubrins exerted potent antifungal activity toward Botrytis cinerea, Mycosphaerella arachidicola, and Fusarium oxysporum. Neither α-basrubrin nor β-basrubrin sexhibited DNAase, RNAase, Lecitin protease activity, indicating that their antifungal action is not due to these activities. The heat shock protein- like peptide and serine–threonine kinase-like protein exhibited a molecular mass of 3 and 30 kDa, respectively. They inhibited neither translation in a rabbit reticulocyte system at concentrations up to50 μM nor HIV-1 reverse transcriptase activity at concentrations up to 400 μM (Annu sharma & Banshidhar behara, 2022).

Anti cancer activity

Fruit extracts of were investigated for their antioxidant and anticanceractivities against human cervical carcinoma (SiHa) cells. The fruits were contained with total betalain contents of 0.34 g/100 g fresh weight and1.9 g/100 g dry weight. Betanin, isobetanin and gomphrenin I were identified as the major pigments along with Phenolic compounds. Both water and aqueous methanol extracts of fruits exhibited significant free radical scavenging potential and ferric reducing antioxidant power. Fruit extracts at 50 mg/mL showed strong (81%) cytotoxic activity against human cervical carcinoma cells. Thus, fruit extracts have potential application for cancer treatments and as nutraceutical or dietary supplements. The plant and leaves are ground with sour buttermilk and salt for preparing a poultice and which is indicated for arbuda (Sandopu Sravan Kumar *et al.,* 2014).

Ethnobotanical uses of B*asella rubra L.*

Fruits:

According to Annu Sharma & Banshidhar Behera 2022.,

- A purple dye from the ripened fruits has been extracted and used to colour or dye the 100% cotton and polyester fibers.
- Deep colouring matter obtained from the ripened fruits is used for colouring the food, to colour pastries or sweets.
- Fresh ripened fruits mixed with alum to obtain maroon colour used to colour the silk and cotton.
- Red dye from the fruit used for official seals and as rouge

Leaves and stems:

According to Akhter *et al.,* 2008

- Cooked leaves and stems have diuretic and febrifuge activity.
- Used in culinary practice.
- Applied for anticancer treatment such as melanoma, leukemia and oral cancer.
- Excellent substitute hot weather spinach and eaten raw in salads.
- Used for antipruritis and burn.
- Used for acne and freckle treatment Colours obtained from the leaves and stems are used for dying fabrics and in paintings.
- A paste of leaves and stem of is applied to cure acne, abscess, and skin diseases.
- Both stem and leaves of are used in Syphilis, intestinal disorders, tumour, acne, leucorrhoea.

Conclusion:

Malabar Spinach is a tropical climbing vine probably native to Indias Malabar Coast or Indonesia. It appears in two forms, green one, called in latin *Basella alba* (alba means white and refer to its white flowers) and *Basella rubra* (rubra means red) with highly ornamental bordo-red stems and veins of leaves. But lack of other differences in its outlook is bringing those names to be interchanged in use for both varieties together. It is quite popular vegetable in many tropical countries of Asia, Africa and South America. But its scientificaly proven medicinal values are mostly unknown and used mainly in traditional medicine**.**

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SIGNIFICANT ROLE OF MULTIFACETED FUNGUS: *PIRIFORMOSPORA INDICA*

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Abstract

Piriformospora indica is one of the most significant root endophytic fungal species. This fungus benefits numerous elements of plant performance and has a wide range of hosts. *P. indica* performs a variety of roles, including promoting plant development and acting as a phytoremediator, bio-fertilizer, immune-modulator, and more. Its hyphae are tightly coiled, frequently cling to one another, and resemble a simple string when entwined. Young mycelia are white and practically hyaline, while other cultures have noted inconspicuous zones. The order Sebacinales (Basidiomycota) contains the axenically cultivable phytopromotional, biotrophic mutualistic root endosymbiont *Piriformospora indica*, which has been shown to imitate the capacities of normal arbuscular mycorrhizal (AM) fungus. *P. indica* has demonstrated to be a very useful endophytic fungus with excellent effectiveness in the field. The need for its widespread cultivation and use is the requirement of the present scenario.

