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Research and Reviews in Botany & Zoology

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Dr. Abhijit Sahasrabudhe
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Dr. Tankala Sreelakshmi

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PREFACE

The biological sciences of Botany and Zoology form the foundation for understanding life in all its diversity, complexity, and interdependence. From the molecular mechanisms governing cellular functions to the ecological interactions shaping ecosystems, these disciplines continue to evolve in response to rapid scientific, technological, and environmental changes. The book *Research and Reviews in Botany and Zoology* is conceived as a comprehensive scholarly platform to present contemporary research, critical reviews, and emerging perspectives that address both fundamental and applied aspects of plant and animal sciences.

This edited volume brings together contributions from academicians, researchers, and practitioners who are actively engaged in advancing knowledge across diverse subfields of Botany and Zoology. The chapters encompass a wide range of themes, including plant physiology, taxonomy, phytochemistry, biodiversity conservation, animal behavior, ecology, genetics, environmental biology, and biotechnological applications. By integrating experimental findings with analytical and review-based approaches, the book aims to bridge the gap between classical biological concepts and modern interdisciplinary research.

In the present era of global challenges such as climate change, habitat degradation, biodiversity loss, and emerging diseases, research in Botany and Zoology has assumed greater significance than ever before. The studies compiled in this volume highlight the role of biological research in sustainable development, environmental management, agriculture, health, and conservation strategies. Special emphasis is placed on regional and indigenous biological resources, innovative methodologies, and translational research with practical relevance.

Research and Reviews in Botany and Zoology is intended to serve as a valuable reference for undergraduate and postgraduate students, research scholars, teachers, and scientists. It is hoped that this book will not only enrich academic understanding but also stimulate critical thinking, inspire further research, and encourage collaborative efforts across disciplines. The editors sincerely acknowledge the contributors and reviewers whose scholarly inputs have made this volume possible and meaningful.

- Editors

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IN VITRO EVALUATION OF ANTIDIABETIC ACTIVITY OF MEDICINAL PLANTS

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Abstract:

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels, which occur either due to insufficient insulin production by the pancreas or the body's inability to utilize insulin effectively. Persistent hyperglycemia can lead to severe complications affecting the heart, blood, vessels, eyes, kidneys, and nerves. Medicinal plants possess significant therapeutic properties and serve as an important source of medicine in both traditional and modern health care systems. Many medicinal plants exhibit antidiabetic activity through various mechanisms, including enhancing insulin secretion, improving glucose utilization, and regulating blood glucose levels. This review summarizes the antidiabetic activity of various medicinal plants. This work stimulates the researchers for further research on the potential use of medicinal plants having antidiabetic potential.

Keywords: Medicinal Plant, Antidiabetic Potential, Diabetes Mellitus

Introduction:

Diabetes mellitus is a multifactorial metabolic disorder characterized by chronic hyperglycemia and glucose intolerance resulting from either inadequate insulin secretion or impaired insulin action, which affects cellular glucose uptake (Grover *et al.,* 2002). It is one of the major public health concerns in developing countries and represents a significant social and economic burden. In India, diabetes has historically been referred to as a "disease of rich man" although its prevalence is now increasing across all socioeconomic groups (Kakkar, 2016).

Persistent hyperglycemia can lead to multiple complications, including dyslipidemia, oxidative stress, diabetic ketoacidosis, nephropathy, neuropathy and cardiovascular disorders, making early management and prevention essential (Buowari, 2013; Chijioke *et al.,* 2010).

Classification of Diabetes Mellitus

The major types of diabetes are type 1 diabetes and type 2 diabetes (Grover *et al.,* 2002). Type 1 diabetes is an autoimmune disorder in which the immune system destroys the insulin producing β-cells of the pancreas, resulting in little or no insulin production. This type typically appears in childhood or adolescence (Folorunso *et al.,* 2013). Type 2 diabetes, on the other hand, occurs when the body develops insulin resistance, meaning that the cells do not respond properly to insulin. As a result, glucose remains in the blood stream instead of being absorbed into the cells for energy (Inzucchi, 2002).

Type 1 Diabetes Mellitus

Type 1 diabetes mellitus is a complex disorder influenced by both genetic susceptibility and environmental triggers that distrupt normal immune pathways. The disease develops through cell-mediated autoimmune destruction of the insulin producing pancreatic β-cells (Folorunso *et al.,* 2013). This destruction is primarily carried out by CD4 T-cells, CD8 T-cells, and macrophages, which mistakenly recognize β-cells as foreign and attack them (Folorunso *et al.,* 2013).

There are four major autoantibody markers used to identify this autoimmune β-cells destruction:

1. Islet cell autoantibodies
2. Insulin autoantibodies
3. Autoantibodies to glutamic acid decarboxylase
4. Autoantibodies to the tyrosine phosphatases IA-2 and IA-2β

These markers help in early detection, diagnosis, and understanding of disease progression in T1DM.

Type 2 Diabetes Mellitus

Type 2 diabetes mellitus is the most prevalent form of diabetes mellitus, accounting for more than 90% of all cases and affecting individuals across all age groups (Kakkar, 2016). The two major hallmarkes of type 2 diabetes mellitus are (Defronzo, 1999; Inzucchi, 2002):

1. Impaired insulin secretion resulting from dysfunction of the pancreatic β-cells
2. Impaired insulin action caused by insulin resistance.

Medicinal Plants with Potential Antidiabetic Activity

Medicinal plants are widely used in traditional medicine because they contain many natural compounds that show therapeutic effects. People prefer these plants as remedies because they generally cause fewer side effects compared to allopathic medicine and are also more affordable. Due to their long history of use and their effectiveness in treating various health problems, medicinal plants continue to play an important role in health care (Grover *et al.,* 2002).

Medicinal plants act as an important source of natural ɑ-amylase inhibitors, which help in controlling blood glucose levels. By inhibiting the ɑ-amylase enzyme, these plants extracts slow down the breakdown of carbohydrates into glucose (Lebovitz, 1997).

Macaranga barteri

Macaranga barteri belongs to the family *Euphorbiaceae*. This family is also known as the spurge family, and the genus *Macaranga* is one of its largest, with over 300 species found in tropical regions of Africa, Asia, and the south pacific. The leaf extracts of *M. barteri* exhibit a strong α-amylase inhibitory effect, which suggests potential antidiabetic properties (Abu *et al.,* 2025).

Lagenaria breviflora

Lagenaria breviflora belongs to the family *Curcurbitaceae*. This family is also known as the gourd or squash family. Methanol extracts of *Lagenaria breviflora* leaves show α-amylase inhibitory effects. It has potential antidiabetic effects (Abu *et al.,* 2025).

Solanum erianthum D. Don

Solanum erianthum D. Don belongs to the family *Solanaceae*. This family is also known as the nightshade or brinjal family. *Solanum erianthum* has potential as a source of natural α-amylase inhibitors, supporting its traditional use in managing conditions like diabetes (Abu *et al.,* 2025).

Spigelia anthelmia

The spigelia anthelmia belongs to the family *Loganiaceae*. Studies have shown that the methanol extract of *spigelia anthelmia* leaves exhibits α-amylase inhibitory effects (Abu *et al.,* 2025).

Uvaria afzelii sc. Elliot

The family for *uvaria afzelii sc*. *Elliot* is *Annonacaea*. This is the family of flowering plants that includes climbing plants and shrubs and the genus *uvaria* is known for its traditional medicinal uses in Africa and Asia (Abu *et al.,* 2025).

Percentage Inhibition (1%) = $\frac{OD\ control - OD\ sample}{OD\ control}$ X 100

Table 1: α-amylase inhibitory activity of plant crude extracts

Plants	Plant Code	Common Name	Family	Part Used	IC_{50} Value ± S.E.M (mg/mL)
Macaranga barteri	MBL	Macaranga	Euphorbiaceae	Leaf	0.54 ± 0.75
Uvaria afzelii sc. Elliot	UAL	Monkey finger	Annonaceae	Leaf	0.68 ± 0.21
Lagenaria breviflora	LBL	Bitter gourd	Curcurbitaceae	Leaf	5.88 ± 0.75
Solanum erianthum D. Don	SEL	Potato tree	Solanaceae	Leaf	4.84 ± 0.56

Many medicinal plants contain natural α-glucosidase inhibitors that help regulate postprandial blood glucose levels by slowing the digestion and absorption of

Carbohydrates. This plant derived inhibitors are being extensively investigated as potential natural alternatives to synthetic antidiabetic drugs such as acarbose (Lebovitz, 997).

Erythroxylum laurifolium

Erythroxylum laurifolium belongs to the family *Erythroxylaceae*. This family is a genus of tropical flowering plants that includes erythroxylum. Methanolic extracts of *Erythroxylum laurifolium* were found to significantly inhibit α-glucosidase, a key enzyme involved in carbohydrate digestion (Picot *et al.,* 2014).

Elaeodendron orientale Jacq

The family for *Elaeodendron orientale Jacq*. is *Celastraceae* also known as the staff-tree family. Methanolic extracts of *Elaeodendron orientale Jacq*. were found to significantly inhibit α-glucosidase, a key enzyme involved in carbohydrate digestion (Picot *et al.,* 2014).

Antidesma madagascariensis

Antidesma madagascariensis is in the family *Euphorbiaceae*. Methanolic extracts of *Antidesma madagascariensis* were found to significantly inhibit α-glucosidase, a key enzyme involved in carbohydrate digestion. The inhibitory effect means the plant could help manage blood sugar levels by slowing down the breakdown of carbohydrates in the small intestine (Picot *et al.,* 2014).

Stillingia lineata

Stillingia lineata belongs to the family *Euphorbiaceae*, commonly known as the spurge family. This species is a member of of the genus stillingia. Which is the native of various regions including parts of the America, the pacific, and the Indian ocean (Picot *et al.,* 2014).

Table 2: α-glucosidase inhibitory activity of plant extracts

Plants	Plant code	family	IC_{50} value (µg/mL)
Erythroxylum laurifolium	EL	*Erythroxylaceae*	(1.02 ± 0.02) (12.00 ±1.57)
Elaeodendron orientale Jacq	EO	*Celastraceae*	(1.75 ± 0.26) (16.72 ± 2.81)
Antidesma madagascariensis	AM	*Euphorbiaceae*	(10.40 ± 0.26) (1.22 ± 0.04)
Stillingia lineata	SL	*Euphorbiaceae*	(19.30 ± 3.59) (185.92 ± 9.00)

Conclusion:

This review highlights the importance of traditional medicinal plants in the management of diabetes mellitus. A wide range of plants has been reported to exhibit antidiabetic, antihyperglycemic and hypoglycemic activities (Grover *et al.,* 2002). Many of these effects are linked to the ability of plant extracts to inhibit key carbohydrate digesting enzymes such as α-amylase and α-glucosidase by slowing down carbohydrate breakdown and glucose absorption, these plants help in improving postprandial blood sugar levels and overall glycemic control (Lebovitz, 1997).

The antidiabetic activity of medicinal plants arises mainly from their rich phytochemical composition. Important bioactive groups include alkaloids, phenolic acids, flavonoids, glycosides, saponins, polysaccharides and tannins (Grover *et al.,* 2002). These compounds act through multiple mechanisms, such as enhancing insulin secretion (Dey *et al.,* 2002), protecting pancreatic β-cells from oxidative damage (Yazdanparast *et al.,* 2007), improving insulin sensitivity (Saito *et al.,* 2016), and reducing intestinal glucose absorption (Lebovitz, 1997).

Medicinal plants serve as a valuable natural resource for discovering new antidiabetic agents. Therefore, further studies should focus on standardization of extracts, isolation of active compounds, mechanistic evaluation, and well-structured clinical trials.

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ORGANIC MANURE PRACTICES AT HOUSEHOLD LEVEL: A CASE STUDY FROM JORHAT DISTRICT, ASSAM

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Abstract:

Organic manure is a key component of sustainable agriculture due to its role in improving soil fertility, enhancing biological activity, and reducing dependence on chemical fertilizers. The present chapter documents household-level organic manure practices followed in selected villages of Jorhat district, Assam. A preliminary survey-based case study was conducted using questionnaires and field observations to record types of organic manures used, their preparation methods, applications, and associated challenges. Practices such as vermicompost (Kesu khar), vermiwash, household compost (Poson khar), egg-based manure, banana peel fertilizer, cattle and poultry manure, and mustard oil cake liquid fertilizer were documented. The use of commercially prepared liquid organic formulations based on traditional principles was also observed. The study highlights the relevance of these practices in maintaining soil health, recycling organic waste, and supporting environmentally sustainable agriculture. However, constraints such as time-consuming preparation and delayed nutrient availability remain challenges for wider adoption.

Keywords: Organic Manure, Sustainable Agriculture, Household Farming, Vermicompost, Jorhat District

Introduction:

Organic manure forms the foundation of sustainable agricultural systems by improving soil fertility, soil structure, and long-term productivity. It is derived from decomposed plant residues, animal dung, kitchen waste, and microbial activity. Unlike synthetic fertilizers, organic manures contribute to the physical, chemical, and biological health of soil and promote environmentally friendly farming practices.

Excessive reliance on chemical fertilizers and the neglect of organic nutrient sources have led to soil nutrient depletion and declining soil health, thereby limiting agricultural productivity. Recycling agricultural and household organic waste and returning it to soil is an effective strategy for maintaining soil organic matter and sustaining soil fertility (Chatterjee *et al.,* 2017). Long-term application of organic manure has been shown to enhance soil organic carbon, microbial biomass, and beneficial soil organisms, contributing to improved soil health (Su *et al.,* 2022). In addition, the use of organic manure can substantially reduce the requirement for chemical fertilizers in cropping systems (Zhou *et al.,* 2022).

The North-Eastern region of India, comprising Assam, Arunachal Pradesh, Manipur, Mizoram, Tripura, Nagaland, and Sikkim, supports several traditional agricultural systems that are largely organic in nature. Practices such as Jhum cultivation, Zabo farming, Apatani farming, and terrace rice cultivation rely on farmyard manure, compost, and indigenous pest management techniques (Tripathi and Barik, 2003). Recognizing this potential, the Government of India has identified the region as a promising zone for organic agriculture, and Sikkim was declared the first organic state of India in 2016.

Objectives

The objectives of the present study were:

1. To document household-level organic manure practices followed in selected villages of Jorhat district, Assam.
2. To record preparation methods and application practices of different organic manures.
3. To assess the perceived benefits and challenges associated with organic manure use in comparison to chemical fertilizers.

Methodology

The present work is a preliminary, questionnaire-based survey conducted in selected villages of Jorhat district, Assam to document household-level organic manure practices. The survey covered Rowmarikhuti, Malowpathar, Borahom Gaon, Bosapathar Boroguri Gaon (Sotai area), Nakachari, and Majkuri Gaon. Data were collected through structured questionnaires, informal interactions with farmers, and field observations.

In addition, one organic farm within Jorhat district was surveyed to understand the production, use patterns, perceived benefits, and challenges of organic manure in comparison to chemical fertilizers. The study focused on qualitative documentation of practices rather than quantitative assessment.

Figure 1: Map showing the surveyed villages in Jorhat district, Assam

Results:

The types of organic manure practices, their modes of preparation and application, and the practical constraints faced by farming households are -

Vermicompost (Kesu Khar)

Vermicompost, locally known as Kesu khar, is widely prepared at household and farm levels using earthworms (*Eisenia foetida*). Organic materials such as banana stems (*Musa paradisiaca*), water hyacinth (*Eichhornia crassipes*), and cow dung are layered in vermibeds under shaded and moist conditions. After a decomposition period of approximately 20–25 days, the compost is harvested and used as an organic soil amendment.

Figure 2: Vermicompost (Kesu khar) preparation and processing: (a) vermibed preparation, (b) earthworms (*Eisenia foetida)*, and (c) vermicompost grinding unit

Vermiwash and Commercial Liquid Organic Formulations

Vermiwash is a liquid extract obtained during vermicompost production by passing water through decomposed organic matter. It is commonly applied as a foliar spray or soil drench and is used by farmers as a plant growth promoter and mild pest deterrent.

The survey further documented the use of commercial liquid organic formulations such as Panchagavya, Jivamrit, Bhoo-Jeevan L, and Khira Pani, which were supplied by a private enterprise (Gattani Organics). These products are based on traditional organic farming principles and contain fermented organic ingredients enriched with beneficial microorganisms. Farmers reported their application after dilution as per manufacturer recommendations, mainly to support crop growth and reduce dependence on chemical inputs

Figure 3: Vermiwash and liquid organic fertilizer preparation practices observed during the survey

Poson Khar (Household Compost)

Poson khar is a household-level compost prepared using kitchen waste such as vegetable and fruit peels, tea waste, cow dung, soil, and dry leaves. Composting is carried out in earthen pots with periodic mixing to ensure aeration. The compost matures within 40–45 days during summer and 60–75 days during winter.

Egg-Based Organic Manure

Egg-based organic manures were frequently observed in the surveyed households. These include egg shell meal, egg amino acid (eggzyme), egg shell liquid, and direct application of egg shells. Egg shells primarily consist of calcium carbonate and are traditionally used to address calcium deficiency in plants.

Figure 4: (a) Direct application of eggshells to plant (b)&(c) Egg Shell meal preparation and application

Banana Peel Fertilizer

Banana peels, a common household organic waste, were used in the form of banana peel water and banana peel powder. Farmers used banana peel-based preparations as supplementary organic inputs within household farming systems.

Cattle and Poultry Manure

Cow dung was the most commonly used organic manure in the surveyed villages due to its availability and traditional acceptance. In some households, goat manure was preferred because of its comparatively higher nutrient concentration and lower susceptibility to pests. Poultry manure was also used after appropriate drying and processing.

Figure 5: (a) Potato and Pumpkin cultivation using cow manure in Malowpathar (b) Onion cultivation using Goat manure in Borahom Gaon.

Mustard Oil Cake Liquid Fertilizer

Mustard oil cake, a by-product of mustard oil extraction, was used as a liquid organic fertilizer after soaking and dilution. Oil cakes are highly valuable in agriculture because they function as nutrient-rich organic manures, soil improvers, and natural pest deterrents. They are traditionally valued for improving soil fertility and structure and help minimize dependence on chemical inputs. In addition, they are used as protein-rich feed for livestock. By adding organic matter, oil cakes improve water-holding capacity, support strong root development, and gradually supply essential nutrients like nitrogen, phosphorus, and potassium for continuous plant nourishment.

Table 1: Observed household-level organic manure practices and their commonly reported functions

Organic manure	Commonly reported function	Mode of application
Vermicompost	Soil fertility improvement	Soil application
Vermiwash	Growth support, pest deterrence	Foliar/soil
Poson khar	Organic waste recycling	Soil application
Egg-based manure	Calcium supplementation	Soil/foliar
Banana peel fertilizer	Supplementary nutrient input	Soil/foliar
Mustard oil cake	Nutrient supply	Soil application

Discussion:

The organic manure practices documented in this preliminary survey broadly reflect the principles of sustainable soil and nutrient management reported in earlier studies. The use of vermicompost (Kesu khar) in the surveyed households aligns with previous findings that vermicomposting enhances nutrient availability and soil biological activity through earthworm-mediated decomposition (Edwards & Bohlen, 1996; Ismail, 2005). Household composting practices, such as Poson khar, facilitate the recycling of biodegradable kitchen waste into organic inputs, consistent with established principles of organic matter addition and soil nutrient replenishment (Bot & Benites, 2005).

The continued use of farmyard manure by villagers aligns with reports that such organic inputs improve soil physical properties, enhance nutrient availability, and stimulate microbial activity (Weil and Brady, 2017). The application of plant-derived organic residues, such as banana peel-based preparations, reflects traditional nutrient recycling practices, as these residues contain essential macronutrients like potassium, which play key roles in plant physiological processes including stomatal regulation and nutrient transport (FAO, 2018; Weil and Brady, 2017). Similarly, the use of egg-based organic manures contributes to soil nutrient balance and supports plant structural development due to their calcium content (Weil and Brady, 2017).

The use of mustard oilcake in the surveyed households reflects its effectiveness as an organic nutrient source. Research in the Upper Gangetic Plain has shown that mustard oilcake application, alone or in combination with farmyard manure and biofertilizers, significantly improved wheat growth and physiological parameters such as plant height, tiller number, spike weight, number of grains per spike, and 1000-grain weight compared to untreated controls (Ghasal ***et al.,*** 2025). Wheat grain yield increased by 31–136.5% over control treatments, and integrating mustard oilcake with bulky organic manure also enhanced economic returns, demonstrating its value in organic farming systems during the conversion period. Overall, the household-level organic manure practices observed in this study are consistent with the reported functions and benefits of organic inputs, underscoring their significance in sustainable agriculture.

Challenges

Despite the benefits, several challenges were identified, including time-consuming preparation, lower immediate nutrient availability, limited production volume, and delayed visible effects when compared to chemical fertilizers. These constraints influence farmer preference and adoption rates.

Conclusion:

The present case study demonstrates that household-level organic manure practices play a significant role in supporting sustainable agriculture in Jorhat district, Assam. These practices contribute to soil health maintenance, organic waste recycling, and environmentally responsible farming. However, wider adoption requires addressing constraints through farmer training, awareness programs, and institutional support. Strengthened collaboration among farmers, researchers, and policymakers will be essential for realizing the long-term potential of organic manure-based farming systems in the region.

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ASSESSMENT OF FRESHWATER ALGAL BIODIVERSITY IN THE LAKES AND PONDS OF SIVAKASI

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Introduction:

Biodiversity is the variability in the life form different level within the specific ecosystem. The various levels of biodiversity working together to form a life on earth. Algal diversity is considered at the levels of richness of species and of higher taxonomic ranks and as the variety of habitats algae dominate and their functional importance in the processes they mediate. Fresh water algae of tamilnadu are based on studies through the various types of algae present in water bodies (Perumal & Anand, 2008). Study of algae diversity is based on their season, depending on the season variation algae appear and disappear (Arulmurugan *et al.,* 2010). So, seasonal variation is combination with ecosystem varied results of algal species biodiversity**.**

Algae are ubiquitous, photosynthetic and diverse eukaryotic (Thituneelagandan, 2015). They act as primary producers and occur in the freshwater includes ponds, oceans, lakes and also present in the rock and soil. According to the Andersen (1992), more or less 40% of global photosynthesis is evaluated by algae. The diversity of algae ranges from the levels of microscopic blue -green to long and complex. In some cases, algae growth associated with other organism called as “Lichens”. The algae occurrence in the water which act as a primary producer of the food chain. The accumulation of phytoplankton in tank waters is one of the best sources of biological detector to measures the amount of pollution in the water (Maya, 2003).

Kumar and amit 2012, investigated that the microalgae are the majority group in prokaryotic and eukaryotic photosynthetic organism in various forms. Algae are abundant and ancient organisms that can be found in virtually every ecosystem in the biosphere (Graham & Wilcox, 2000). Eutrophication is the phenomenon of nutrient overload resulting in highly turbid conditions and algal blooms (Toner *et al.,* 2005). The measurement of algal cell size is recognised as an important aspect in algal studies (Wetzel, 2001). This is because cell size is related to cell biovolume (the total area of an algal cell when measured) and this is an important measurement when studying the role of algae within an aquatic habitat (Sun & Dongyan, 2003). Four species that can function as bio monitors are the unicellular green algae *Selenastrum capricornutum* and *Scenedesmus subspicatus* as well as the marine diatoms *Skeletonema costatum* and *Phaeodacytlum tricornutum* (Graham & Wilcox, 2001).

Microalgae represent an extraordinarily diverse but highly specialized group of microorganisms adapted to various ecological habitats. Their main habitats are freshwater, brackish and marine ecosystems. They are oligeonous microorganisms whose biomass could be converted to potential products such as pigments, fine chemicals, bioactive molecules, and most importantly for biofuels (Spolaore *et al.,* 2006). In this work mainly focused on the biodiversity of algae surrounding the sivakasi region.

Review of Literature

Alifha Severes, (2018) his study was aimed to assess the microalgal diversity of 6 water bodies of Dakshina Kannada region, Mangalore, India and also determine their distribution and abundance. The water samples collected were preserved and the physico-chemical parameters were analysed using standard protocols. Microalgae belonging to Bacillariophycea, Chlorophycea, Cyanophycea and Euglenophycea were identified. Bacillariophytes were dominant in all sites and *Navicula* and *Nitzchia* sp.

Jadhav, (2022), studied 5 classes of algae and 50 different algal groups were found under the class of Chlorophyceae, Charophyceae, Bacillariophyceae, Euglinophyceae and cyanophyceae. These types of algal group members used to help in maintain the healthy ecosystem in the water body. He concluded through his study, algal diversity in lake and various water bodies play a crucial role in conservation of the diversity between the period of February 2017 to January 2018.

Reena *et al.,* (2020) studied the algal diversity of south Rajasthan. Their collides collects algae in the period of July to august 2019. There are 15 different types algal groups are identified belongs to the class of Chlorophyceae is a major part of the planktonic algae.

Magar *et al.,* (2007) observed algal flora of Kasura Reservoir, he found 53 species belonged to 28 genera identified, of which 27 species under 11 genera belonged to Cyanophyceae, 19 species under 12 genera belonged to Chlorophyceae and 7 species under 5 genera belonged to Bacillariophyceae. Jadhav *et al.,* 2007, studied algal diversity of Salim Ali Lake, Aurangabad. A total of 56 species belonged to 39 genera were identified. Which are belonged to Cyanophyceae, Chlorophyceae, Bacillariophyceae and Euglenophyceae. He also studied algal flora of world famous Lonar crater, at their selected sites. A total 25 species belonged to 19 genera were identified. Algal members belonging to Cyanophyceae, Chlorophyceae, Balcillariophyceae and Euglenophyceae were identified. Cyanophyceae members were dominated algal flore of Lonar crater.

According to Mane, (2007) the microbiological examination of water has significance in case of pollution studies. It is generally believed that every water capable of accepting certain minimum amount of pollution without any adverse effect on itself due to natural biological cycles and self-purification capacity. Any undesirable change characteristics of water brings about pollution, which is assessed by studying the biological features of aquatic biota. Study of diversity of any microorganisms is very much essential in any ecosystem. Algae is one of the eukaryotic microorganisms found commonly in water. Algae uses phosphorus and nitrates as major source of nutrient, these are entering into the water body through by sewage, agricultural runoff, industries, residential area and rain waters. The most expanding field of biology is the biodiversity. Study, many researchers worked on many types of diversity and reveals importance of study of biodiversity

Some of the researchers worked on algal diversity are Zimmerman and Cardinale (2013) on algal diversity in North America lakes Ramesh and Aruna, (2015), on diversity of fresh water algae in Trivenisangamam of Nizamabad district, Telangana state, India. Vijayan *et al.,* (2014) on depiction of microalgal diversity in Gundur lake, Tiruchirappali district, Tamil Nadu, South India., Costelloe *et al.* (2005) on algal diversity in lake Eyre Basin. on biodiversity of freshwater algae and cyanobacteria on deglaciated northern part of James Ross Island, Antarctica. All these researchers paper highlighted the diversity of algae and its effect on freshwater.

This Minor project mainly focused on the study of algal diversity in Cracking city of Sivakasi.

Materials and Methods

Study Area

The geographical location of sample collection site 9.45º N 77.8167º E. Surrounding of sivakasi area there is huge amount lakes are present, the water sample collected from the Retta Palam, sivakasi. Amathur and 2 water sample were collected from the lake area of Ayya Nadar Janaki Ammal College, Sivakasi.

Sample Collection

The floating and submerged region of microscopic algal sample was collected in sterile plastic water container. Sample were brought to the laboratory and stored in formalin for further studies (APHA, 2005). The collected water sample was further observed in the light microscope. Through this observation various types of algal groups are predicted with the help of research article. There are 6 algal sample were collected from various places such as Periyakulam, Retta palam, Sengulam, Satchiyapuram, Saraswathi pond and east block of Ayya Nadar Janaki Ammal College, Sivakasi.

Observation and Identification of Algae

Collected water samples was placed in a clean slide and observed thoroughly in the light microscope and morphological features are identified standard book include prescott (1951) and research article (Ganesh, 2022).

Results:

Collections of Samples

Various samples were collected and observed through the microscope and recorded. Based on observation of the water sample there are six different group of algal groups were identified.

Figure1: Periyakulamkanmaai, Retta palam

Figure 2: Sengulam, Satchiyapuram

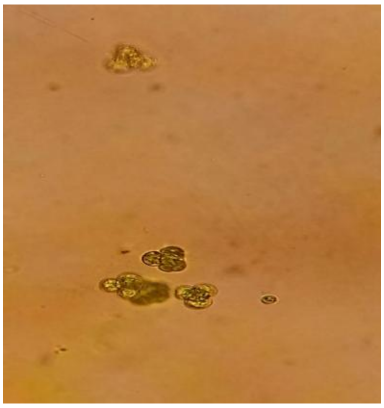
Figure 3: Saraswathi pond Ayya Nadar Janaki Ammal College, Sivakasi

Figure 4: East block pond in Ayya Nadar Janaki Ammal College, Sivakasi

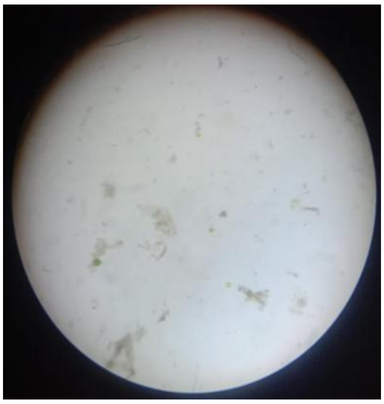
Figure 5: Amathur pond water

Figure 6: Sivakasi pond water

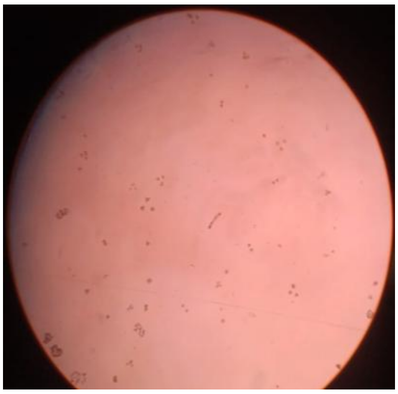
Observation and Identification Algae:



A. *Volovox* Sample collected



B. Sample Collected from

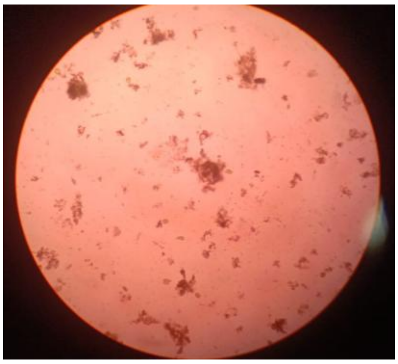


C. Sample collected from

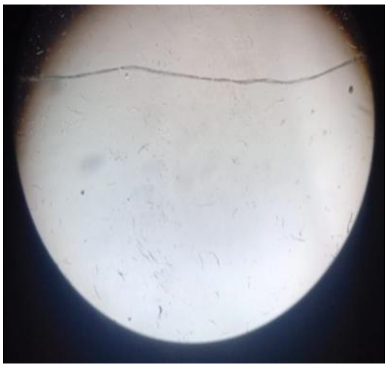
from Satchiyapuram

Ayya Sengulam, Nadar Janaki Ammal College, Sivakasi

Amathur Pond



D. Sample collected from Saraswathi pond



E. Sample collected from Periyakulam kanmaai *Retta palam*

F. *Navicula* sp. Sample Collected *from the Sivakasi*

Identification five different group of algae groups were identified under the 3 different class of algae. Fig. A- *Aphanotheacae* sp (Cyanophyceae), Fig. B- *Chlorella* sp (Chlorophyceae), Fig. C- *Chlorella* sp (Chlorophyceae), Fig. D- *Chlorococcum* sp (Chlorophyceae), Fig. E- *Phormidium* sp (Cyanophyceae), Fig. F- *Navicula* sp (Bacillariophyceae). Based on identification the different algae groups were identified such as Cyanophyceae, Chlorophyceae and Bacillariophyceae.

Cyanophyceae:

Cyanophyceae is a group of primitive algae, contain 150 genera and about 2500 species. In India, this algal group divided by 98 genera and 833 species. Members of the class of Myxophyceae are commonly called as blue green algae. This member of algae group having a dominant pigment C-Phycocyanin called blue green pigment. Additionally, these algae having a other pigments such as Chlorophyll-a, C-Phycoerythrin (Red colour), ß- Carotene and various Xanthophylls are also present. These algae are simplest living autotrophic prokaryotes.

Ex: *Nostoc*, *Oscillatoria*, *Spirulina*, and *Anabaena*.

Chlorophyceae:

Chlorophyceae (Chloros means- Green and Phyceae means- algal organization) is commonly known as ‘Green algae’. Fritsch concluded green algae under the class Chlorophyceae. The name given green algae due to the presence of dominant pigments such as chlorophyll a, Chlorophyll b, carotenoids and Xanthophylls. The cells are eukaryotes. The members of the Chlorophyceae generally grow in fresh water about 90% water and additionally occurs in the saline water, terrestrial habitat etc. The fresh water members such as *Volvax*, *Oedogonium* and *Spirogyra*. Grow in ponds, pools and lakes.

This class consist of 425 genera and about 6500 species but later Prescott (1966) reported that the number of species may be as many as 20,000.

Ex: *Chlamydomonas, Chlorella, Dunaliella*, *Oedogonium* and *Volvax*.

Bacillariophyceae

Bacillariophyceae is a large group of algae containing of 200 genera and over 10,000 species out of which 92 genera and about 569 species are reported from India. They are commonly known as diatoms. The diatoms are most important microscopic algae due to their structure and their cell walls. They occur in various like fresh water and saline water and also epiphytes along with algae, leaf of forest tress, mostly present in tropical rain forests. Depending on the mode of nutrition they may be photosynthetic autotrophs or photosynthetic symbionts or heterotrophs.

Ex: *Cyclotella*, *Fragilaria*, *Rutilaria*, *Pinnularia* and *Navicula*.

Discussion:

Various Water sample collected from surrounding area of sivakasi region. In this present study 5 types of algal genera are identified, such as *Aphanotheacae* sp, *Chlorella* sp *Chlorococcum* sp, *Phormidium* sp and *Navicula* sp. Under the class of Cyanophyceae, Chlorophyceae and Bacillariophyceae. The collected water samples enriched with a good diversity of green algal species which is used to biofuel production.

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A STUDY ON BIODIVERSITY AND CONSERVATION OF THE BAIRLUTY DIVISION OF THE NALLAMALA FOREST AREA, ANDHRA PRADESH

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Abstract:

The present study evaluates the phytosociological structure and vegetation composition of the Nandyal Forest Division, located in the south-eastern part of Kurnool District, Andhra Pradesh. The region experiences a dry tropical climate with an average annual rainfall of 914 mm and supports diverse soil types, including black cotton, alluvial, brown loamy, and red soils. Quantitative analysis revealed a rich floristic diversity comprising 26 tree species, 16 shrubs, 23 herbs, and 15 climbers. Phytosociological parameters such as frequency, density, abundance, basal area, relative density, relative frequency, relative dominance, and Importance Value Index (IVI) were assessed to determine species structure and ecological significance. The findings highlight the ecological complexity and conservation value of this dry deciduous forest ecosystem.

Keywords: Nandyal Forest Division, Phytosociology, Frequency, Density, Basal area, Relative dominance, Importance Value Index (IVI)

Introduction:

Biodiversity encompasses the total variety of life forms present on Earth, including species, populations, communities, and ecosystems, both wild and domesticated, that collectively sustain the biosphere. It represents heritable variation at multiple hierarchical levels of biological organization, ranging from genetic diversity within local populations to species diversity within communities and ecosystem diversity across landscapes. Biodiversity is not restricted to the presence of distinct species alone but also includes the ecological interactions, processes, and cycles that link organisms with one another and with their physical environment. Globally, biodiversity is unevenly distributed, with the highest concentration occurring in equatorial and tropical regions due to favorable climatic conditions such as high temperature and abundant rainfall. Tropical rainforests of Central and South America, Equatorial Africa, and Southeast Asia are recognized as biodiversity hotspots supporting extraordinary levels of species richness and endemism. Communities with greater biological diversity tend to be more resilient, stable, and functionally efficient, enabling them to withstand environmental disturbances and maintain ecosystem services. Thus, biodiversity plays a critical role in ecological sustainability, climate regulation, nutrient cycling, and the long-term survival of life on Earth.

India is one of the world's most biologically diverse countries, despite occupying only about 2–2.5% of the Earth's total land area. Remarkably, it harbors approximately 7.8–8% of the world's known biodiversity, placing it among the twelve globally recognized mega-diverse nations. This exceptional biological wealth is attributed to India's vast geographical extent, diverse topography, and wide climatic gradients, which together support a broad spectrum of ecosystems ranging from tropical rainforests and mangroves to alpine meadows, deserts, wetlands, and coastal marshes. Biodiversity in India includes not only millions of species of plants, animals, and microorganisms but also the genetic variation within species and the ecological processes that sustain complex ecosystems. The country contains nearly all of South Asia's major ecological zones, reflecting its ecological representativeness (Rodgers & Panwar, 1988). According to the Food and Agriculture Organization of the United Nations (FAO), tropical forests are broadly categorized into eight types, including tropical wet deciduous, tropical dry forest, tropical shrubland, tropical desert, tropical mountain, subtropical mountain, and temperate mountain forests. Of these, tropical dry deciduous forests, tropical moist deciduous forests, and tropical shrublands are dominant in much of peninsular India, forming the backbone of its forest biodiversity.

Andhra Pradesh, located in the southeastern part of India, exhibits substantial ecological and biological diversity owing to its varied terrain, climate, and vegetation types. The state spans landscapes ranging from the Eastern Ghats and Nallamala hill ranges to the fertile river valleys and the coastal plains along the Bay of Bengal. Based on floristic and faunal composition, the forests of Andhra Pradesh are broadly divided into four major biotic provinces: the Deccan Plateau, the Central Plateau, the Eastern Highlands, and the East Coastal Plains. Champion and Seth (1968) classified the forests of the state into five major forest type groups and twenty forest types, with dense tropical forests predominating in the Eastern Ghats and sparse shrub vegetation occurring in the rain-shadow regions of the Deccan Plateau. Dry deciduous forests dominate large areas, characterized by species such as *Terminalia*, *Dalbergia*, *Pterocarpus*, *Anogeissus*, and the economically significant teak (*Tectona grandis*). The endemic Red Sanders (*Pterocarpus santalinus*), known for its distinctive color and grain, represents a unique and highly valued component of the state's forest flora. Andhra Pradesh has also been a pioneer in Joint Forest Management (JFM), with nearly one-third of its forest area managed under participatory conservation programs, reflecting a strong emphasis on sustainable forest governance.