Keywords: Fungus, Root Endophyts, Arbuscular mycorrhiza

Introduction:

Piriformospora indica, is a symbiotic-plant growth promoting fungus as well as improve nutrient availability. It belongs to member of the Sebacinales (Basidiomycota) and have pear shaped chlamydospores. This fungus is axenically cultivable on synthetic media (Verma *et al.*, 2012). Originally, this fungus is found in rhizosphere of *Prosopsis juliflora* and *Zizyphus nummularia* which are woody shrubs of Thar Desert of Rajasthan, India during the screening of Arbuscular mycorrhiza fungi collected sample (Verma *et al.*, 1998; Singh *et al.*, 2000). It simply promotes overall growth in various plant species through root association like AM fungi (Singh *et al*., 2003).

Root colonization:

Piriformosapora indica has a board host range, it interacts with many vascular plant like *Arabidopsis thaliana* and *Hordeum vulgare* but showed effective colonization in mosses also

(Deshmukh *et al.,* 2006; Qiang *et al.,* 2011). Dense population of *P. indica* around the rhizosphere, covered roots with extracellular hyphae, invades root-cortex and colonizes living cells by direct penetration but not reached into the vascular tissue (Jacobs *et al.,* 2011).

P. indica proves its role in plant growth, as well as colonized plant is resistance to fungal pathogen and showed abiotic stress tolerance (Harman, 2011). Lots of beneficial characteristics are in *P. indica* pockets like they improve the secondary metabolites of many economically important plant, bio-regulator, immune modular enhance anti-ageing agent, anti-cancer drug, phyto-remediator, anti-oxidant system and tremendous biological hardening agent.

Genome and Fungal Morphology

The *P. indica* genome size is 24.97 Mb. The estimated DNA content of *P. indica* nuclei ranges from 15.3 to 21.3 Mb. (Zuccaro *et al.,* 2009). Its hypahe are looked like intertwined cord because highly interwoven and stick together. Hyphae are thin walled with 0.7 to 3.5nm diameter. Young branches are irregular. Its single compartment contains one or more nucleus with irregular septation.

Media

Fungus can grow axenically and best growth is reported on Hill and Kaffer medium, among tested media. (Varma *et al.,* 1999, Qiang *et al.,* 2011). When nutrient composition is change, it grows significantly and some morphological changes are detected with no negative effect on plant (Kumar *et al.,* 2011). It also grows on different type of media also that affect growth and morphology.

Applications

P. indica proves its role in plant growth and their lots of beneficial characteristics are in pockets like they improve the secondary metabolites of many economically important plant, bio-regulator, immune modular enhance anti-ageing agent, anti-cancer drug, phyto-remediator, anti-oxidant system, Bio-fertilizer, Bio-protector, permit to survive under water stress, temperature stress, salt stress, resistance to toxin and tremendous biological hardening agent (Unnikumar *et al.,* 2013, Bagde *et al.,* 2014, Prasad *et al.,* 2008a, 2008b).

It amazingly provides a natural platform for plant growth and overall biomass production in board host range, such as medicinal plant, herbaceous, monocotyledons, dicotyledons and other economically important plants (Shrivastava and Varma, 2014).

Remarkably, formulation of effective biofertilizer of *P. indica* is simple because it's easy to culture in bioreactors (Singh *et al.,* 2003, Bagde *et al.,* 2010). Inocula of *P. indica* are very useful for commercial applications to different crops. As its efficiency to improve seed

germination, biomass production, plant growth and development and crop productivity as a powerful tool for sustainable Agriculture (Ansari *et al.*, 2014).

Some Plants listed below is benefited by *Piriformospora indica*

Host plants/plants	Beneficial role	References
T. aestivum	Protection against *B. graminis f. sp. Tritici etc.*	Serfling *et al.*, 2007
Z. mays	Bio-protection against root parasite	Kumar *et al.*, 2009
Oryza sativa	Phyto-promotional effect with increased biomass, early germination, increased number of seeds	Prasad 2008; Kumari *et al.*, 2004a
Phaseolus vulgaris	Phyto-promotional effect with increased biomass	Prasad 2008
Abrus precatorius	Phyto-promotional effect with increased biomass	Prasad 2008
T. procumbans	Phyto-promotional effect with increased biomass, early germination, increased number of seeds	Prasad 2008; Kumari *et al.*, 2004a
Abrus precatorius	Phyto-promotional effect with increased biomass	Prasad 2008
Trigonella foenumgraecum	Increased biomass, secondary metabolites	Sharma *et al.*, 2011
Cicer arietinum, Phaseolus aureus, P. mungo, Pisum sativum and *Glycine max*	Promote growth in tropical legumes	Varma *et al.*, 2012b
A*rtemisia annua, Bacopa monniera, Abrus precatorius, Stevia rebaudiana, Linum album, Trigonella sp., Coleus forskohlii, Spilanthes calva, Withania somniferra, Chlorophytum tuberosum* and *Curcuma longa*	Promote growth and increase secondary metabolites in several folds In medicinal plants	Varma *et al.*, 2012

Hordeum vulgare	Increase seed viability, vegetative and grain yield	Harrach *et al*.,2013
Halienthus annus	Increased seed yield with oil content	Bagde *et al.,* 2011
Cyclamen persicum	Increase number of microspores, viable pollen, ovules and flowers	Ghanem *et al.,* 2014
Lycopersicon esculentum (Tomato)	Increase seedling growth	Anith *et al*.,2015
Oryza staiva	Improve root and shoot length	Jogawat *et al.,* 2013
Aelo vera	Improve micropropagation, growth and phytochemical content	Sharma *et al.,* 2014
Herbal medicinal plant	Increase vegetative growth, quality and quantity of herbal medicine	Das *et al.,* 2012
Chlorophytum borivillium, withanian sominifera	Improve vegetative growth , yield if early flowering	Varma *et al.,* 2013, Prasad *et al*., 2008
Jatropha and Populus	Early seed germination and seed yield	Varma *et al.*,2013
A. thaliana	Significant reduction in *Verticillium dahalia* – mediated disease development	Sun *et al.,* 2014
H. vulgare	Protection against Rhizoctonia root rot	Qiang *et al.,* 2012
L. esculentum	Protection against Fusarium wilt and black root rot caused by *Fusarium oxysporum* and *Thielaviopsis basicola*	Fakharo *et al.*,2010
Nicotiana tabacum	Enhance cadmium tolerance	Hui *et al.,* 2015
Solanum lycopersicum	Osmotic stress and chloride toxicity	Al-Absi and Al-Ameiri, 2015
T. aestivum	Increase in cadmium stress tolerance	Shahabivand et al., 2012
H. vulgare	Increase crop yield under low temperature	Murphy *et al.*,2014

Triticum aestivum	Protection against *Fusarium* head blight disease isolates and mycotoxin contamination	Rabiey *et al.,* 2015
A. thaliana, Brassica rapa	Growth promotion and increase seed yield	Sherameti *et al.,* 2005; Shahollari *et al.,* 2007

***P. indica* with *In vitro* micropropagated plants**

In vitro generated plantlets easily cultured with *P. indica* that increase number of shoot and phytochemical content. *P. indica* treated plantlets acclimatized in natural environmental condition increases survival rate about 90-100% compare to untreated plantlets (Sahay 1999; Sudha 1999).

Some medical plant like *Bacopa monniera, Nicotiana Xanthi, Azardirachta indica* colonized with *P. indica* in Tissue culture conditions showed improved biomass production (Varma *et al.*,2012). Even small quantity of *P. indica* mycelium associate with *in vitro* regenerated plantlets demonstrated significant increase in root and shoot growth. Micropropagated *Chlorophytum sp*. with *P. indica* increases plantlet survival, P content and nutrient acquisition in Biotization (Gosal *et al.,* 2010) and also improved iron, zinc and magnesium uptake by treated plantlets.

This fungus play a key role to improves biosynthesis of phyto-hormones especially auxin and cytokinin which concerned in vascular development, apical dominance, cell division, stress tolerance and decrease leaf senescence in colonized Arabidopsis roots and also increases *P. indica* treated Arabidopsis plant growth because of biosynthesis of trans-zeatin (Vadassery *et al.,* 2008).

It also plays some extended role in defense system only in initial stage of time of *P. indica* root colonization. During the early period of interaction with plant it demonstrates pathogenesis related genes, ethylene-targets transcription factors, ethylene signaling components etc (Camehl *et al.,* 2010).

Conclusion:

Piriformospora indica is endophytic Mycorrizal fungus interact with wide range of host plant which provide lots of opportunities to improve and increase overall growth (Phytochemical content, shoot length and number, root length number etc.) of Agricultural and Horticultural crops. *P. indica* interaction with various important and major crops of Agriculture provides a

way for sustainable and organic farming, which reduced the uses of chemical fertilizer and improve quality and quantity of crop which play crucial role in economy also.