The Nallamala Hills, a prominent physiographic feature of the Eastern Ghats, represent one of the most ecologically significant regions of Andhra Pradesh and are renowned for their rich biodiversity (Rao, 1998). Stretching approximately 430 km from the Palnad basin in the north to the Tirupati basin in the south, with an average width of about 30 km, the Nallamala range covers an area of nearly 7,640 km² (Anon, 1965). Elevations range from 200 to 950 m, and the region is characterized by rugged terrain, steep cliffs, deep valleys, plateaus, and gorges. The soils are predominantly black in cultivated plains with low humus content (Krishnan, 1956). Vegetation mainly comprises southern tropical dry deciduous and southern tropical moist deciduous forest types interspersed with shrub growth (Champion & Seth, 1968). Climatically, the region experiences hot and dry conditions, with summer temperatures rising to 43–45°C and winter temperatures falling to about 8°C, while annual rainfall varies between 600 and 1000 mm. The Nagarjunasagar–Srisailam Tiger Reserve (NSTR), located within the Nallamala landscape, epitomizes this vegetation complexity and supports remarkable floral and faunal diversity. The reserve hosts approximately 1,521 angiosperm taxa belonging to 149 families, along with 29 grass species and 353 medicinal plant species. Despite several earlier studies on species composition and diversity, there remains a critical need to examine biodiversity patterns in relation to rainfall variability and ecological gradients, underscoring the importance of the present work.

The most predominant trees found here are:

Local Name	**Scientific name**
Nallamaddi	*Terminalia tomentosa*
Chirumanu	*Anogeissus latifolia*
Billudu	*Chloroxylon swietenia*
Yepi	*Hardwickia binata*
Peedayegi	*Pterocarpus marsupium*
Gumpena	*Lannea coromandelica*
Anduga	*Boswellia serrata*
Pachaari	*Dalbergia paniculata*
Gotti	*Ziziphus xylopyrus*
Chennangi	*Lagerstroemia parviflora*
Tellamaddi	*Terminalia arjuna*


Tellamaddi
(Terminalia arjuna)


Gumpena
(Lannea coromandelica)


Pachaari
(Dalbergia paniculata)


Nallamaddi
(Terminalia tomentosa)

Gotti
(Ziziphus xylopyrus)

Billudu
(Chloroxylon swietenia)

Anduga
(Boswellia serrata)

Chirumanu
(Anogeissus latifolia)

Chennangi
(Lagerstroemia parviflora)

Peedayegi
(Pterocarpus marsupium)

Yepi
(Hardwickia binata)

Andhra Pradesh supports a wide range of plant communities owing to its remarkable physiographic and ecological diversity. The state encompasses varied habitats extending from the hill ranges of the Eastern Ghats and Nallamala to the fertile coastal plains along the Bay of Bengal. Based on floristic and faunal characteristics, the forests of the state are grouped into four major biotic provinces: the Deccan Plateau, the Central Plateau, the Eastern Highlands, and the East Coastal Plains. According to Champion and Seth (1968), the forest vegetation of Andhra Pradesh is classified into five major forest type groups and twenty distinct forest types. Dense tropical forests dominate the Eastern Ghats region, while areas lying beyond the Ghats on the Deccan Plateau are characterized by sparse shrub and dry deciduous vegetation. The dry deciduous forests are largely composed of species belonging to the genera *Terminalia*, *Dalbergia*, *Pterocarpus*, and *Anogeissus*, along with the widely distributed teak (*Tectona grandis*). Among these, the endemic Red Sanders (*Pterocarpus santalinus*), valued for its deep crimson colour and distinctive grain, is of exceptional ecological and economic importance. Nearly one-third of the forest area of Andhra Pradesh is managed under Joint Forest Management (JFM), highlighting the state's leadership in participatory forest conservation.

India, situated in southern Asia between latitudes 6°–38° N and longitudes 69°–97° E, occupies a total geographical area of about 329 million hectares, making it the sixth largest country in the world. It is bounded by the Himalayas in the north, the Bay of Bengal in the east, the Arabian Sea in the west, and the Indian Ocean in the south, and possesses an Exclusive Economic Zone (EEZ) of approximately 2.02 million km² extending up to 200 nautical miles from its coastline. The country experiences a tropical monsoonal climate, receiving rainfall primarily from the south-west and north-east monsoons. Rainfall distribution is highly variable and uneven, with regions such as Goa, Maharashtra, Karnataka, Kerala, West Bengal, and Assam receiving over 2,000 mm annually due to the influence of the Western Ghats. In contrast, the Vindhya ranges receive 1,000–2,000 mm, while the upper Gangetic plains, north-western Deccan, and south coastal plains receive 500–1,000 mm annually. The arid zones of Rajasthan and Gujarat receive as little as about 100 mm of rainfall per year.

Materials and Methods

Reconnaissance surveys were carried out across the district to identify community groups, assess their spatial distribution and socio-cultural status, and document their traditional lifestyles and indigenous knowledge systems. Selected villages were visited at regular intervals, and systematic data were collected on the role of herbs in daily life, healthcare practices, and cultural traditions. Ethnobotanical information was obtained through structured and semi-structured interviews with knowledgeable informants, as well as through direct field observations of plant use. Information related to the medicinal and cultural applications of specific herbs was cross-verified through repeated inquiries and indirect validation with multiple respondents to ensure reliability and accuracy.

Following the Andhra Pradesh Reorganization Act of 2014, Andhra Pradesh emerged as the eighth largest state in India, covering an area of 162,440 km², while Telangana was formed as the 29th state of India on June 2, 2014. Andhra Pradesh is bounded by Telangana to the northwest, Chhattisgarh and Odisha to the north, the Bay of Bengal to the east, Tamil Nadu to the south, and Karnataka to the west. The state has 36,914.7 km² of classified forest area, constituting 22.73% of its total land area, and a population of 49.66 million as per the 2011 Census. Geographically, the state lies between latitudes 12°41′ and 22° N and longitudes 77° and 84°40′ E, with a coastline extending 974 km. It comprises three major physiographic zones: hilly regions, plateaus, and river deltas. The climate is predominantly arid to semi-arid, with temperatures ranging from 8°C to 50°C and annual rainfall between 500 and 1,300 mm, mainly from the southwest and northeast monsoons.

Bairluty Division

Bairluty is a tribal village located in Atmakur Mandal of Kurnool District, Andhra Pradesh. It lies approximately 15 km from Atmakur, 47 km from Dornala, 85 km from Kurnool, 96 km from Srisailam, and about 300 km from Hyderabad. The village forms part of an ecologically significant landscape and has been identified as one of the eco-tourism initiatives promoted by Andhra Pradesh Tourism. Owing to its natural surroundings and accessibility, Bairluty has emerged as a popular weekend destination for visitors from nearby urban centers, particularly Hyderabad.

The climate of the region is predominantly arid to semi-arid in nature. Temperatures generally range from 8°C during the cooler months to about 50°C in peak summer. The area receives an average annual rainfall of 500–1,300 mm, mainly from the southwest and northeast monsoons. The dominant soil types in and around Bairluty include red soil, black cotton soil, alluvial soil, and sandy soil, which together support a mosaic of dry deciduous vegetation.

Nandyal Division

The Nandyal Forest Division is situated in the south-eastern part of Kurnool District, Andhra Pradesh. Geographically, it lies between latitudes 14°53′58.56″ and 15°40′58.08″ N and longitudes 78°20′27.6″ and 78°46′40.08″ E. The division covers an area of approximately 2,600 km^2, accounting for about 14.73% of the total geographical area of the district. The region experiences a predominantly dry climate, with an average annual rainfall of around 914 mm, most of which is received during the southwest monsoon. Temperatures typically range between 20°C and 40°C. The major soil types of the division include black cotton soil, alluvial soil, brown loamy soil, and red soil, which collectively influence the vegetation structure and forest composition of the area.

Conclusion

The present study documents the biodiversity of the Nandyal Division of the Nallamala Forest, Andhra Pradesh. The findings reveal extensive use of local herbs by indigenous communities for medicinal and cultural purposes, reflecting a strong dependence on forest resources. Analysis of the frequency and distribution of tree, shrub, herb, and climber species highlights rich floristic diversity. However, several valuable species are under threat, emphasizing the urgent need for conservation and domestication efforts.

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DIVERSITY OF SEED-BORNE MYCOFLORA IN CEREALS, PULSES AND OILSEEDS FROM MARATHWADA REGION

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Abstract:

Seed-borne fungi play a significant role in reducing seed quality, germination, and crop productivity. The present study investigates the diversity of seed-borne mycoflora associated with Jowar (*Sorghum bicolor*), Gram (*Cicer arietinum*), and Groundnut (*Arachis hypogaea*). Seed samples were collected from local markets and analyzed using standard blotter paper and agar plate methods. A total of 21 fungal species belonging to 11 genera were isolated. The dominant genera included *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus*, and *Alternaria*. Jowar seeds showed maximum fungal diversity followed by gram and groundnut. The presence of toxigenic fungi such as *Aspergillus flavus* and *Fusarium oxysporum* indicates potential risks to seed health and food safety. The results highlight the influence of crop type and storage conditions on fungal diversity. The study emphasizes throughs light on the need for regular seed health monitoring and improved storage practices to ensure sustainable crop production and make fit for consumption. The study highlights the need for regular seed health testing and improved storage practices.

Keywords: Seed-Borne Fungi, Mycoflora, Jowar, Gram, Groundnut, Biodiversity

Introduction:

Seeds are vital natural units responsible for the propagation and continuity of plant species. Apart from serving as planting material, seeds also act as carriers of several microorganisms, including fungi, bacteria and viruses. Among these, seed-borne fungi are of major concern due to their ability to reduce seed viability, impair germination and transmit diseases to the growing crop. Cereals, pulses and oilseeds constitute major agricultural commodities worldwide. Cereals supply carbohydrates, pulses serve as primary protein sources, and oilseeds provide edible oils essential for human nutrition. However, seeds of these crops are often exposed to fungal contamination during harvesting, processing and storage. Fungi associated with seeds may be categorized as field fungi, which infect seeds before harvest, and storage fungi, which proliferate during storage under favourable moisture and temperature conditions.

Several studies have reported the dominance of genera such as *Aspergillus, Fusarium* and *Penicillium* in seed samples. These fungi not only affect seed quality but may also produce mycotoxins, posing serious health hazards. Despite their importance, comparative studies on seed-borne mycoflora across cereals, pulses and oilseeds remain limited. Therefore, the present study was undertaken to assess the diversity of seed-borne mycoflora associated with these crop groups and to understand their implications for seed health. Seeds are the primary means of crop propagation and act as carriers of several microorganisms, including fungi. Seed-borne fungi are responsible for seed deterioration, reduced germination, seedling diseases, and transmission of pathogens to the field. These fungi may be pathogenic, saprophytic, or toxigenic in nature. Jowar (*Sorghum bicolor*) is an important cereal crop, Gram (*Cicer arietinum*) is a major pulse crop, and Groundnut (*Arachis hypogaea*) is a widely cultivated oilseed crop. All three are highly prone to fungal infestation due to their nutrient composition and storage conditions, especially in tropical climates. Despite their economic importance, comparative studies on seed-borne mycoflora of these crops are limited. Hence, the present study was undertaken to evaluate and compare the diversity of seed-borne fungi associated with these crops.

2. Materials and Methods

Collection of Seed Samples

Healthy, untreated seeds of: Jowar (*Sorghum bicolor*), Gram (*Cicer arietinum*) and Groundnut (*Arachis hypogaea*) were collected from local markets and farmer storage facilities. Samples were collected in sterile polythene bags and stored under laboratory conditions until analysis.

Isolation of Seed-Borne Fungi

a) Standard Blotter Paper Method: Seeds were surface sterilized with 1% sodium hypochlorite, placed on moist blotter paper, and incubated at **25 ± 2°C** for 7 days.

b) Standard Agar Plate Method: Seeds were plated on Potato Dextrose Agar (PDA) supplemented with antibiotics and incubated for fungal growth. Agar plate method, 20 ml of PDA was poured in glass Petri plates of 9 cm diameter. In each case, five seeds, treated and untreated, were used and replicated four times. The Petri plates were incubated at 25-27o C±2 under 12h alternating of fluorescent light and darkness for a week. Fungi were identified on the basis of their typical structure and basic characters as suggested by Barnett (1960) and Melone & Masket (1964). Emerging fungal colonies were sub-cultured for identification.

Identification of Fungi: Fungal isolates were identified based on Colony morphology, Spore structure, microscopic characteristics using standard mycological manuals. The seed samples were examined for fungal association using standard seed health testing methods recommended by the International Seed Testing Association (ISTA).

Statistical Analysis: Percentage frequency of occurrence**,** Shannon–Wiener diversity index (H′) and Simpson’s diversity index (D)

Observation:

Diversity of Seed-Borne Mycoflora

A total of 21 fungal species belonging to 11 genera were isolated from all three crops.

Fungal Species Isolated

Sr. No.	Fungal Genera	Jowar	Gram	Groundnut
1.	*Aspergillus flavus*	+++	++	+++
2.	*Aspergillus niger*	++	++	+++
3.	*Fusarium oxysporum*	++	+	++
4.	*Penicillium* spp.	++	++	+
5.	*Penicillium* spp.	++	++	+
6.	*Rhizopus stolonifera*	+	++	++
7.	*Alternaria alternata*	+	+	–
8.	*Curvularia lunata*	++	–	–
9.	*Mucor* spp.	+	+	–
	(+++: High, ++: Moderate, +: Low)			

Diversity Indices

Crop	Shannon Index (H′)	Simpson Index (D)
Jowar	2.78	0.91
Gram	2.34	0.86
Groundnut	2.12	0.83

Results:

The present investigation revealed a considerable diversity of seed-borne mycoflora associated with Jowar, Gram, and Groundnut seeds. Fungal isolation through blotter paper and agar plate methods resulted in the recovery of 21 fungal species belonging to 11 genera.

Discussion:

The highest fungal diversity recorded in Jowar seeds may be due to their high starch content and longer storage duration. The dominance of *Aspergillus* and *Fusarium* species agrees with earlier studies on seed-borne mycoflora of cereals and pulses. Gram seeds showed moderate fungal diversity, while groundnut seeds were highly infected with *Aspergillus flavus*, which is a known aflatoxin producer. The presence of toxigenic fungi raises concerns regarding food safety and post-harvest losses.

Conclusion:

The present investigation reveals significant diversity of seed-borne mycoflora in Jowar, Gram, and Groundnut seeds. Jowar exhibited maximum fungal diversity followed by gram and groundnut. Regular seed health testing, proper storage, and seed treatment measures are essential to prevent fungal contamination and improve crop productivity. The study demonstrates that seeds of cereals, pulses and oilseeds carry diverse fungal communities comprising both pathogenic and storage fungi. Storage fungi were predominant in all crop groups, while pulses exhibited a higher incidence of field fungi. Regular seed health testing, proper drying and improved storage practices are essential to minimize fungal contamination and ensure high-quality seeds.

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POLLEN GERMINATION IN SELECTED ANGIOSPERMS: A COMPARATIVE STUDY

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Abstract

Pollen germination is a critical physiological process in the sexual reproduction of angiosperms, serving as a prerequisite for successful fertilization and seed development. The ability of pollen grains to germinate and produce a functional pollen tube is influenced by intrinsic genetic factors as well as external environmental and nutritional conditions. The present investigation evaluates in vitro pollen germination and viability in selected angiosperm species namely Tithonia, Hibiscus, Dahlia, Impatiens balsamina, Ocimum tenuiflorum, and Catharanthus roseus using a sucrose-based nutrient medium. Fresh pollen grains collected from newly dehisced anthers were cultured in 10% sucrose solution and examined microscopically to assess germination percentage. Considerable interspecific variation in pollen germination was observed, indicating species-specific physiological responses to the nutrient medium. Impatiens balsamina exhibited the highest pollen viability, whereas Ocimum tenuiflorum showed comparatively lower germination. The findings highlight the importance of pollen physiology, nutritional requirements, and adaptability in reproductive success. This comparative study provides baseline data useful for plant reproductive biology, horticulture, and plant breeding research.

Keywords: Pollen Germination, Pollen Viability, Angiosperms, Sucrose Medium, Plant Reproduction

Introduction:

Sexual reproduction in flowering plants is a complex and highly coordinated process that ensures genetic continuity and variation across generations. Among the various stages involved, pollen germination represents a crucial transitional phase between pollination and fertilization. Following successful pollination, pollen grains deposited on a receptive stigma undergo hydration, metabolic activation, and germinate to produce pollen tubes that transport male gametes to the ovule. Any disruption in this process can significantly affect fertilization efficiency and seed set.

Pollen viability and germination capacity are considered reliable indicators of male fertility in plants. These parameters are influenced by genetic constitution, pollen wall structure, enzymatic activity, and environmental factors such as temperature, humidity, and nutrient availability. In vitro pollen germination techniques have been widely employed to evaluate pollen viability, as they offer controlled conditions for understanding pollen behavior independent of stigma-related variables.

Sucrose-based media are commonly used in pollen germination studies because sucrose serves both as an osmotic regulator and an energy source for pollen tube growth. Comparative studies involving different plant species help elucidate species-specific pollen requirements and reproductive strategies. The present study focuses on selected angiosperms commonly found in ornamental and garden settings, aiming to compare their pollen germination behavior under uniform in vitro conditions.

Objectives

- To study in vitro pollen germination in selected flowering plant species.
- To compare pollen viability percentages among different taxa.
- To understand the influence of sucrose medium on pollen tube development.
- To document species-specific variations in pollen germination behavior.

Review of Literature

Extensive research has been conducted on pollen biology, emphasizing the structural and functional aspects of pollen grains. Shivanna and Johri highlighted the role of pollen wall composition and cytoplasmic constituents in determining pollen viability. Heslop-Harrison emphasized the significance of pollen–stigma interactions in regulating pollen hydration and germination. Studies by Stanley and Linskens provided comprehensive insights into the biochemical requirements of pollen germination, including the role of sugars, calcium ions, and boron.

Taylor and Hepler demonstrated that pollen tube growth is a highly polarized process dependent on cytoskeletal dynamics and nutrient availability. Several researchers have reported species-specific responses to different sucrose concentrations, suggesting that optimal germination media vary among taxa. Comparative in vitro studies have been successfully applied in horticulture and plant breeding programs to assess fertility status and hybridization potential. However, limited comparative data are available for commonly cultivated ornamental angiosperms, which justifies the present investigation.

Materials and Methods

Plant Material

Freshly opened flowers were collected from healthy plants growing under natural conditions. The selected species included Tithonia (Asteraceae), Hibiscus (Malvaceae), Dahlia (Asteraceae), Impatiens balsamina (Balsaminaceae), Ocimum tenuiflorum (Lamiaceae), and Catharanthus roseus (Apocynaceae). These species were chosen due to their availability, contrasting floral morphology, and taxonomic diversity.

Reagents and Apparatus

- Sucrose solution (10%)
- Distilled water
- Cavity slides and cover slips
- Dropper and fine brush
- Petri dishes with moist blotting paper
- Compound microscope

Methodology

A 10% sucrose solution was prepared by dissolving 10 g of sucrose in 100 ml of distilled water. A drop of the solution was placed on a clean cavity slide. Fresh pollen grains collected from dehisced anthers were gently transferred to the sucrose drop using a fine brush. The preparation was covered with a cover slip and incubated in a moist chamber at room temperature to prevent desiccation. After 15–30 minutes of incubation, the slides were observed under a compound microscope. Pollen grains showing pollen tube emergence longer than the pollen diameter were considered germinated.