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CONSERVATION OF MICROBIAL DIVERSITY

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Abstract:

Biodiversity refers to the diversity in species of plants, animals and microorganisms in a given habitat. Microbial diversity is a part of biodiversity that refers to the distribution and occurrence of microorganisms in biosphere including land, water and air. The microflora broadly includes bacteria, fungi, archaea, algae, protozoa and protists. It is an integral part of biodiversity but is less spoken about or addressed. CBD (convention on biological diversity) gives the much-needed attention to microbial diversity. Many organisms have extinct and some are on verge of extinction due to increased human interference and pollution. So, it is important to study microbial diversity and conserve it. Conserving microorganisms is important because microorganisms play various roles in nature and have a variety of biotechnological application including production of novel biocatalysts, drugs, biofertilizers etc. Much research has been done and much microflora on earth is needed to be explored to find its use for benefit of mankind.

Introduction:

Microorganisms are widespread in nature and constitute a large part of our ecosystem and in turn the biosphere. Microorganisms have developed their metabolism in such a way that they can thrive in almost every ecosystem. Thus, it can be said that they are the pillars of biosphere. They also play important role in cycling of nutrients and are part of all the biogeochemical cycles occur in biosphere. It is estimated that only a small fraction (less than 15%) of microbes is discovered till now. This simply implies that if any living organism is abundant on the earth, it is microorganism. Microorganisms are diverse. Biodiversity refers to the richness, evenness and differences of species and microorganisms fulfil all the mentioned criteria. Thus, microbial diversity is the largest biodiversity. Microorganisms are diverse in term of their genetic as well as phenotypic characters. How these microorganisms are so widespread, it is because of the fact that they can disseminate by the means of air and water very easily. Whiteman *et al.,* 1998 found that the number of prokaryotes in terrestrial sub surfaces is 0.25-2.5x 10^{30} in ocean it is 2.6x 10^{29}

and in soil it is approximately 20.6x 10^{29}. Archaea are microorganism that can thrive in extreme of environment. About one third of this microorganism occurs in sea at great depth (Weinbauer and Hofle, 1998). The microbes are so abundant that if we observe a single particle of soil, it contains so many different kinds of fungi and bacteria (Nee, 2002; Steage and Zaft, 2002; Morin, 2000). About 2-3 tons of microorganisms including fungi and bacteria are present in top soil and approximately 10,000 species of bacteria be present in 100gm of soil as studied by Torsvik *et al.,* 1990. Why studying microbial diversity and its conservation is important? It is important because microorganisms can be utilized as experimental systems and sustain the other life forms on earth. The diversity present among microorganism can be a source for new genes and thus provide a tool to biotechnology. All the more it is observed that microbes may change patterns in accordance to the changing environment, thus monitoring weather changes. Boucher and Dolittle, 2002 reported presence of microorganisms thriving in underwater hot vent whereas Miskin *et al.,* 1998 reported bacteria in post glacial freshwater sediments.

Distribution of microbial diversity

To understand the concept of microbial diversity it is important to know their occurrence and distribution. The evolution of occurrence of types of microbes is their special ecological niches and with their preferred substitutes may be a good perspective to study microbial diversity. Recognizing microbial diversity provided an insight and adds to the microbial taxonomy and physiology. It also helps identify potentially endangered species of prokaryotes that can be identified on the basis of microbiological habitats and their capability to metabolize nutrients, thus they can be measured and identified when plated on culture plates, called as plate count anomaly (Wonderoth and Reber, 1999). On the other hand, eukaryotes possess morphological and identifiable features as compared to prokaryotes (Lopze Gracia *et al.,* 2001; Moon-Van der staay *et al.,* 2001). Selective media can be used to isolate pure culture of undiscovered microorganisms. The use of selective media with certain antibiotics or chemicals that inhibit the more prevailing form and let the slow glowing / less abundant species to thrive. Other than use of selective media, physical methods can used. Modern molecular biological technique can also be used to identify and study microbial diversity. Techniques such as Fluorescence in situ hybridization (FISH), reverse transcription, polymerase chain reaction (RT – PCR), density gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T – RFLP) are some modern methods that can analyse the diversity of microorganisms. Moreover, the interaction of abiotic factors with microbial population can be studied using DNA – based methods (Arias *et al.,* 2005). Process like mass spectrometry is among one of the rapid identification techniques used to identify microorganisms. Bull *et al.,*

1992 uses this technique to discover new approx. 4288 ORF'S in *E. Coli* genome. Metagenomics is the study of assessing the whole genome in natural habitat of Microorganism. So, Metagenomics can provide an insight to the undiscovered world of microorganisms.

One other approach for occurrence and recognition of microbial diversity involves the investigation of the conserved sequences in Prokaryotes and Eukaryotes. Conserved RNA sequences can be used to identify novel microorganism as well as allows comparisons between microorganisms. The microbial distribution in natural communities can be surveyed using RNA gene sequencing (Pace, 1997; Head *et al.,* 1996). Ribotyping can be a better method for isolation of novel bacteria (Kulkarni, 2002). Novelty of microbes (about 90 Microbes) was determined by Watve *et al.,* (2000) using ribotyping. Bach *et al.,* (2001), discussed the in vitro amplification of conserved sequences using Universal primers through PCR based procedures. Isolation of individual types of genes by separation and sequencing of gene was studied by (Tobin – Janzen *et al.,* 2005).

DNA fingerprinting, determination of G+C content of total soil DNA, flow cytometry and analysis of phospholipid fatty acid have been proved to be powerful tool in identification of microbial diversity existing in a particular niche or ecosystem. (Niemi *et al.,* 2001)

Global and indian scenario of microbial conservation

In United nation, there is a legal agreement on biodiversity called as convention on biological diversity (CBD). It was enforced in 1993 after much discussion and negotiations on from 1987 to 1992. The international union of biological service (IUBS) and international union of microbiological societies made a multidisciplinary commission called as world federation for culture collection (WFCC) that aims to promote and support the culture collection establishment. It enables a smooth interface between collection centres and user. The (WFCC) has developed an international database, world centre for Microorganism (WDCH) to maintain the collection of microbial cultures. The world directory of culture collections has data of about 758 culture collections from nearly 76 countries with a total of 2,963856 microbes.

To create a reliable and user-friendly network and initiative called WFCC global catalogue of microorganism is made that help culture collection to interchange and manage information. In India, WDCM identified thirty-two microbial culture collection. Some culture collection centres are given in table given below.

Some Microbial Centres in India		
State/City	Collection name	WDCM
Pune, Maharashtra	National Collection of Industrial Microorganisms, CSIR-National Chemical Laboratory.	WDCM 3
Pune, Maharashtra	National Fungal Culture Collection of India, ARI.	WDCM 932
Trivandrum, Kerala	NII Microbial Culture Collection, National Institute for Interdisciplinary Science and Technology.	WDCM 961
Jalgaon, Maharashtra	972 North Maharashtra Microbial Culture Collection Centre, North Maharashtra University	WDCM 972
Bangalore, Karnataka	Culture Collection, Microbiology and Cell Biology Laboratory, Indian Institute of Science	WDCM 107
Mumbai, Maharashtr	Food and Fermentation Technology Division, University of Mumbai,	WDCM 562
Santiniketan, West Bengal	Vishva-Bharati Culture Collection of Algae, Visva Bharati Central University,	WDCM 931

Types of microbial conservation

1. *In-situ* conservation

It refers to the conservation of species of microorganism in their natural habitat by prolonged preservation of ecosystem and population under continuing adaptation condition. As now days many wild life sanctuaries, reserve forests, biosphere reserves, eco park etc have been created that leads to destruction of the natural habitat of organisms. Microbial species are very complex and play critical role in ecological process but in spite of their importance there are numerous microorganisms present in ecosystem that are yet to be discovered (Stewart, 2012). Stewart 2012 suggested that the monophyletic group of undiscovered microorganisms are found at every level of bacterial phylogeny and are playing major role in biogeochemical cycle produce novel products, but traditional methods of cultivating microorganisms does not seem to be efficient to grow ecologically and phylogenetically more relevant microorganisms that may be slow grower, or fastidious microorganisms. In situ management can be of two types- species centric approach or ecosystem-based approach. The species centric approach targets the population of selected species whereas ecosystem approach targets the whole ecosystem appropriate statistics and data are necessary to a certain the advantage of in- site conservation approach. A more adaptable basis of in situ conservation are protected area (CBD, 1992).