Calculation

Below is the formula used to calculate Percentage of Pollen viability

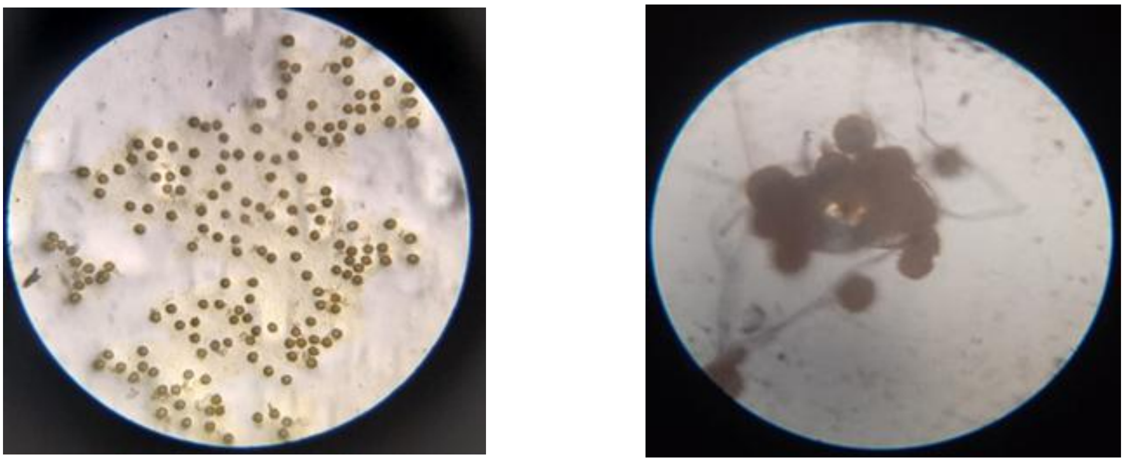
Pollen sterility percentage can be obtained by subtracting Pollen viability with 100 (as percentage is considered)

Percentage of pollen germination=Total number of pollen grains observed/Number of germinate pollen grains×100

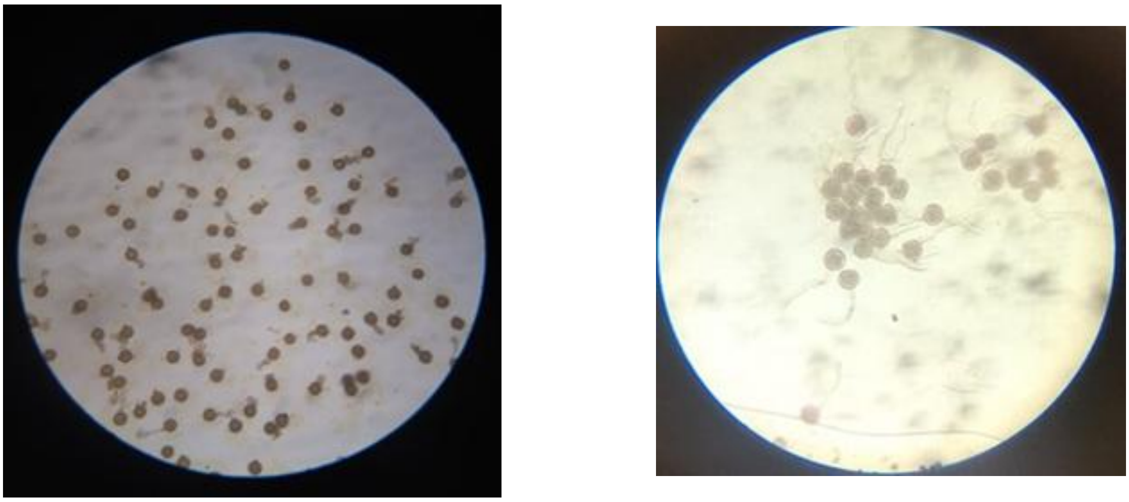
Results

Species	**Total Pollen Count**	**Germinated**	**Non-germinated**	**Viability (%)**	**Sterility (%)**
Tithonia	20	17	3	85	15
Hibiscus	50	33	17	66	34
Dahlia	20	14	6	70	30
Impatiens balsamina	20	18	2	90	10
Ocimum tenuiflorum	20	9	11	45	55
Catharanthus roseus	20	10	10	50	50

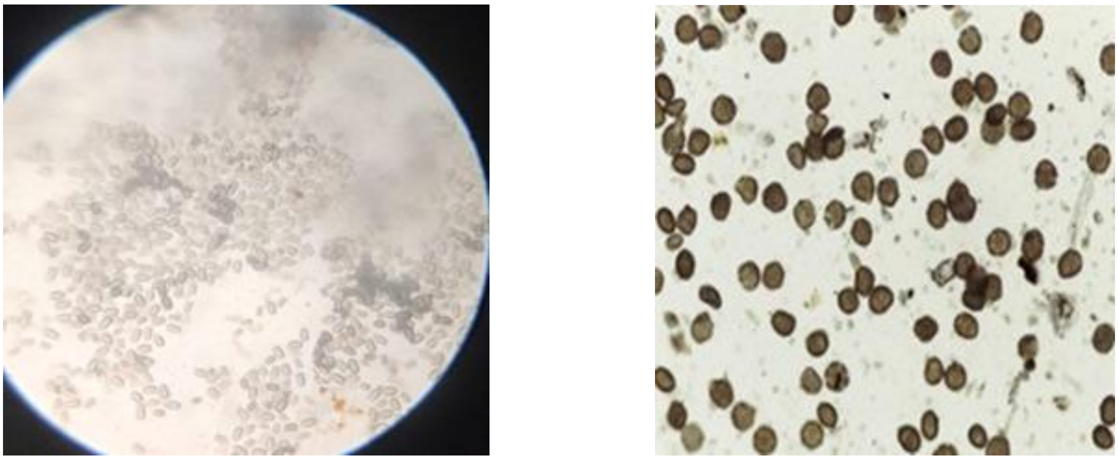
Pollen germination in



Tithonia Hibiscus



Dahlia Catharanthus roseus



Impatiens *balsamina Ocimum tenuiflorum*

Results:

The results of in vitro pollen germination revealed marked variation among the studied species. Impatiens balsamina exhibited the highest pollen germination percentage, indicating high pollen viability and favorable response to the sucrose medium. Tithonia and Dahlia showed moderate to high germination percentages, suggesting efficient utilization of the nutrient medium. Hibiscus displayed moderate pollen viability, while Ocimum tenuiflorum and Catharanthus roseus showed comparatively lower germination percentages.

The observed differences in pollen germination reflect variations in pollen physiology, wall composition, and metabolic activity among species. The results are summarized in the table showing total pollen count, germinated pollen grains, non-germinated pollen grains, viability percentage, and sterility percentage for each species.

Discussion:

The variation in pollen germination observed in the present study highlights the species-specific nature of pollen physiology. High pollen viability in Impatiens balsamina may be attributed to efficient osmotic regulation and rapid metabolic activation in response to the sucrose medium. Similar observations have been reported in other members of Balsaminaceae, known for high reproductive efficiency.

Moderate germination in Hibiscus could be associated with its wet stigma type, which normally facilitates rapid pollen hydration in vivo. The comparatively lower germination observed in Ocimum tenuiflorum and Catharanthus roseus may be due to differences in pollen wall structure, sporopollenin composition, or specific nutrient requirements not fully met by sucrose alone.

These findings support earlier reports that while sucrose is essential for pollen germination, additional factors such as calcium and boron may be required for optimal pollen tube growth in certain species. The study reinforces the importance of standardized in vitro techniques for comparative pollen viability assessment.

Conclusion:

The present investigation demonstrates significant interspecific variation in pollen germination among selected angiosperms under uniform in vitro conditions. The results emphasize that pollen viability and germination efficiency are influenced by species-specific physiological traits. In vitro pollen germination using sucrose medium proves to be a simple and effective method for assessing pollen viability. The findings contribute to a better understanding of plant reproductive biology and have practical implications in horticulture, plant breeding, and educational laboratory studies.

Future Scope:

Future studies may focus on evaluating the effects of different sucrose concentrations, temperature regimes, and additional nutrients such as calcium and boron on pollen germination. Such studies would provide deeper insights into the nutritional and environmental requirements of pollen tube growth across diverse angiosperms.

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ENDOPHYTES AS NATURAL AGENTS FOR PLANT GROWTH PROMOTION AND PATHOGEN DEFENSE

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Introduction:

Endophytes are endosymbionts which are most commonly bacteria or fungi—that inhabit plant tissues for part or all of their life cycle without causing any detectable disease symptoms. These microorganisms are widespread and have been documented in every plant species studied so far. Although the mechanisms behind many plant–endophyte interactions remain incompletely understood, such associations are frequently observed to be mutually beneficial.

The association between plants and endophytes has evolved for over 300 million years. This long co-evolution is reflected in the strong host specificity seen in many endophytes. For instance, several *Lophodermium* species are known to colonize only particular hosts within the plant family Pinaceae. The term *endosymbiont* was first introduced by Heinrich Anton de Bary in 1866 to describe an organism that resides within the body or cells of another organism.

Bacterial Endophytes

Common bacterial endophytes

Endophytic bacteria	Host plant
Azorhizobium caulinodans	Rice
Azospirillum brasilense	Banana
Bacillus cereus	Rice
Bradyrhizobium japonicum	Rice
Chromobacterium violaceuma	Rice
Citrobacter sp.	Banana
Enterobacter sakazakiia	Soybean
Herbaspirillum rubrisulbabicans	Sugarcane
Methylobacterium mesophilicuma	Citrus
Pseudomonas cichori	Grapes
Rhizobium radiobacter	Carrot, Rice
Xanthomonas species	Grapes

Bacterial endophytes play a crucial role in promoting plant growth by producing essential phytohormones such as auxins and gibberellins, fixing atmospheric nitrogen, enhancing phosphate availability, and improving overall nutrient uptake. They also help plants withstand various abiotic stresses, including drought and salinity. In addition, bacterial endophytes contribute to disease control by synthesizing antibiotics, enzymes, and other bioactive compounds that suppress soil-borne pathogens. Through these symbiotic interactions, endophytic bacteria enhance plant physiological functions, resulting in improved growth, vigor, and resilience.

Fungal endophytes

Endophytic fungi colonize both the intercellular and intracellular regions of healthy plant tissues, establishing close and often long-lasting symbiotic relationships with their hosts. These fungi produce a diverse array of antibiotics, enzymes, and other bioactive compounds that not only help them survive in microbe-rich environments but also influence the plant's internal ecosystem. In addition, the secondary metabolites secreted by endophytic fungi can protect the host from bacterial and fungal pathogens, enhance tolerance to environmental stresses, and even stimulate plant growth by modulating nutrient uptake, hormone production, and overall physiological activity. Such multifunctional interactions make endophytic fungi key contributors to plant health and productivity.

Common fungal endophytes

Endophytic fungi	**Host plant**
Alternaria spp.	Soybean
Colletotrichum magna	Tomato
Colletotrichum boninense	Bugweed
Fusarium culmorum	Tomato
Trichoderma homatum	Wheat
Streptomyces hygroscopicus	Rice
Fusarium oxysporum	Cucumber
Penicillium oslonii	Wheat

Transmission of Endophytes

Endophytic microbes can spread through vertical or horizontal transmission:

1. Vertical transmission

This pathway involves the transfer of endophytes from parent plants to their offspring. It typically occurs when fungal hyphae penetrate developing seeds or when they are carried through the plant's pollen.

2. Horizontal transmission

In this mode, endophytes are acquired from the environment through asexual conidia or sexual spores that colonize plant tissues during growth.

Role of Endophytes in Soil

Endophytes—microorganisms that inhabit internal plant tissues without causing disease—play a vital role in soil health and plant productivity. They establish beneficial associations with their host plants and significantly contribute to nutrient cycling, stress tolerance, and overall ecosystem functioning. Their roles can be broadly categorized into plant growth promotion, biosynthesis of bioactive compounds, biocontrol activity, and phytohormone production.

1. Plant Growth Promotion

Endophytic microorganisms enhance plant growth through a combination of direct and indirect mechanisms. These interactions help plants adapt to diverse environmental conditions while improving their growth, productivity, and resilience.

a) Direct Mechanisms

Production of Phytohormones

Many endophytic microbes synthesize key plant hormones such as auxins, gibberellins, and cytokinins. These molecules regulate various physiological processes, including root and shoot elongation, cell division, and tissue differentiation. By influencing these growth pathways, endophytes contribute to stronger roots, healthier shoots, and improved biomass accumulation.

Nitrogen Fixation

A number of endophytic bacteria possess the ability to fix atmospheric nitrogen (N_2) into ammonia (NH_3), which plants can readily absorb. This process significantly enhances soil fertility and reduces the dependence on synthetic nitrogen fertilizers. *Examples include: Azospirillum*, *Herbaspirillum*, *Azoarcus*, *Burkholderia*, *Bacillus*, and *Enterobacter* species.

Phosphate Solubilization

Certain endophytes can convert insoluble forms of phosphate into bioavailable forms through organic acid production and enzyme secretion. This facilitates improved phosphorus uptake, an essential element for plant metabolism, energy transfer, and reproductive growth. *Common examples: Bacillus* spp., *Pseudomonas* spp.

Enhanced Nutrient and Water Uptake

Endophytes often produce enzymes such as cellulases, pectinases, and proteases that support colonization and create microchannels within plant tissues. This enhances nutrient flow and root surface area. Some strains also promote the formation of fine roots and root hairs, improving water uptake and helping plants withstand drought conditions.

Stress alleviation and metabolic support

Many endophytes aid in the synthesis of osmoprotectants and stress-responsive molecules that allow plants to maintain physiological functions under saline, drought, or temperature stress.

They may also influence the expression of plant genes associated with metabolism, nutrient transport, and defense pathways.

Together, these direct benefits position endophytes as sustainable biological alternatives to conventional chemical fertilizers.

b) Indirect Mechanisms

Endophytes also promote plant growth indirectly by enhancing plant defense and maintaining a healthier rhizosphere environment.

Induced Systemic Resistance (ISR)

Endophytic microorganisms activate plant defense pathways, increasing resistance against pathogens and environmental stresses. This helps plants allocate more energy toward growth and development.

Suppression of Pathogens

By colonizing plant tissues and occupying ecological niches, endophytes limit the entry and spread of harmful microbes. Their metabolic activities help create unfavorable conditions for pathogenic organisms, thereby reducing disease incidence.

2. Bioactive Compounds Produced by Endophytes

Endophytes synthesize a wide variety of secondary metabolites, including quinolones, tannins, alkaloids, phenolic acids, terpenoids, saponins, and xanthones. These bioactive compounds exhibit antimicrobial, antioxidant, antifungal, and growth-promoting properties.

In plants, these metabolites:

- Protect against fungal and bacterial pathogens
- Enhance tolerance to oxidative stress
- Facilitate improved nutrient acquisition
- Promote growth and developmental processes

Beyond agriculture, these compounds hold significant potential in pharmaceutical, food, cosmetic, and biotechnological industries due to their therapeutic properties.

3. Endophytes as Biocontrol Agents

Endophytes act as effective natural biocontrol agents by producing antibiotic-like molecules that inhibit the growth of pathogenic microorganisms. They also modulate plant defense signaling pathways, particularly through the induction of jasmonic acid (JA) and ethylene, which are critical regulators of resistance:

- Ethylene regulates processes such as fruit ripening, flower opening, leaf abscission, and stress responses.
- Jasmonic acid (JA) contributes to defense against necrotrophic pathogens and regulates plant responses to both biotic and abiotic stresses.

Additionally, many endophytes synthesize siderophores, which bind iron and increase its availability to beneficial microbes. This competitive advantage helps them colonize the root environment effectively while suppressing harmful organisms.

Through these combined actions, endophytes significantly reduce disease pressure and support sustainable crop protection.

4. Phytohormone Production by Endophytes

Phytohormone production is one of the most important contributions of endophytic microorganisms. These hormones regulate plant growth, development, and stress responses.

Auxins (Indole-3-Acetic Acid, IAA)

IAA regulates cell elongation, root initiation, shoot development, and fruit set. Endophytic production of IAA enhances root system architecture, improving nutrient and water uptake.

Gibberellic Acid (GA)

GA promotes cell division, seed germination, stem elongation, and fruit development. It also helps break seed dormancy, ensuring proper plant establishment.

Cytokinins

Cytokinins control cell differentiation, delay leaf senescence, and promote root and shoot growth. Their balanced interaction with auxins determines overall plant morphology.

Other Hormones

Many endophytes also produce abscisic acid (ABA) and salicylic acid (SA), which play key roles in stress tolerance, stomatal regulation, and pathogen defense.

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PRESENT AGRICULTURAL PRACTICES AND THEIR EFFECT ON THE ENVIRONMENT

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Abstract:

The modern science and technological activities are yielding disruptive results in the environment.The key reasons of environment pollution are urbanisation, irregular and rapid industrialisation, misuse of agricultural lands and wrong agricultural applications, use of pesticides and chemical fertilisers as well as plant hormone applications contribute to degradation of soil making it unhealthy for human health and environment.Deforestation for agriculture expansion, monoculture farming and reliance on fossil fuels also contribute to climate change and biodiversity loss. So, adopting sustainable agricultural practices is crucial to mitigate the negative impacts of farming on the environment and human health such as integrated pest management, crop diversification, promoting agro ecology and precision agriculture etc. Demand for agricultural commodities is rising rapidly as the world's population grows. Agriculture's deep connections to the world economy, human society and biodiversity make it one of the most important frontiers for conservation around the earth.

Keywords: Environment, Agro Ecology, Monoculture Farming, Biodiversity, Sustainable Agriculture

Introduction:

The environmental impact of agriculture is the effect that different farming practices have on the ecosystems around them, and how those effects can be traced back to those practices. The environmental impact of agriculture varies widely based on practices employed by farmers and by the scale of practice. Farming communities that try to reduce environmental impacts through modifying their practices will adopt sustainable agriculture practices. The negative impact of agriculture is an old issue that remains a concern even as experts design innovative means to reduce destruction and enhance eco-efficiency. Animal agriculture practices tend to be more environmentally destructive than agricultural practices focused on fruits, vegetables and other biomass. The emissions of ammonia from cattle waste continue to raise concerns over environmental pollution.

Present Agricultural Practices

1. Use of Chemical Fertilizers and Pesticides:

- Chemical fertilizers (NPK - Nitrogen, Phosphorus, Potassium) are widely used to boost crop yields.

- Pesticides and herbicides are employed to control pests and weeds.
- Though effective, their overuse has led to soil and water contamination.

Chemical fertilizers and pesticides have played a major role in increasing agricultural productivity, especially after the Green Revolution. However, their excessive and improper use has caused significant harm to human health, soil, water, and ecosystems.

2. Monoculture and Intensive Farming:

- Monoculture involves growing a single crop over large areas, often without crop rotation.
- This depletes specific nutrients from the soil and makes crops more vulnerable to pests.

Examples:

- Growing only wheat in Punjab.
- Tea plantations in Assam.
- Oil palm plantations in Malaysia.

What is Intensive Farming?

Intensive farming (also called **industrial agriculture**) is a high-input, high-output method of agriculture where large amounts of **c**hemical fertilizers, pesticides, irrigation, and machinery are used to maximize crop or livestock yields from a small area.

Examples:

- Green Revolution farming techniques.
- Hydroponic or greenhouse farming with artificial inputs.
- Commercial poultry or dairy farms with high-density animal populations.

3. Mechanization and Use of Fossil Fuels:

- Tractors, harvesters, and irrigation pumps reduce human labor but increase fossil fuel use.
- These machines release greenhouse gases, contributing to air pollution and climate change.

4. Irrigation Practices:

- Large-scale use of water-intensive irrigation methods such as flood irrigation is common.
- This leads to waterlogging, salinization, and over-extraction of groundwater.

5. Genetically Modified (GM) Crops:

- GM crops are engineered for higher yields and resistance to pests.
- Although beneficial, they raise concerns about biodiversity, seed sovereignty, and long-term ecological balance.

6. Stubble Burning:

- After harvesting, farmers burn crop residues to clear fields quickly.
- This releases particulate matter and greenhouse gases into the atmosphere, degrading air quality.