2. *Ex situ* conservation method

This type of conservation method involves conservation of species outside their natural habitat. It is an efficient way for conserving biodiversity. It plays a vital role in microbiology and comprises of gene banks, culture collections and microbial resource centres (Colwell, 1997). Conservation by this method help in prevention of repetition of expensive and time-consuming re-isolation protocols (Day, 1996; Day *et al.,* 1998). Ex situ Conservation is preferred and encouraged by various association such as (WFCC) world federation for culture collection and directory of collection of culture of microorganisms, Oceanic and atmospheric administration for marine microbial diversity, National Institute of Health for deciphering the emerging microbial pathogen diversity, American society of microbiology and American Phyto pathological society. In India, this work is done and included under Ministry of Environment and forestry and the ministry of science and technology. Various departments under above mentioned functions for microbial conservation such as department of agricultural research and education, Indian Council of Forestry Research and Education, DBT. The well-known microbial type culture collection section of IMTECH Chandigarh is now an International Depository Authority (IDA). That maintain and distributes pure cultures internationally.

The Various method that are used for ex situ conservation of microorganisms include repeated sub cultivation, preservation on agar beads, storage in sterile soil, mineral oils, silica gel storage, spray drying, freezing, desiccation, anhydrobiosis, vitrification gelatine discs, water - salt solution etc. Cryopreservation and lyophilization are the most efficient method. They help in long term storage of important microorganism. The conservation of microorganism by Ex situ method is a more practical approach. The *ex situ* collection of microorganisms integrated by various modern method such as molecular evolution systematic etc. can be very beneficial for future research programs.

Why studying microbial diversity

There are four major applications of microbial diversity. Firstly, microbes play role in biogeochemical cycles such as Carbon, Phosphorus, Oxygen, Nitrogen and Sulphur etc. They have remarkable activity to degrade and metabolize a wide variety of complex organic compound (Grine, 1997). Haack *et al.,* 1995 have studied the utilization of carbon sources by microbial communities and factors that affects the reproducibility and accuracy. The continuous recycling of various elements is necessary for the proper functioning of biosphere (Maire *et al.,* 1997)

Secondly, Microbes also help to maintain the fertility of soil, thus play vital role in terms of agricultural activities. They are natural indicators also. The indicates the presence or absence of certain nutrients, minerals and also type of pollution. Microbiota can be used in combination for the improvement of sustainability (Dilly and Blume, 1998). The microbial reaction in soil

improves the soil fertility but human intervention causes a disturbance in this process by addition of pollutant to soil such as fertilizers (Magu, 1998). Ramakrishan and Sethunathan, 1998 discussed the inhibition of nitrogen fixation, oxidation of sulphur and nitrification by application of pesticides. Mycorrhizal symbiosis helps in restoration of semiarid and other ecosystems (Requena, 1997).

Thirdly, various microbial products can be used for agricultural purposes. Agricultural practices involve use of various harsh chemical fertilizers. The use of biofertilizers is a good alternative. The biofertilizers are usually comprise of a mixture of microbes such as *Rhizobium, Azotobacter, Azospirillum* and thus when applied to fields will help in increasing growth and yield of crops (Peoples *et al.,* 1995).

Fourth, Microbes also help in biodegradation of xenobiotics. Xenobiotics are chemical compounds that are present in much higher concentration than usually present. Microorganisms have capability to destroy xenobiotics such as DDT present in our environment. Halogens such as chlorine in the form of chlorinated aromatic compounds, nitroaromatics and other compounds that happens to cause contamination can be restored by use of microbial cultures. *Spingomonas paucimobilis* BPSI-3 was found to degrade biphenyls and PAHs and was isolated from contaminated Soil (Davison *et al.,* 1999). *Ralstonia sp* aids in degradation of polycyclic aromatic hydrocarbon as studied by Samanta *et al.,* (2002). According to Pointing (2001) White-rot fungi can be used for bioremediation. Heavy metals are a major contaminant and deteriorate the quality of soil and natural soil resource as stated by Muller *et al.,* 2002.

Lastly, soil microflora is the most valuable resource of products that have commercially important applications. So, the more the new microorganisms are discovered the more are the chances to get novel products that will benefits the mankind. The microbes have biosynthetic ability to produce useful products in economically feasible processes that is otherwise too expensive to be made industrially via other sources. Of immense importance is gene mining. This technique is based on the screening of genetic libraries that are formed from the soil metagenomic studies and it provide a way to explore and discover the microbial diversity in terms of genetics and metabolic pathways. A number of microbes has been extracted from soil that plays important role food processing, biocontrol agents, medicines, development of biocides. Keeping this in view pharmaceutical companies spend a lot of money on the screening and isolation of industrially useful microorganisms.

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