Environmental Effects of Modern Agriculture

1. Soil Degradation

- Loss of organic matter and beneficial microbes due to chemical input.
- Erosion from over-ploughing and loss of fertility from continuous monocropping.

2. Water Pollution and Depletion

- Fertilizer runoff causes eutrophication in water bodies, leading to algae blooms and aquatic life death.
- Overuse of groundwater, especially in Punjab and Haryana, is leading to falling water tables.

3. Air Pollution

- Farm machinery emits carbon dioxide.
- Pesticide sprays contribute to air contamination.
- Stubble burning is a major source of smog and particulate pollution in North India.

4. Loss of Biodiversity

- Monocultures reduce habitat variety, affecting insects, birds, and animals.
- Pesticides kill not only pests but also pollinators like bees and beneficial predators.

5. Climate Change Contribution

- Agriculture contributes nearly 20–30% of global greenhouse gas emissions.
- Methane from rice fields and livestock, and nitrous oxide from fertilizers are potent GHGs.

Steps Towards Sustainable Agriculture:

1. Organic Farming:

- Avoids chemical inputs; uses compost, green manure, and natural pest control.
- Improves soil health and reduces water contamination.

Organic farming is a method of agriculture that emphasizes the use of natural inputs and processes to grow crops and raise animals. It avoids the use of synthetic fertilizers, pesticides, genetically modified organisms (GMOs), and growth hormones. Instead, it promotes ecological balance, biodiversity, and soil health.

Principles of Organic Farming

i. **Health:** Maintain and improve the health of soil, plants, animals, and humans.
ii. **Ecology:** Work with natural systems and cycles rather than trying to dominate them.
iii. **Fairness:** Build relationships that ensure fairness with regard to the environment and life opportunities.
iv. **Care:** Manage farming practices in a precautionary and responsible way to protect future generations.

2. Crop Rotation and Diversification:

- Helps maintain soil fertility and reduces pest infestations.

Crop rotation is the practice of growing different types of crops in the same field in a planned sequence over several seasons. For example, a farmer might grow wheat in year one, legumes in year two, and vegetables in year three on the same piece of land.

3. Drip and Sprinkler Irrigation:

- Reduces water usage and prevents waterlogging and salinization.

Drip irrigation delivers water directly to the root zone of plants through a network of valves, pipes, tubing, and emitters.

Key Features:

- Water is supplied slowly and precisely.
- Ideal for row crops, orchards, and vegetable gardens.
- Minimizes evaporation and runoff.

Advantages:

- Saves up to 30–70% water compared to traditional methods.
- Reduces weed growth, as water is targeted to specific areas.
- Enhances plant health by maintaining consistent moisture levels.
- Suitable for areas with limited water supply.

Sprinkler irrigation simulates rainfall by spraying water into the air, allowing it to fall on crops from above.

Key Features:

- Water is distributed through a system of pipes and sprayed through nozzles.
- Can be used on a variety of terrains and crop types.
- Suitable for large-scale farming.

Advantages:

- Uniform water distribution.
- Can be used for cooling crops and protecting them from frost.
- Reduces soil erosion.

4. Integrated Pest Management (IPM):

- Combines biological, physical, and chemical tools to control pests sustainably.

Integrated Pest Management (IPM) is an environmentally sensitive approach to controlling pests using a combination of techniques. Instead of relying solely on chemical pesticides, IPM integrates biological, cultural, mechanical, and chemical methods to manage pest populations in a way that minimizes harm to people, animals, and the environment.

5. Agroforestry and Permaculture:

- Combining agriculture with tree planting to improve biodiversity and carbon storage.

Agroforestry is a land-use system that integrates trees and shrubs into crop and animal farming systems. It combines agriculture and forestry to create more productive, diverse, and sustainable land-use systems.

6. Conservation Tillage:

- Reduces erosion and increases soil moisture retention.

Conservation tillage is a farming practice that minimizes soil disturbance and maintains crop residues on the soil surface. Unlike conventional tillage (which involves plowing and turning the soil), conservation tillage reduces erosion, conserves moisture, and improves soil health.

In conclusion, while modern agricultural practices have significantly boosted food production and ensured food security, they have also led to serious environmental consequences. Excessive use of chemical fertilizers and pesticides, monoculture farming, over-irrigation, and deforestation have degraded soil quality, polluted water bodies, reduced biodiversity, and contributed to greenhouse gas emissions. The long-term sustainability of agriculture is now under threat due to these unsustainable practices.

To address these challenges, there is a growing need to shift toward eco-friendly and sustainable agricultural methods such as organic farming, crop rotation, conservation tillage, integrated pest management, agroforestry, and precision agriculture. These approaches aim to balance productivity with environmental conservation, ensuring food security for future generations while protecting our natural resources.

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PLANT-BASED NUTRACEUTICALS AND FUNCTIONAL FOODS FOR THE TREATMENT OF RESPIRATORY INFECTIONS

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Abstract:

Respiratory infections represent a major global health challenge, caused by viral pathogens such as influenza viruses and respiratory syncytial virus (RSV), as well as bacterial agents including *Streptococcus pneumoniae* and *Haemophilus influenzae*. Increasing antimicrobial resistance, drug-associated adverse effects, and limited accessibility to healthcare have encouraged the exploration of complementary approaches such as plant-based nutraceuticals and functional foods. Bioactive phytochemicals including curcumin, quercetin, catechins, allicin, and gingerols exhibit antiviral, antibacterial, antioxidant, anti-inflammatory, and immunomodulatory properties relevant to respiratory health. Clinical studies have demonstrated the effectiveness of elderberry in reducing influenza symptom duration and ginger in alleviating cough and throat irritation. Functional food formulations such as herbal teas, fortified beverages, and nutraceutical supplements provide feasible dietary strategies with improved compliance. This chapter critically examines the phytochemical profiles, mechanisms of action, clinical evidence, safety considerations, and future prospects of plant-based nutraceuticals, highlighting their integration into evidence-based respiratory infection management.

Keywords: Respiratory Infections, Plant-Based Nutraceuticals, Bioactive Phytochemicals, Antimicrobial Resistance, Functional Foods.

1. Introduction:

Respiratory infections affect the upper and lower respiratory tract and remain among the leading causes of morbidity and mortality worldwide (WHO, 2023). Viral respiratory infections, including influenza, RSV, rhinovirus, and coronavirus infections, are highly contagious, whereas bacterial infections such as pneumonia and bronchitis are often associated with severe complications (Sharma *et al.,* 2017). Conventional treatment strategies rely on antivirals, antibiotics, and symptomatic management; however, the emergence of antimicrobial resistance and drug-related adverse effects necessitates alternative or adjunctive approaches (Sharma and Singh, 2016).

Nutraceuticals are food-derived products that provide health benefits beyond basic nutrition, while functional foods are consumed as part of the daily diet to promote health and prevent disease (Sharma, 2015). Plant-based nutraceuticals, widely used in traditional medicine systems, are gaining scientific attention due to their immunomodulatory and antimicrobial potential in respiratory infections (Rondanelli *et al.,* 2018).

2. Phytochemical Profiles of Plant-Based Nutraceuticals

Plant-based nutraceuticals contain diverse bioactive compounds responsible for their therapeutic actions (Sharma *et al.,* 2017).

a) Curcuminoids

Curcumin, the principal bioactive constituent of *Curcuma longa* (turmeric), exhibits strong anti-inflammatory, antioxidant, and antiviral properties. It modulates inflammatory cytokines and suppresses viral replication pathways involved in respiratory infections (Aggarwal and Sung, 2009).

b) Flavonoids

Quercetin, found abundantly in onions, apples, and berries, demonstrates antiviral activity by inhibiting viral entry and replication. Additionally, it stabilizes mast cells and reduces histamine release, which is beneficial in allergic and inflammatory respiratory conditions (Sharma *et al.,* 2017).

c) Organosulfur Compounds

Allicin from *Allium sativum* (garlic) exhibits broad-spectrum antimicrobial activity against respiratory viruses and bacteria, contributing to reduced incidence of common colds (Sharma and Singh, 2016).

d)Phenolic Compounds and Terpenoids

Gingerols and shogaols present in *Zingiber officinale* (ginger) exert antitussive, anti-inflammatory, and antimicrobial effects, supporting the traditional use of ginger in respiratory ailments (Grzanna *et al.,* 2005).

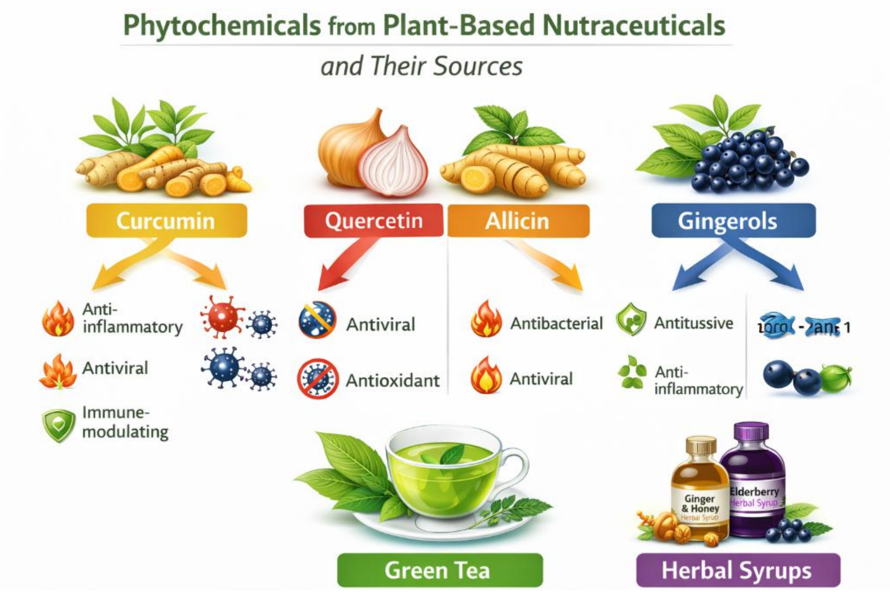

Figure 1: Major Phytochemicals from Plant-Based Nutraceuticals and Their Sources

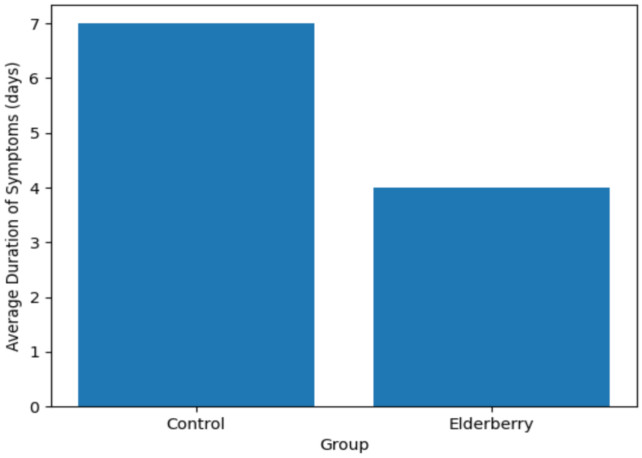

Figure 2: Effect of Elderberry on Influenza Symptom Duration

3. Mechanisms of Action

Plant-based nutraceuticals exert therapeutic effects through multiple biological pathways (Sharma, 2015).

a) Antiviral and Antibacterial Mechanisms

Several phytochemicals inhibit viral replication enzymes, prevent viral attachment to host cells, and disrupt bacterial cell membranes, thereby limiting pathogen survival (Sharma *et al.,* 2017).

b) Immunomodulatory Effects

Nutraceuticals enhance innate immune responses by activating macrophages and natural killer cells while regulating adaptive immune pathways and cytokine production (Rondanelli *et al.,* 2018).

c) Anti-inflammatory and Antioxidant Activity

Suppression of nuclear factor-kappa B (NF-κB) signaling and reduction of oxidative stress help mitigate inflammation-induced lung damage during respiratory infections (Aggarwal and Sung, 2009).

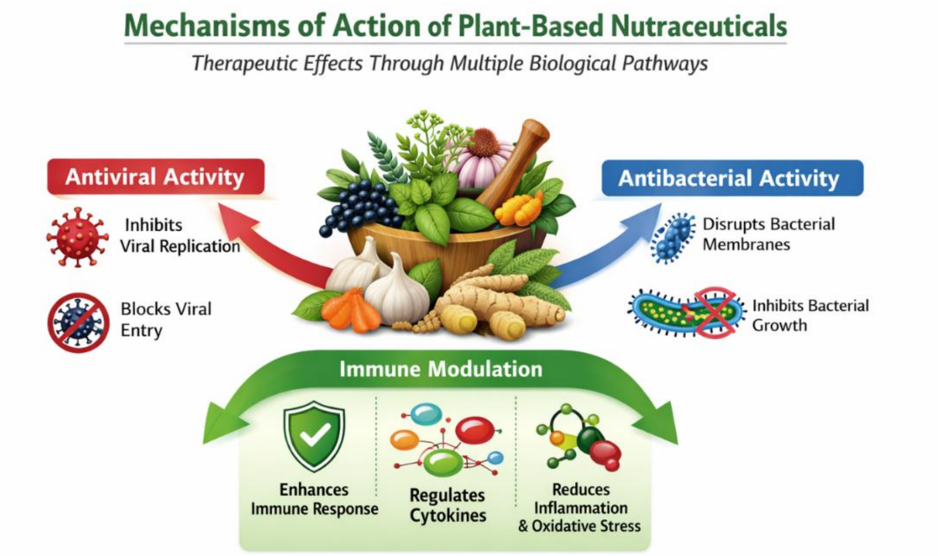

Figure 3: Mechanisms of Action of Plant-Based Nutraceuticals in Respiratory Infections

4. Clinical Evidence Supporting Plant-Based Interventions

Clinical studies provide evidence for the efficacy of selected plant-based nutraceuticals. Elderberry (*Sambucus nigra*) supplementation has been shown in randomized controlled trials to significantly reduce the duration and severity of influenza symptoms (Zakay-Rones *et al.,* 2004). Ginger (*Zingiber officinale*) has demonstrated effectiveness in relieving cough, throat irritation, and bronchial inflammation in respiratory infections (Grzanna *et al.,* 2005). Garlic (*Allium sativum*) intake has been associated with reduced incidence and severity of common cold episodes (Sharma and Singh, 2016). Green tea catechins have also been linked to decreased risk of upper respiratory tract infections due to their antiviral and immune-enhancing properties (Rondanelli *et al.,* 2018).

5. Functional Foods for Respiratory Health

Functional foods offer a practical approach to delivering bioactive compounds in daily diets (Sharma, 2015). Common formulations include herbal teas prepared from ginger, tulsi, and green tea; fortified smoothies containing turmeric, citrus fruits, and berries; honey-based herbal syrups; and fermented plant-based beverages. These food forms enhance bioavailability and improve consumer adherence (Sharma *et al.,* 2017).

6. Safety, Dosage, and Drug Interactions

Although plant-based nutraceuticals are generally considered safe, excessive intake may result in gastrointestinal disturbances or herb–drug interactions (Sharma and Singh, 2016). Certain phytochemicals may interact with anticoagulants, antiplatelet agents, or immunosuppressive drugs. Standardization of dosage, quality control, and medical consultation—particularly for pregnant women, children, and immunocompromised individuals—are essential for safe use (Rondanelli *et al.,* 2018).

Figure 4: Common Functional Food Formulations for Respiratory Health

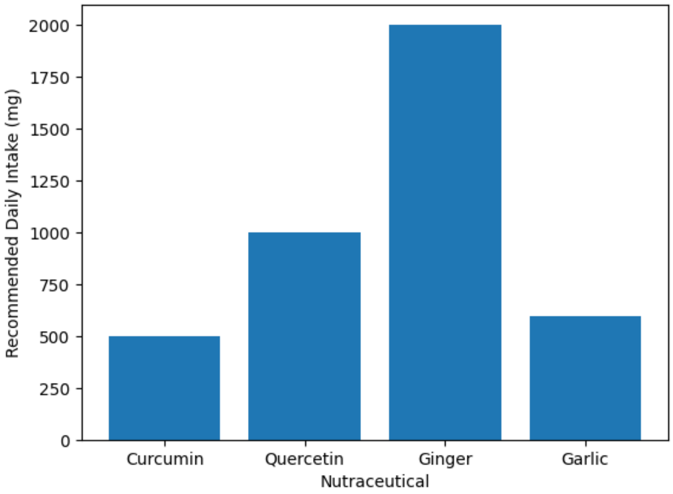

Figure 5: Recommended Daily Intake Ranges of Common Plant-Based Nutraceuticals

7. Conclusion and Future Perspectives

Plant-based nutraceuticals and functional foods represent promising complementary strategies for the prevention and management of respiratory infections. Their antiviral, antibacterial, anti-inflammatory, and immunomodulatory mechanisms, supported by clinical evidence, justify their integration into conventional therapeutic regimens (Sharma, 2015; Sharma *et al.,* 2017). Future research should emphasize large-scale clinical trials, standardized formulations, and synergistic combinations to strengthen evidence-based application in respiratory healthcare.

Table 1: Plant-Based Bioactives: Compounds, Mechanisms, and Recommended Intake

Plant Source	**Primary Bioactive Compound**	**Mechanism Summary**	**Suggested Dietary/Supplemental Intake**
Turmeric (*Curcuma longa*)	Curcuminoids (Curcumin)	Inhibits NF-kB and pro-inflammatory cytokines (IL-6, TNF-α).	500–1,000 mg/day (often with piperine for bioavailability).
Garlic (*Allium sativum*)	Allicin / S-allyl cysteine	Antimicrobial; stimulates Natural Killer (NK) cell activity.	1–2 cloves of raw garlic or 600–1,200 mg aged garlic extract.
Ginger (*Zingiber officinale*)	Gingerols & Shogaols	Bronchodilatory effects; inhibits leukotriene biosynthesis.	1–3 g of dried powder or 5–10 g of fresh rhizome in infusions.
Onion (*Allium cepa*)	Quercetin (Flavonoid)	Mast cell stabilizer; reduces histamine release in allergies.	250–500 mg (as supplement) or high inclusion in daily diet.
Green Tea (*Camellia sinensis*)	EGCG (Catechins)	Antioxidant; disrupts viral attachment to respiratory mucosa.	3–5 cups/day (standardized to ~250 mg catechins).

Future Perspectives

Despite the promising data, several hurdles remain for the global adoption of nutraceuticals in mainstream respiratory care:

- **Standardization:** There is an urgent need for standardized extraction protocols to ensure consistent concentrations of "marker compounds" across different batches.
- **Bioavailability:** Future research should focus on advanced delivery systems, such as **nano-emulsions** or **lipid-based carriers**, to enhance the systemic absorption of hydrophobic compounds like curcumin.
- **Clinical Validation:** While *in vitro* results are robust, large-scale, double-blind, placebo-controlled human trials are essential to establish definitive dosage guidelines and safety profiles.
- **Regulatory Frameworks:** Harmonizing the regulatory status of these products across different regions will facilitate international trade and consumer trust.

In conclusion, the strategic inclusion of plant-based nutraceuticals in the daily diet offers a sustainable and cost-effective strategy to bolster respiratory resilience and reduce the global burden of chronic pulmonary diseases.

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THE ENTOMOLOGICAL STUDY EXPLORES THE FRONTIERS OF THE COMPLEMENTARY AND SUPPLEMENTARY ROLES OF THE BIOLOGICAL PROCESSES OF THE SILKWORM *BOMBYX MORI* LINN.

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Abstract:

The life cycle of the *Bombyx mori* insect, which is very important for the silk industry, depends entirely on humans. Its entire genome has been mapped out. It lives for a short time, is easy and cheap to care for, has fewer ethical issues, and has several genes that are very similar to genes involved in human diseases. Because of these features, it has been recognized as a possible alternative model organism for studying life sciences. In the past ten years, it has been used in many scientific areas including human disease research, environmental monitoring, epigenetic studies, and drug discovery. Like other traditional model organisms such as fruit flies and roundworms, the silkworm has shown promising results in its short lifespan and has a good future ahead. The use of silkworms as a model species in health and disease research can help us better understands biological processes, even though they can't fully replace mammals as model organisms. In short, using silkworms as a model in life science can help advance research in many areas by offering new perspectives. This might even open up new areas of study that were not possible before.

Keywords: *Bombyx mori*, Human Diseases Research, Epigenetic and Drug Discovery.

Introduction:

A major economic insect is the silkworm, *Bombyx mori*. It is also a well studied member of the Lepidoptera group. The body of the silkworm is made up of segments called somites, which are divided into three parts: the head, thorax, and abdomen. The head, also called the cephalosome, is a complex structure that contains the most advanced endocrine system in insects. It includes the brain, sub oesophageal ganglion, and corpora allatum, which control various biological processes in insects. In addition to the antennae, ocelli, mouthparts, and spinnerets, the silkworm's head has other connected organs. Because of the variety of organs it contains, the head is a multi functional part of the body. The silkworm, *Bombyx mori*, is famous for producing silk thread and is the base of the silk industry. Unlike many other Lepidoptera insects that eat a variety of foods, the silkworm is monophagous, meaning it only eats mulberry leaves during its different larval stage. After mating, adult female silkworms lay eggs that hatch into small, hairy black first instar larvae. These larvae go through four molts, creating five different growth stages. From a cocoon made by the fifth stage larva, an adult emerges, finds a mate, and reproduces. After mating, females lay eggs. The eggs are creamy white when laid, but they turn black if they are fertile. The life cycle of the silkworm is shown in a diagram. Animal models help scientists understand various scientific challenges. For this reason, animals like mice, rabbits, and monkeys are often used. However, using these higher animals brings several problems, including ethical concerns, animal rights issues, and the high cost of keeping them. Because of their widespread use in research, scientists have been trying to create an alternative model organism to reduce the burden on these higher order animals. Although invertebrate models like *Drosophila melanogaster* and *Caenorhabditis elegans* have helped reduce the need for higher animals, their small size makes it hard to handle them and inject precise amounts of medicine. *Bombyx mori*, an invertebrate insect, has qualities that make it ideal as a model organism. These include a larger body size compared to other invertebrates, a short life cycle, fewer ethical issues, lower breeding costs, a clear genetic background, and a large number of genes that are similar to human genes. Researchers have started studying this insect not only as a silk producer but also as a possible model for life science research because of its special features. The use of silkworms has increased a lot in areas like drug screening, environmental studies, testing for anti-pathogenic drugs, antiviral research, and toxicological studies. This chapter covers various areas related to human health and diseases where *Bombyx mori* has been used or could be used as a model organism, and it also adds new information to support other published chapters in this field.

Metabolic disorders of *Bombyx mori*:

Infections from pathogens, especially the *Bombyx mori* Nucleo polyhedrovirus (BmNPV), greatly affect how the body uses amino acids. When the diet lacks important amino acids, certain amino acids build up in the blood, and too much nitrogen waste like urea and uric acid is produced. A shortage of glycine, which is needed to make silk protein, leads to lower silk production. Poor quality diets can also slow down how the body breaks down carbohydrates. Important chemicals like trehalose, glucose, and parts of the TCA cycle (like fumaric acid and succinic acid) often go down, which means the body can't make enough energy for normal growth and fighting off infections. If the diet doesn't provide enough sterols or essential fatty acids, lipid metabolism can be messed up, which affects hormone production, like ecdysone, and lowers cholesterol levels, slowing down development. BmNPV infection can also harm oxidative phosphorylation, the main way the body makes ATP. Silkworms that resist disease show higher levels of metabolites involved in energy production through this process, showing that strong energy metabolism is connected to disease resistance. Diets that lack important vitamins, like thiamine, biotin, riboflavin, and alpha tocopherol, can weaken the immune system, mess up the metabolism of carbohydrates, fats, and amino acids, and make the silkworm more likely to get sick.

(i) Uric acid metabolism:

A major and growing health issue around the world is hyper uricemia. Even though we don't fully understand how mulberry leaves help in treating this condition, they have been used for a long time in traditional diabetes treatments because they are both a medicinal and edible plant. This study used a hyper uricemic rat model and network pharmacology to predict the active ingredients and key targets of mulberry leaf extract (MLE), and also checked its effects. The study looked at how MLE affects uric acid metabolism, the body's internal pathways, and gut bacteria by combining metabolomics, molecular docking, and 16S rRNA sequencing. According to the network pharmacology results, MLE works through a multi-target and multi-component method. Experimental data showed that MLE lowered serum uric acid levels, increased urate excretion through the kidneys, reduced high lipid levels and kidney inflammation, and improved liver antioxidant activity. Mechanistic studies found that MLE effectively regulates uric acid metabolism by inhibiting xanthine oxidase (XOD) activity in the liver and reducing glucose transporter 9 (GLUT9) expressions in the kidneys and intestines. Metabolomic research showed that MLE can help with lipid metabolism problems caused by hyperuricemia and identified XDH/XOD as key targets. Molecular docking confirmed that nine main components of mulberry leave have high binding affinity to XDH/XOD proteins. 16S rRNA sequencing revealed that MLE helps restore gut bacteria balance by increasing populations of butyrate-producing bacteria, such as Lachnospiraceae NK4A136 and Oscillospiraceae UCG-005. Met Origin analysis and correlation studies further emphasized the important connection between host and microbial co-metabolism in hyperuricemia. Overall, these findings suggest that MLE might be a natural treatment for hyperuricemia and provide a strong scientific foundation for its use in clinical settings. Monosodium urate crystals build up in the joints of patients, causing the painful condition known as gout. Hyperuricemia, which happens when the body doesn't excrete enough uric acid (UA), is closely related to gout. Mice are often used as a model for gout because their physiology is similar to humans, but instead of UA, mice produce allantoises in as the end product of purine metabolism because they have the uricase enzyme, which breaks down UA into allantoin. In contrast, humans don't have this enzyme, so UA is the end result. A study on the silkworm, *Bombyx mori*, found that urate granules are mainly found in the skin, giving it a white color. If the silkworm is treated with a gout medicine that lowers UA levels, the skin color can change from white to transparent. Researchers tested this by feeding fifth instar silkworm larvae mulberry leaves treated with allopurinol and sodium bicarbonate, two gout drugs. The result showed the skin turned from white to transparent as uric acid levels dropped (Zhang *et al.,* 2012). Unlike other invertebrate models such as D. melanogaster and C. elegans, where there is no clear sign of UA metabolism, this feature gives the silkworm a natural advantage. In C. elegans, hyperuricemia is induced by treating with xanthine, UA, and hypoxanthine, but in silkworms, there's no need for such treatment because the white epidermis directly shows UA buildup. While D. melanogaster is also used to model hyperuricemia and related conditions, the ability to screen drugs by simply looking at the epidermis, as in silkworms, isn't possible in D. melanogaster. Thus, silkworms are a better model for testing gout drugs compared to traditional invertebrate models like D. melanogaster and C. elegans.

(ii) Gout model:

Gout is a type of inflammatory arthritis that occurs when there is too much uric acid in the blood, leading to the formation of monosodium urate (MSU) crystals in the joints and soft tissues. Researchers have created various animal models of gout, either by injecting MSU crystals into joints or by feeding animals a high fat diet. However, these models do not accurately reflect the full picture of gout in humans. In this study, we looked at a new model that combines both MSU crystal injection and a high-fat diet to see if it better represents the disease. Gout is a common metabolic condition that causes high levels of uric acid in the blood, which then forms MSU crystals in the joints and soft tissues. These crystals trigger inflammation, leading to symptoms like swelling, redness, heat, pain, and severe discomfort. While high uric acid levels are a major risk factor for gout, some people with gout have normal blood uric acid levels. This means that while high uric acid is needed for gout to develop, it isn't enough on its own. In recent years, the number of people with high uric acid and gout has been increasing. Both conditions have become significant public health issues, causing a lot of economic and social problems. Because of this, there is a lot of interest in understanding how gout develops and in finding new treatments. Creating accurate animal models is an important part of this research because they help scientists test drugs and study the causes of the disease. There are two main types of gout models: acute gout models and hyperuricaemia models. The acute model is made by injecting MSU crystals into a joint, which causes sudden pain and swelling (Abdelli, N. *et al.,* 2018). This model mimics a gout attack but doesn't show the longer term effects or the buildup of uric acid due to issues with purine metabolism. The hyperuricaemia model is created by feeding animals a high fat diet or using drugs that block an enzyme called uricase, which normally breaks down uric acid. However, most animals have an enzyme that helps them break down uric acid, making it hard to create a stable model of high uric acid. Because of this, these models don't fully represent the complex causes of gout. This study aimed to create a better model by combining MSU crystal injection with a high fat diet in male C57BL/6 mice. To assess how effective this model was, we looked at the changes in symptoms, levels of inflammation causing chemicals, and the gut microbiome in these mice. Earlier research has shown that the gut microbiome plays a big role in gout. Our findings could help develop a more accurate model of gout, which could be useful for future research and treatment development.

(iii) Hyperglycemic model:

Mulberry, or *Morus alba* L, is part of the Moraceae family and is commonly grown in Asia. People usually eat mulberry fruits fresh, make them into jams or juices. These fruits have a lot of active ingredients that might help with health in different ways. Because of this, mulberry has been used in traditional medicine for a long time. Research shows that mulberry fruits contain bioactive components like alkaloids and flavonoids, which are linked to activities such as antioxidant effects. One key compound in mulberry is anthocyanins, which are water-soluble and belong to the polyphenol group. Studies suggest that mulberry can offer health benefits like lowering cholesterol, reducing obesity, and protecting the liver, which may be due to these bioactive compounds. However, there are not many human studies on how effective mulberry is for health. Future research should look more into how eating mulberry affects human health and identify the exact compounds involved. This chapter gives a general overview of the health benefits of mulberry fruits. Mulberry is tasty and has few calories (Cabreiro, F., *et al.* 2013). It has a tangy flavor with a pH lower than 3.5, making it strong in taste and good for making juice or eating fresh. Mulberry has several health benefits, such as reducing cholesterol, helping control diabetes, offering antioxidant protection, and reducing obesity (Bhattacharyya, P., *et al.,* 2016). These benefits come from polyphenols, including anthocyanins. However, mulberry fruits of the same species can have different levels of anthocyanins depending on their color. Cyanidin-3-rutinoside and cyanidin-3-glucoside are the main types of anthocyanins in mulberry fruits. Even if mulberry plants are genetically the same, their nutritional and health benefits can vary (Custodia, N., *et al.,* 2001). The purpose of this work is to review the possible roles of mulberry fruits (*Morus alba* L.) and their active compounds in promoting health. It also briefly discusses how these compounds might work in the body. We hope this work will be a useful resource for future research in this area. Literature reviews show that mulberry fruits contain a high amount of polyphenols and antioxidants (Ruiz, X. & Almanza, M. 2018). This means there are many opportunities for the food and healthcare industries to explore the health benefits of mulberry fruits, as there is a growing market for them. However, the amounts of bioactive components like anthocyanins, alkaloids, flavonoids, and polyphenols depend on the type of mulberry plant. Though these compounds might work together to improve health, more studies are needed to show the exact link between eating mulberry and health benefits. There aren't enough studies with clear data to say for sure that mulberry is good for human health, especially for managing and preventing diseases like diabetes and cardiovascular disease. Most studies that show benefits are in animals. These studies use different types of mulberry, solvents, and preparation methods, making it hard to compare results and leading to inconsistent findings. Therefore, larger, well-designed, randomized controlled trials are needed to better understand how eating mulberry affects human health. Like other plants and foods **(**Sekimura, T., **2005)**, it's also important to study how polyphenol compounds are processed in the body, especially after being changed by gut bacteria. Further research should also focus on identifying the structures of active ingredients in mulberry and how they contribute to their health benefits.

(iv) Human phenylketonuria model:

Mulberry fruits, like most other fruits and vegetables, have a low amount of phenylalanine and can be part of a controlled PKU diet. Studies have shown that eating low-Phenylalanine fruits and vegetables doesn't harm metabolic control in PKU patients, but it doesn't cure the disease. Mulberry extracts have been studied for other health benefits, like antioxidant and anti-inflammatory properties, and possibly neuroprotective effects in diseases like Parkinson's and Alzheimer's. But they don't cure PKU itself. Generally, chemicals from medicinal plants can help prevent or cure some diseases. Neurodegenerative diseases are marked by the slow loss of nervous system function, affecting either the central or peripheral nervous system. Oxidative stress might be a major factor in the death of nerve cells and the development of these disorders. Among these active plant chemicals are phenolic compounds, or polyphenols, which are among the most common substances in plants. Polyphenols have many biological effects due to their antioxidant properties. They can neutralize a range of harmful reactive species like hydroxyl radicals, peroxyl radicals, hypochlorous acid, and superoxide radicals. Mulberry, or Morus (L., Moraceae), is a genus with around 10 to 16 species found worldwide. It grows in various climates, from temperate to subtropical areas in the Northern Hemisphere and tropical regions in the Southern Hemisphere, adapting to different weather, landscape, and soil conditions. Mulberry has been used in traditional and folk Chinese medicine, and many studies suggest it may offer certain health benefits. Mulberry extracts contain various phytochemicals and bioactive compounds, contributing to different pharmacological and biological effects. Besides antibacterial and antiviral properties, mulberry has also been linked to antitussive, blood sugar-lowering, blood pressure-lowering, anti atherogenic, cholesterol lowering, diuretic, astringent, antioxidant, and alpha-amylase inhibitory effects. Recent research has highlighted that phytochemical rich mulberry extracts can offer protective and therapeutic roles in the development of brain diseases **(**Panthee, S.**,** ***et al.,*** **2017)**. This protection is likely due to the presence of phenolic compounds in mulberry. Additionally, many of the pharmacological benefits of mulberries are connected to the amount and type of phenolic compounds, especially flavonoids and anthocyanins, and a strong link exists between antioxidant activity and the presence of these compounds. Several studies have shown that mulberry extracts may have potential in neuroprotection and preventing brain damage. Their neuroprotective effects have been documented. This review focuses on the neuroprotective abilities and mechanisms of mulberry extract against neurodegenerative diseases. Mulberry extract and its related phenolic compounds may help alleviate the symptoms of degenerative diseases like Alzheimer's and Parkinson's and could help prevent age-related neuro degeneration. Mulberry extract has strong antioxidant and anti-inflammatory properties and can also help with anti-convulsive effects, reducing depression, improving memory loss in the elderly, and supporting cognitive function in aging individuals. There is growing evidence that dietary polyphenols may help reduce oxidative stress and prevent neurodegenerative disorders **(**Ota, M.**, *et al.,* 2019)**. Mulberry's rich polyphenol content makes it a promising option for neuro protection and treating brain disorders caused by oxidative stress. The bioactive components in mulberry extract show strong antioxidant potential, protecting against brain damage. The effects of mulberry extract in animal models and patients with brain disorders such as Alzheimer's and Parkinson's will be examined, highlighting the role of antioxidants in treating neurodegenerative disorders in humans. Phenylketonuria is a genetic metabolic condition that leads to a decrease in the metabolism of the amino acid phenylalanine. Silkworms have a mutant strain, called 'albino', caused by a mutation in the BmPTS gene, which prevents the production of tetrahydrobiopterin (BH4), a cofactor involved in melanin synthesis. Researchers found that the development of the 'al' silkworm is similar to PKU in patients who lack BH4. This property can be used to study the disease using silkworms as a model, which is beneficial for ongoing PKU research, since traditional insect models like Drosophila melanogaster cannot accurately represent this condition **(**Ramadan, D.E.**, *et al.,* 2020)**. Sepiapterin reductase deficiency (SRD) is an inherited disease in humans. Sepiapterin reductase is involved in the production of tetrahydrobiopterin (BH4). When this enzyme isn't functional, BH4 production is affected. Most organs have alternative pathways for BH4 production, but the brain relies entirely on sepiapterin reductase. BH4 is essential for the production of neurotransmitters like dopamine and serotonin **(**Ramya, M.N.**, *et al.,* 2018)**. A lack of these neurotransmitters can cause movement problems, leading to dopa responsive dystonia, which involves involuntary muscle contractions, tremors, and other uncontrollable movements. Researchers have identified a mutant strain of silkworm called 'lemon' due to the loss of the BmSPR gene. This disrupts BH4 production and leads to abnormal melanin pathways, causing xanthine B1 accumulation in skin cells. The symptoms are similar to those seen in human SRD. The 'lem' mutant has been found to follow a pathogenic pathway similar to SRD, making it the only model for the disease. Therefore, silkworms are greatly needed for studying this condition.

(v) Human sepiapterin reductase deficiency model:

The phrase "human sepiapterin reductase deficiency model removed by mulberry" refers to research that found a silkworm mutant named 'lemon' which eats mulberry leaves could be an insect model for human sepiapterin reductase deficiency (SRD). SRD is an inherited disease caused by mutations in the SPR gene and is a type of disorder affecting monoamine neurotransmitters. In this study, researchers looked into whether the lemon mutant could be used as a model for SRD. A single point mutation in the BmSPR gene caused a deletion of five amino acids at the end of the protein in the lemon mutant. The lemon mutant also showed similar symptoms to human SRD patients, like normal levels of phenylalanine, lower levels of dopamine and serotonin, and higher levels of neopterin **(**Shen, L. & Ji, H.F. 2013 and Smith, D.L., *et al.,* 2010**)**. When researchers gave the lemon mutant l-dopa, their dopamine levels increased. The lemon mutant also had negative behavioral traits. These findings suggest that the lemon mutant has the right genetic background and meets the biochemical needs to be a model for SRD. Therefore, the lemon mutant could be a candidate animal model for SRD, which might help in better diagnosis and treatment of the disease. SRD is an autosomal recessive disease in humans. Sepiapterin reductase is involved in making Tetra-Hydro-Biopterin (BH4). If sepiapterin reductase doesn't work, BH4 can't be made. Most organs in the body have alternative ways to make BH4 without sepiapterin reductase. However, the brain doesn't have any other pathway and depends entirely on sepiapterin reductase. BH4 is needed for the production of neurotransmitters like dopamine and serotonin. A lack of these neurotransmitters leads to problems with movement, such as involuntary muscle contractions, tremors, and other uncontrolled movements, which is called dopa-responsive dystonia (Arrabal *et al.,* 2011; Nevill *et al.,* 2005). Researchers found a mutant silkworm, named lemon (lem), which was caused by the loss of the BmSPR gene. This leads to difficulty in making BH_4 and changes in melanin pathways, causing the accumulation of xanthine B_1 in skin cells. The symptoms of the lemon mutant are similar to those of human SRD. So far, the lemon mutant is the only known model for SRD (Meng *et al.,* 2009). Therefore, studying this disease using silkworms is very important. Kidney stones, also called nephrolithiasis, are hard masses made of minerals and salt that form inside the kidneys. These can come from many factors, including diet, medical conditions, weight gain, and some supplements or medications. Some kidney stones, like struvite, UA, and cystine stones, are linked to urinary tract infections. Calcium stones are the most common type and are mostly made of calcium oxalate. Nephrolithiasis is now understood as a complex disease that's connected to other health issues instead of being just a simple urinary problem. Many studies have shown that nephrolithiasis is linked to diseases such as obesity, metabolic syndrome (Wong *et al.,* 2016), diabetes, high blood pressure, cardiovascular disease, and chronic kidney disease (Stamatelou & Goldfarb, 2023). Silkworms are good models for studying calcium oxalate crystal formation in their malpighian tubules because they build up a lot of calcium oxalate crystals during larval development from first to fifth instar without harming themselves. This unique trait makes them useful for research into kidney stone formation. In one study, when exudates from the malpighian tubules were collected and analyzed using GC-MS, many proteins and amino acids found in these exudates were also common in human urine. This suggests that silkworms could be useful in understanding the factors that influence calcium oxalate crystallization. Additionally, malpighian tubules, which are like mammalian kidneys, are similar to kidney epithelium **(**Wyman & Webb, 2007). Since silkworms are easy to manipulate, their environment can be changed to study how it affects kidney stones. Also, drugs or chemicals that can reduce calcium oxalate crystal formation can be tested using this model. However, the physiology of silkworm malpighian tubules is different from that of human kidneys, which limits how much silkworms can be used in this area. Nevertheless, this model still provides a good base for further research. One major issue with *Bombyx mori* is the difference in how filtrate is formed. In humans, filtrate is created through a glomerulus, which filters blood to form tubular fluid through ultra-filtration, absorption, and secretion. In silkworms, the process is different, which is a key limitation. Other invertebrate models like D. melanogaster have also been used for studying kidney stones and assessing drug effects (Ali *et al.,* 2018), as well as looking at how genetic variations, environmental changes, and diet affect stone formation (Miller *et al.,* 2013). Silkworms can offer researchers a new way to study this disease and may provide new ways to understand and treat it.

Potential for Kidney Stone Research:

Mulberry has a dual capacity for kidney stone studies: the physical presence of mulberry shaped debris (mulberry cells/our bodies) in kidney stones and the therapeutic ability of mulberry leaf extracts. The mulberry cells are shaped from pink blood cells adhering to and interacting with calcium oxalate crystals, suggesting a function in stone formation that would be studied for prevention or early diagnosis, as they are additionally observed in certain sicknesses like Fabry sickness. One by one, studies have proven that mulberry leaf extracts have and other properties which could help save you or deal with kidney stones, making them a goal for in addition medicinal studies. The goal of this text became to study some of the brand new and generally used medicinal flora within the treatment of kidney stones; emphasizing medicinal makes use of, pharmacological hobby, and studies have a look at of medicinal flowers. Renal stones are a common trouble international with huge morbidities and financial fees. The excessive economic burden related to kidney stones is linked specifically with healthcare prices. In vitro and in vivo studies on alternative treatment of kidney stones have been searched via the use of phrases which includes phytotherapy of kidney stones, medicinal plant life used in kidney stones, and lithotriptic interest of vegetation. Articles reviewed showed that medicinal vegetation has lithotriptic ability. Medicinal vegetation may be useful in remedy of kidney stones. This examine was aimed to explore and difficult the efficacy and availability of opportunity remedy for kidney stones and to offer safe, fee effective, and efficacious management alternatives in addition to lessen the weight of disease. Price effectiveness may allow upgrades in remedy efficiency which could benefit patients and the healthcare machine. Further randomized clinical trials ought to be conducted to assess the protection and efficacy of medicinal flowers. Urolithiasis is a common ailment and affects a tremendous variety of people global. Globally, 12% of populace suffers from kidney stone at a level of lifestyles. Throughout the beyond a long time, prevalence of kidney stones increased each in developing as well as advanced international locations especially due to sedentary existence style, consuming conduct, and international warming issues. According to a file, US population suffers from kidney stones 1 out of every 11 humans and in Asian countries about 1%–19.1% of the populace suffers from urolithiasis consisting of in India 12% of general population suffers from urolithiasis and half of them have consequent failure of kidney especially because of high price of treatment. In China and United Arab Emirates, occurrence of kidney stones is 6.four% and 6.8 %, respectively. A massive financial burden is related to kidney stones, with annual estimate exceeding US$5 billion in the US. A topic that emerges from current research is that the complexity of minerals and electrolyte imbalance concerning different body systems can lead to urinary stone formation. Medicinal plant lives are used as urolithotriptic agents. All through latest decades, medicinal floras have emerged as extra effective in stone disorder. Medicinal flowers lessen ache of kidney stones and save you lithogenesis. Medicinal vegetation expels the stones from the kidney. Medicinal plant life is commonly used for treatment of kidney stones (calcium oxalate, uric acid, struvite and cysteine) (Ames, B.N., *et al.,* 1981). Natural medicines is an indispensable part of the development of modern-day civilization. numerous medicinal vegetation is utilized in urolithiasis and their effect is documented. Medicinal flora having urolithiasis interest encompass Cynodon dactylon, Holarrhena antidysenterica, Berberis vulgaris, Rubus idaeus, Matricaria chamomilla, Abutilon indicum, Terminalia arjuna, Herniaria hirsute, Aerva lanata, Solanum surattense, Saxifraga ligulata and Asparagus racemosus that are local to Pakistan and discovered international. All medicinal floras are documented and a number of them experimentally documented, however more or much less historically can be viewed as the opportunity supply as therapeutics for one of kind sorts of kidney stones (calcium oxalate, uric acid, struvite and cysteine). The toxicity is minimum, and whilst there are aspect consequences of conventional medicinal drug herbal remedy can be a good opportunity. there is evidence in treating kidney stones with herbal medicaments. The plant species listed have potential active compounds and might useful resource researchers in their have a look at of herbal products, which can be useful to deal with kidney stones (calcium oxalate, uric acid, struvite and cysteine). In gift have a look at, we've got documented medicinal plant usage in sufferers with urolithiasis. Compound isolation is recommended to enhance the lithotriptic moves of flowers. in addition, randomized medical trials need to be performed to evaluate the safety and efficacy of medicinal plants. Manufacturing of appropriate formulations of these medicinal flowers by using pharmaceutical industry may be a thrilling and economically useful prospect. A substantial economic burden is related to kidney stones, with annual estimates exceeding US$5 billion in the USA. Therefore supplying safe fee effective and efficacious options of treatment with medicinal flora referred to in this evaluation can reduce the burden of sickness. Value effectiveness can also enable upgrades in remedy performance that could benefit patients and the healthcare device. Kinds of renal stones are Calcium oxalate stones, Calcium phosphate stones, Uric acid stones, Cysteine stones, Xanthine stones. These stones are fashioned due to calcium oxalate and are popularly called mulberry stones. It's far protected with sharp projections, which can also bleed early. These are typically single. Because of its irregularity, it produces signs early. These stones are commonly very hard. In cross-section, it's far visible as concentrically placed layers. The crystals can be seen in urine and identified by means of their envelope like form. These may additionally arise as calcium phosphate, ammonium phosphate or triple phosphate (calcium, ammonium, and magnesium). Those stones are smooth and dirty white in color. The stone enlarges in alkaline urine. It simplest gives signs and symptoms when stone is large in size. Those are hard, smooth, and radiolucent. They a couple of and faceted, and yellow to reddish brown in shade. These may be smooth and flexible. These might also appear in the patient with cystinuria which may additionally occur within the patient with reduced reabsorption of cysteine from the kidney. those are soft stones. On exposure to air, they exchange their shade into green. This greenish colour seems because of the presence of sulfur in them. Those cysteine crystals are hexagonal, translucent, and seem in acidic urine. Some patients with small-sized stones do now not produce any signs. pain is the leading symptom in seventy five% of cases; ache is fixing, and located in renal perspective. In case of ureteric colic, while stones radiate to ureter from the right or left lumbar location after which to the groin, there may be additionally vomiting and sweating because of excessive ache? On stomach exam, there may be tenderness in the stomach muscles and if hydronephrosis has been produced, then the kidney will become palpable. There's pyuria and hematuria. Mulberry (Morus spp.) has a protracted history of use in conventional remedy, especially in East Asian and Ayurvedic systems, for a ramification of ailments such as kidney stones. Traditional practitioners regularly use mulberry fruits, leaves, or root bark as part of herbal remedies meant to "clean heat," sell diuresis (urination), and aid kidney health. The reason is generally based totally at the belief that mulberry can assist flush out small stones or prevent their formation with the aid of increasing urine output and reducing infection. However, scientific validation for those specific makes use of is constrained. a few preclinical research have proven that mulberry extracts own antioxidant, and moderate diuretic effects, that could theoretically benefit kidney health. Though, there is a loss of robust medical trials at once examining mulberry's efficacy in stopping or treating kidney stones in people. Most to be had evidence comes from animal research or check-tube experiments, and those do not offer sturdy support for medical tips. In precis, even as the use of mulberry for kidney stones is supported by way of conventional medicinal systems and anecdotal reviews, cutting-edge clinical proof is sparse and not definitive. All people thinking about mulberry for kidney stones need to seek advice from a healthcare professional, as greater studies are needed to establish its protection and efficacy for this purpose. Mulberry refers back to the trees and shrubs of the Morus genus, specifically *Morus alba* (white mulberry), *Morus nigra* (black mulberry), and *Morus rubra* (red mulberry). Those deciduous bushes are local to Asia, Europe, and North the United States relying at the species and are valued for his or her fit for human consumption berries, medicinal leaves, and rapid-growing timber. The fruit is good and wealthy in nutrition C, anthocyanins, resveratrol, rutin, and different polyphenols, whilst the leaves include bioactive compounds which include 1-deoxynojirimycin (DNJ), a natural alpha glucosidase inhibitor known for its blood sugar-lowering properties. In contemporary natural medicinal drug, mulberry is used for supporting metabolic health, regulating blood glucose, improving cardiovascular feature, reducing oxidative stress, and enhancing liver cleansing. The berries are eaten up sparkling, dried, or in extract form for antioxidant and immune guide. The leaves are widely used as teas or supplements to assist manipulate diabetes, hyperlipidemia, and hypertension. The plant also has moderate antimicrobial results, making it suitable for broader well being applications. Historic Use: Mulberry has been an critical a part of traditional chinese language medicinal drug (TCM) for over 2,000 years. The leaves (Sang Ye) are used to clean lung warmness, alleviate coughs, cool the blood, and improve vision. They may be additionally hired to decrease fevers and blood stress, and to treat cold signs and eye irritation. The fruit (Sang Shen) is taken into consideration a tonic that nourishes the yin and blood, and is used to deal with dizziness, insomnia, premature graying of hair, and constipation. The basis bark (Sang Bai Pi) is used to lessen edema, clean lung warmth, and calm wheezing. In Ayurvedic remedy, mulberries are less usually featured but are used as cooling, nourishing end result to aid liver characteristic and increase energy. The plant's antioxidant houses make it useful for pitta-associated imbalances, which include pores and skin infection, warmness intolerance, and immoderate thirst. In European herbalism, in particular with *Morus nigra* (black mulberry), the fruit become traditionally used as a gentle laxative, and throat treatment. Syrups made from the berries were prescribed for tonsillitis, sore throats, and mouth ulcers, whilst mulberry wine become consumed as a tonic for blood and vitality. The leaves and bark were every now and then used as poultices for pores and skin infections or decoctions for digestive problems. In North American Indigenous traditions, *Morus rubra* (purple mulberry) become utilized by numerous tribes as a meals supply and remedy, with applications starting from fever and dysentery treatments to topical wound care the use of bark infusions. Nowadays, mulberry stays a staple in useful foods, teas, and integrative fitness supplements, mainly for its position in supporting glucose metabolism, cardiovascular well-being, and cell protection. Its long records across continents reflect its wide-ranging software as both a nutritive and therapeutic plant. Kidney stones are tough, crystalline mineral deposits that shape in the kidneys while urine becomes too focused, allowing minerals like calcium, oxalate, and uric acid to crystallize and bind. They are able to vary in length from tiny grains to large stones that obstruct urine float, and can pass overlooked or purpose extreme ache and urinary symptoms.

Silkworm *Bombyx mori* as Antimicrobial Drug Screening and Pathogenicity Model:

Over the past few decades, the way antibiotics have been used and misused has led to the widespread problem of antibiotic resistance, which today is a major threat to human health. Because of this, finding new anti-infective agents is very important. Testing these new antibiotics in real-life conditions, known as in vivo studies, is essential. Insect models are becoming more popular as an alternative to using mammals, mainly because of the high costs and ethical issues involved with mammals. Among these insects, the silkworm has shown promise. In this study, we created an infection model using silkworm larvae by exposing them to Staphylococcus epidermidis, a common hospital-related infection-causing bacterium. We then tested the effectiveness of three important glycopeptide antibiotics vancomycin, teicoplanin, and dalbavancin which are used to treat infections caused by drug-resistant Gram-positive bacteria. As more bacteria become resistant to existing antibiotics, there is a growing need to develop new antimicrobial treatments and strategies. Using insects like silkworms for drug screening is considered a promising approach to speed up the discovery process and reduce the use of mammals. For the first time, we used the silkworm *Bombyx mori* as an in vivo model to test the effectiveness of these three glycopeptides against *S. epidermidis*. To mimic the human body's temperature, the infection was carried out at 37°C, and we monitored the larvae's survival and their immune response markers, such as hemocyte activity, prophenoloxidase system activation, and lysozyme activity. All three antibiotics were effective in curing the infected larvae, significantly lowering their death rate and reducing the activation of their immune system. These findings support the use of the silkworm model for studying antimicrobials that target staphylococcal infections. Antibiotics are one of the most significant medical breakthroughs of the 20th century and remain crucial in modern medicine for treating and preventing infections in high-risk patients. However, the overuse or misuse of these drugs has contributed to the rise and spread of resistant bacteria. The urgent need to discover and develop new antibacterial compounds is due to the growing challenge of antibiotic resistance. Testing new antibiotics in living organisms can help identify promising candidates more quickly, helping prioritize drugs that offer good effectiveness with minimal toxicity. Using large numbers of mammalian animals, like mice and rats, at the earlier stages of drug development is not practical due to high costs and ethical issues. This is why the European directive on animal protection, which encourages

Replacement, Reduction, and Refinement of animal use (the 3Rs), recommends limiting their use. To find suitable alternatives, different invertebrate models have been introduced recently, such as amoebas and nematodes (Aronoff, S.L., 2004). Among these, holometabolous insects are promising because they are affordable to raise and their larvae are easy to handle (Arrabal, L., *et al.,* 2011). Using insects also avoids ethical issues (8), and while they don't have acquired immunity, their innate immune responses are similar to those of mammals in both function and evolution (Blau, N. & Bonafe, L. B. 2001). Several insect species, including Drosophila melanogaster, Galleria mellonella, and *Bombyx mori*, have been used to study new antimicrobials and test their effectiveness (Bogdanov, M., 2008). A big advantage of using silkworms as an infection model is access to germplasm banks, where B. mori is kept in genetic collections. These centers raise the insects on artificial food, helping to keep the supply consistent and reliable. Also, the genome of *Bombyx mori* (Bonafe, L., *et al.,* 2001) has led to the creation of molecular tools and gene editing techniques, such as RNA interference, transposon-based gene insertion, and CRISPR/Cas9 (Bove, J., *et al.,* 2005). These have been used to make customized disease models to study how drugs work. Silkworm infection models have already been used to test antimicrobial drugs against Gram-negative and Gram-positive bacteria in the ESKAPE group, like Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiella pneumoniae (Brunet, A., *et al.,* 2001), as well as other bacteria such as Francisella tularensis, Listeria monocytogenes, Bacillus cereus, and Mycobacterium abscessus (Buhroo, Z.I., *et al.,* 2018), and fungi like Candida spp. and Aspergillus fumigatus (Carmona, E.R., *et al.,* 2015). However, so far, no study has used coagulase-negative Staphylococcus epidermidis, which is one of the most common causes of hospital-acquired infections. S. epidermidis is normally harmless and lives on human skin and mucous membranes. But it can become harmful if it enters the body through medical devices, such as catheters or implants. This can lead to bacteremia, and S. epidermidis can form biofilms that are hard to treat with the body's defenses or antibiotics (Cheng, L., *et al.,* 2017). In this study, the effects of three important glycopeptide antibiotics vancomycin, teicoplanin, and dalbavancin used to treat serious infections caused by Gram-positive bacteria were tested in *Bombyx mori* larvae infected with a clinical strain of S. epidermidis. By tracking larval survival and the responses of three key immune markers hemocyte metabolic rate, lysozyme activity, and phenoloxidase (proPO) system activity we showed the effectiveness of the three antibiotics, confirming that the silkworm infection model is a strong tool for testing antimicrobial compounds. In conclusion, this study suggests that using silkworms as a reliable infection model could help in developing new antimicrobials that are needed to fight antibiotic resistance. In addition to tracking larval survival, two more immune markers were validated hemocyte viability and lysozyme activation while a third marker, the proPO system, did not work well for this purpose. Using these markers could help in better understanding how silkworms respond to antimicrobial treatments, although their behavior at different temperatures needs to be checked before using them in screening tests. Also, using fewer insects and less time for analysis could help in faster identification of new tools for screening antimicrobials on a large scale. Our hope is that this insect infection model can help speed up the discovery and development of new compounds that are urgently needed to combat antibiotic resistance.

Silkworm *Bombyx mori* as a Model for Periodontal Disease:

Bombyx mori silkworms are a promising model for studying periodontal disease because they have a short life cycle, are low cost to raise, and are relatively large in size. Their innate immune system is similar to that of humans, which makes them useful for research. Scientists have found that silkworms can be infected with periodontal pathogens like *Porphyromonas gingivalis* and *Streptococcus mutans*. By observing how silkworms respond to infections and potential treatments, researchers can study the effects of drugs on these infections. However, silkworms do not have complex tissues like joints and gums, so they cannot fully replicate the human disease process. In this study, changes in the hemolymph protein and trehalose levels in *Bombyx mori* L. due to infection with Streptococcus mutans and antibiotic treatment were evaluated. When S. mutans was successfully grown inside the larvae, it caused a significant decrease in protein levels in the haemolymph by 69.60% and 50.21%. At the same time, trehalose levels increased dramatically, reaching 60.22% and 65.13% compared to healthy larvae. This was due to infection through both the hemocoel and oral routes. Antibiotics commonly used to treat dental caries were found to reduce bacterial infection in silkworm larvae. This led to an increase in protein levels and a decrease in trehalose levels. The antibiotic Advent was particularly effective, leading to a recovery of protein synthesis at 133.79% and 92.62%, while trehalose levels dropped to 34.30% and 40.31% compare to healthy larvae. Taxim also showed some improvement, increasing protein content by 106.73% and 78.99%, while reducing trehalose by 24.47% and 33.70%. Despite showing dose, concentration, and drug-dependent effects, Advent was more effective than Taxim against S. mutans infection. This suggests that silkworms could be a valuable model for evaluating new drugs for dental caries before clinical trials. Streptococcus mutans is a gram-positive, spherical bacterium commonly found in human dental caries. It thrives at temperatures between 18-40 degrees Celsius and ferments sugars, creating an acidic environment that harms tooth structure. This bacterium is not only responsible for oral infections but is also linked to various systemic diseases. It can move from the mouth into the throat and intestines due to the weak immune defenses of the oral mucosa. It can even enter the bloodstream, causing serious conditions like endocarditis and bacteraemia **(**Choi, H.**, *et al.*, 2005)**. Since multiple bacteria are involved in the development of dental caries, understanding the disease biology is key to developing effective treatments. Silkworms, specifically *Bombyx* *mori*, have long been used as a model organism to study various biological processes including genetics, molecular biology, physiology, biochemistry, and pathology. They have become one of the most studied Lepidopteran insects and are considered a central model for this group of insects. Recently, they have been used as infection models for various human pathogens like Staphylococcus aureus, Pseudomonas aeruginosa, Vibrio cholerae, and Stenotrophomonas maltophilia, except for S. mutans, which opens up new opportunities for using silkworms as a model for dental caries. Protein synthesis in silkworms is heavily influenced by their nutritional status during the larval stage **(**Church, W.H. *et al.,* 1994**)**. Proteins are essential for development, metamorphosis, and maintaining various physiological functions. Trehalose, a major sugar in the larval hemolymph, is produced in the fat bodies from various sugars derived from the diet. These molecules are crucial for normal growth and development of silkworms and their life cycle. Hemolymph cells play a role in the immune response through phagocytosis and signaling, and they recognize self and non-self through mechanisms independent of microbial patterns. The study investigated the multiplication of S. mutans in the host system and the effectiveness of antibiotics against this pathogen, along with the corresponding biochemical changes in the infected tissues of *Bombyx mori* larvae. The main goal of this research was to establish strong biochemical evidence that silkworm larvae could serve as a promising model for human dental caries caused by S. mutans. The results clearly showed that S. mutans infection negatively affects protein and trehalose levels in silkworms and antibiotic treatment can regulate these levels, although the effect depends on the type, concentration, dosage, and drug used. Based on these findings, the study suggests that similar systematic research should be conducted on humans with dental caries, using a silkworm model developed by the research group, before moving on to clinical trials. In humans, periodontal disease, also known as gum disease, is a group of inflammatory conditions that affect the tissues surrounding the teeth. Gingivitis is an early stage of the disease, where the gums become swollen, red, and bleed. Porphyromonas gingivalis is one of the bacteria responsible for this condition. A study used silkworms to look at how P. gingivalis affects the body. When these bacteria were injected into the silkworms, they died. The silkworms' body fluid, called hemolymph, turned black, and there was more activity of enzymes called caspases in the hemolymph. When antioxidants like glutathione and catalase, or caspase inhibitors, were given, it slowed down the silkworms' death. In another experiment, using antibiotics didn't help reduce the silkworms' death. Even when the bacteria were killed by heat, they still caused death. A version of the bacteria that lacked a key harmful part also led to silkworm death. When a substance called peptidoglycan from P. gingivalis was injected, the silkworms died. But when an enzyme called mutanolysin was used to break down the peptidoglycan, it stopped the death. This showed that the body's natural immune response became too strong, creating too much harmful chemicals called reactive oxygen species (ROS), and causing cells to die through a process called apoptosis, which led to the silkworms' death (Ishii *et al.,* 2010). It is widely believed that in humans, death from P. gingivalis causing gum disease is due to a strong immune reaction known as a cytokine storm, similar to what happens in the silkworm model. Thus, the findings from the silkworm study match what happens in humans (Ramadan *et al.,* 2020). Because of this, a new model using silkworms has been created to study P. gingivalis. This model can help us learn more about the disease and also support research using other invertebrate models.

Silkworm *Bombyx mori* as a Model to Study Drug Induced Tissue Injury:

Researchers use rats and mice to study how drugs can damage tissues or organs in the body. This kind of damage is known as drug-induced tissue injury. However, there are challenges with this approach, like high costs and ethical concerns, which need to be addressed. Other small organisms like fruit flies and nematodes are used in many areas of life science, but their tiny size makes it difficult to give them the right doses of chemicals. This problem can be fixed by using silkworms as a model. Silkworms have similar ways of handling drugs in their bodies, such as absorbing, distributing, breaking down, and removing them, just like mammals (Hamamoto *et al.,* 2009; Nwibo *et al.,* 2015). When toxic chemicals are injected into silkworms, it raises the level of alanine aminotransferase (ALT), which is a key indicator of tissue damage in their body fluid, called hemolymph (Inagaki *et al.,* 2016). ALT is also a reliable sign of liver or cell damage in humans. Scientists have even created a transparent silkworm that glows green because of a protein called green fluorescent protein (GFP). This allows them to observe the silkworm without drawing hemolymph to measure ALT. By injecting toxic drugs into the silkworm's hemolymph and watching the glow, they can see how it fades over time and with different doses. This fading shows cell damage because the GFP leaks out when cells are harmed, changing the brightness of the glow (Hamamoto *et al.,* 2009). Because of this, silkworms are a good alternative model for studying drug-induced tissue injury. They can also be used to test various compounds that might help reduce such injuries. The silkworm, *Bombyx mori*, is a great model for studying drug induced tissue injury because it shares similar metabolic and physiological processes with mammals. For example, it has a liver like organ called the fat body that helps eliminate toxins. Using silkworms avoids the ethical and financial issues that come with traditional models like mice and rats. Silkworms also have an open circulatory system, where their internal organs are surrounded by hemolymph, making them more sensitive to drug toxicity. This allows for early detection of tissue damage, the use of biomarkers like ALT, and the visualization of injury through methods like transgenic reporter systems. In mammals, including humans, chemical-induced tissue injury can lead to serious conditions like sudden liver failure. That's why it's important to assess how chemicals affect tissues during drug development. In the process of creating new medicines, using animal models in live experiments is necessary to predict how harmful substances might affect humans. Mice and rats are often used to test the toxicity of new compounds and natural medicines. However, using mammals for experimental models brings problems like high costs and ethical dilemmas. An alternative animal model is needed to solve these issues. The findings of this study show that silkworm larvae are a good model for assessing drug-induced tissue injury, based on measuring ALT levels in their hemolymph. In humans, ALT levels in the blood are considered a very sensitive and specific indicator of cell or liver damage. Because of this, ALT is used in many pharmaceutical studies to evaluate liver damage caused by natural products or new chemicals. Here, we showed that injecting various toxic drugs into the body cavity of silkworm larvae caused an increase in ALT levels. These results suggest that we can develop a new experimental model to evaluate tissue injury using silkworm larvae. Silkworms have become a useful model in scientific research (30). Models of bacterial and fungal infections in silkworms are used to test antibiotics and find new genes that cause disease **(**Edwards, N., 2008). A model of high blood sugar in silkworms is also helpful for developing diabetes treatments (Hamamoto, H., 2009). These studies show that silkworms can replace mammals like mice and rats in pharmacological research for developing new medicines. Additionally, silkworms and mammals share similar metabolic processes involving enzymes like cytochrome P450 and conjugation enzymes. The effects of toxins on tissues and the release of marker enzymes from damaged cells happen in similar ways in both silkworms and mammals. In this study, we found that injecting CCl4 into silkworms increased ALT levels in their hemolymph. Also, pretreatment with NAC decreased this increase, showing that NAC can reduce tissue damage caused by CCl4. NAC is a substance that neutralizes harmful substances and is used to treat liver damage from medications like acetaminophen (Hayashi, Y., 1962). The results showed that NAC also works in silkworms. This silkworm model is useful for analyzing the harmful effects of chemicals and for discovering drugs that can protect against such damage. Even though we have shown the distribution of ALT activity in silkworms, the exact mechanism of how tissue injury happens when ALT levels rise is still unclear. The silkworm model can quickly assess tissue damage, but it doesn't fully explain the specific targets of the drugs. More research is needed to clarify how tissue injury occurs in silkworms. Predicting drug-induced liver damage is essential in drug development. Although small mammals like mice and rats are commonly used to test liver toxicity, they come with problems such as high costs and ethical issues. In an effort to address these issues, in vitro systems using human liver cells have been developed. Toxicogenomic systems, which analyze gene activity, are also considered useful for predicting liver toxicity based on how genes respond to harmful substances **(**Inagaki, Y., 2016). The collection of mammalian cells as a material and the differences between in vitro tests and real-life body conditions are still challenges in these lab based tests. The new silkworm tissue injury model created in this study is a fresh way to study histotoxicity. Based on where ALT activity is found in the body, the gut has the highest level of ALT activity among other parts in the silkworm. So, in the silkworm, higher ALT levels seem to happen when the gut is injured. This model could be very helpful in checking the histotoxicity of new chemicals before using mice or rats. We think using this silkworm model first could help reduce the number of mammals used in drug development. The study found that injecting different toxic drugs increases ALT activity in the silkworm's blood. This model can be used to test the toxicity of new chemicals. This method is more sensitive than tests that just count how many silkworms survive after being given a sample. Even though more research with different kinds of chemicals is needed, using this silkworm model before using mammals can help with the ethical and money issues that come with using animals in experiments.

Silkworm as a Model in Environmental Monitoring Concerning Animal & Human Health:

Chemicals like pesticides, nanoparticles, harmful substances, heavy metals, and many others have harmed the environment because of industrialization, fast development, and people's strong focus on quick economic growth. Because of this, there is a need to find a model organism that can help monitor the environment and show us how dangerous these chemicals might be *Bombyx mori*, or the silkworm, can be useful in this area because it is sensitive to environmental pollutants such as pesticides, heavy metals, and other harmful chemicals (Hamamoto *et al.,* 2009). In one study, the survival rate of silkworms exposed to cadmium was looked at, and it was found that when the amount of cadmium in their artificial diet reached 10 ppm, their survival rate dropped a lot, showing how sensitive silkworms are to heavy metal pollution (Sekimura, 2005). Silkworms react in different ways to various types of nanoparticles, which can affect their growth, development, and health. Low levels of nano TiO2 have been found to increase silkworm weight, while high levels are toxic (2015; Li *et al.,* 2016). This shows that the effect of TiO2 NPs depends on the amount, and it's important to consider the concentration when looking at their impact. Similarly, copper nanoparticles have been shown to harm silkworm development, feed efficiency, and cocoon shell weight (Cheng *et al.,* 2017). In contrast, green-synthesized silver nanoparticles, which are considered less harmful, have been found to increase the survival rate of larvae and boost their weight at different life stages (Jaculine *et al.,* 2017). This suggests that how nanoparticles are made can influence their effect on silkworms, with green-synthesized nanoparticles being less harmful. Other nanoparticles, such as zinc oxide nanoparticles, have been found to harm silkworms by lowering hemocyte viability, increasing ROS production, and causing cell death and changes in hemocyte structure (Mir *et al.,* 2020). These varying effects of different nanoparticles on silkworms show how important it is to consider the specific nanoparticle, its production method, and its concentration when studying their impact on human health and the environment. Silkworms are a great model for monitoring the effects of harmful chemicals and environmental pollutants. Their different responses to nanoparticles can give us important clues about the risks and benefits of these materials. The effects of polystyrene microplastics (PS-MP) and polystyrene nanoparticles (PS-NP) have also been studied, with short-term exposure affecting the molecular, biochemical, and physical aspects of silkworms (Muhammad *et al.,* 2021). Parenti *et al.* (2020) showed that ingesting 0.5 micrometers of polystyrene nanoparticles led to their presence in different body parts and caused behavior changes, such as strange movements and problems with chemical attraction, in *Bombyx mori* larvae. Besides their reactions to nanoparticles and microplastics, silkworms have also been studied for how they respond to environmental stress, like temperature and lack of food. A study found that when larvae were starved before being tested for cold tolerance, it acted as a positive stressor and improved their ability to withstand cold. However, it acted as a negative stressor for heat tolerance. These findings suggest that silkworms can be used to study how starvation affects an organism's ability to handle heat in stressful situations. Other invertebrate model organisms like D. melanogaster and C. elegans are also used for environmental monitoring. D. melanogaster is used to assess genotoxicity linked to air pollution, nanotoxicity (Ong *et al.,* 2015), and nano genotoxicity (Demir *et al.,* 2015). C. elegans is used to detect endocrine disrupting chemicals (EDS) in groundwater and to monitor bacterial air quality (Duclairoir Poc *et al.,* 2011). These two models have been used in biological research for a long time and are widely used. However, silkworms are not used as much as these models, as they are still in the early stages of being used as a research model.

Scope of Silkworm as an Ageing Model:

As people age, their bodies gradually lose their ability to function properly. This decline affects all parts of the body, including cells, molecules, and biological systems. Along with this loss of function comes an increased risk of diseases and a higher chance of death. Scientists have found some common genetic pathways that control the ageing process by studying different model organisms such as fruit flies, nematodes, mice, and yeast. These findings might help us find ways to live longer. In these organisms, reducing the activity of certain pathways like the insulin/IGF signalling (IIS) and Target of Rapamycin (TOR) pathways has been shown to increase lifespan. Since ageing is a complex process, using more model organisms can help scientists better understand how it works. Silkworms are a good choice as a model for studying ageing. They have many genetic variations, are easy to raise, have a clear genetic background, and cause fewer ethical issues. Their life stages are easy to observe, and they have a short life cycle, making them ideal for testing how external factors affect lifespan. It's also simple to create genetically modified silkworms by introducing new genes into their eggs. Under normal conditions, silkworms take about 5 to 7 weeks to complete their life cycle, which is much faster than many other organisms, making them easier to study. Silkworms are also useful for examining how fasting affects lifespan, even though adult silkworms don't eat. They still have enough energy for mating, reproduction, and survival for about 1 to 2 weeks. Interestingly, during their larval stage, silkworms have a functional midgut that can absorb nutrients, and it can recycle molecules from other parts of their body. This finding shows that the silkworm's midgut undergoes complex changes during the transition from larva to adult, suggesting that adult feeding habits may not be as simple as previously thought. Therefore, when using silkworms as an ageing model, scientists must take into account these dynamic changes in the midgut. Using silkworms as a model can help in early testing of drugs that extend lifespan. For instance, metformin, a drug commonly used for type 2diabetes, has been shown to increase lifespan in various animals like worms, mice, and rats. When tested on silkworms, metformin increased the lifespan of male silkworms and boosted antioxidant levels, helping them resist environmental stress. Another study showed that a Chinese medicine called Astragalus polysaccharide increased the lifespan of silkworms by extending the adult phase. Resveratrol, another drug, also increased the lifespan of both male and female silkworms by improving their ability to handle heat and boosting antioxidant activity. It also activated a specific pathway linked to antioxidants. These findings show that silkworms are valuable for understanding how drugs can promote longevity. However, using silkworms as a model for ageing has its limits. Since they are not closely related to mammals, some genes found in silkworms may not have similar counterparts in mammals or humans. Therefore, while silkworms can offer important insights into the process of ageing, scientists should be cautious when applying these findings to mammals and humans. Despite these limitations, silkworms remain a promising model for studying ageing and should continue to be studied alongside other organisms to better understand this complex process.

Aging and lifespan have always been key areas of study in biology. Today, the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* are the most common invertebrates used in aging and lifespan research. Because the mechanisms behind aging are complex, it's essential to use a variety of model organisms to understand how these processes work across different species. Also, animals with simpler biology, such as invertebrates, are easier to study and can help scientists make discoveries more quickly than mammals. The silkworm, *Bombyx mori*, has recently become a popular choice for some aging and lifespan studies, especially when looking at how longevity genes work, how signals are passed through the body, and how effective drugs are. Here, we will look at why the silkworm is a good model for studying aging and lifespan, as well as the challenges that researchers might face. Animal models are crucial in many areas of study, such as biology, medicine, and pharmacy. However, current invertebrate models do not fully meet the needs of understanding the complex mechanisms that control aging and lifespan. Therefore, there is a need to identify and develop more experimental models. The shared pathways for controlling lifespan across different species provide a good basis for studying aging using simpler animals. The silkworm is one of the most important insects in agriculture and has also been used in classical genetics research. Additionally, it has several features that make it a strong candidate for studying aging and lifespan. The silkworm has been bred by humans for over five thousand years, giving it unique biological traits and cultural significance. With the increasing demand for new model organisms in biological and medical research, the silkworm is becoming a preferred choice for studying the molecular regulation of aging and longevity. Several laboratories, including our group, are using silkworms to explore aging and lifespan. Although there is less research on aging in silkworms compared to *C. elegans, D. melanogaster*, and *M. musculus*, the silkworm is becoming a new model for aging and lifespan research, expanding the options available to scientists. One key difference between silkworms and mammals is their long evolutionary divergence. Silkworms are poikilotherms, meaning they do not regulate their body temperature, and they have a much more distant evolutionary relationship with humans. This is because silkworms lack several important physiological systems, especially the acquired immune system. Also, the aging mutations studied in silkworms are mostly related to development, such as voltinism and moultinism, which have little connection to mammalian aging. Another challenge is that some genes in humans that are important for aging and lifespan are not found in silkworms. The silkworm genome contains around 14,623 genes, with about 8,469 having direct human counterparts. However, the remaining 6,154 genes, which make up 42% of its total genes, are without human homologs and might be involved in aging and longevity. For example, sirtuin 1, a gene involved in calorie restriction, stress response, and DNA repair, is not present in silkworms. This means that the effects of such genes on aging and lifespan can't be studied in silkworms. These limitations put some constraints on using silkworms as a model for aging and lifespan research. Despite these challenges, silkworms are emerging as a valuable model for studying the mechanisms of aging and lifespan. They could also serve as an alternative to traditional laboratory animals, offering early insights into potential drugs that extend lifespan and their effects. There's potential for silkworms to become more widely used in aging and lifespan research. We hope that in the future, the strengths of the silkworm as a model for aging and lifespan will be further explored, and some limitations can be overcome with the continued efforts of researchers in the field. Studies using silkworms to uncover the molecular mechanisms behind aging and lifespan will provide new ideas and insights.

Usefulness of Silkworm as Parkinson's Disease Model:

Silkworms are helpful for studying Parkinson's disease (PD) because they can be changed genetically or treated with harmful chemicals that make them show similar symptoms as humans, like difficulty moving, low dopamine levels, and increased stress from free radicals. This makes them a good model for looking into how PD starts, testing new medicines, and checking many different substances quickly and cheaply, including natural ones. In humans, Parkinson's is a type of brain disease that causes damage to the nerve cells that make dopamine, a chemical needed for movement. This damage leads to problems with the body's waste removal system, which creates more stress and causes dopamine levels to drop in the brain's substantia nigra (Devine *et al.,* 2011; Dias *et al.,* 2013). Environmental stress is a big part of why some people get PD, with oxidative stress playing a major role in the brain (Bove *et al.,* 2005). In the body, uric acid (UA), which is a byproduct of breaking down purines, helps fight off oxidative stress (Ames *et al.,* 1981). Studies have shown that lower levels of UA in the blood are linked to more advanced stages of PD (Shen & Ji, 2013). High UA levels, like in a condition called hyperuricemia, might reduce the risk of PD but can also increase the risk of heart disease. In silkworms, UA is mainly made in the fat cells and carried through the body's fluid to the outer layer, where it builds up as granules and makes the skin look white. UA also helps protect against oxidative stress in silkworms, as seen by the lower survival rate of larvae exposed to UV light and given allopurinol, which stops UA from being made (Matsuo & Ishikawa, 1999). A special strain of silkworm called 'op translucent' can't make UA because it lacks the enzyme XO, leading to abnormal UA granules and a see-through look (Komoto, 2004). This strain also shows a decrease in the gene PARK7/Dj-1, leading to less XO and more oxidative stress, which harms tissues and nerves. Researchers found that the 'Op translucent' mutant helps explore the role of UA in PD (Tabunoki *et al.,* 2013). Studies also found that human UA levels drop as PD progresses, though the exact reason for this is still unclear. The 'Op translucent' mutant is a great way to study this. Other invertebrates like fruit flies *D. melanogaster* and roundworms *C. elegans* are also used for studying PD. Fruit flies help look at the genetic causes of PD, showing symptoms when certain genes are changed (Xiong & Yu, 2018). Similarly, roundworms can be made to show PD-like symptoms through genetic changes (Cooper & Ruamsdink, 2018). However, no single model can fully replicate the complexity of the human brain, so mammalian models are still needed for comprehensive study. Parkinson's disease (PD), the second most common brain disease worldwide, requires new animal models to better understand its causes and find better treatments. Animal models are vital for exploring the disease and testing new therapies. In this study, we looked at how a chemical called rotenone affects silkworm cells using a technique called transcriptome sequencing. We found that rotenone lowers the energy levels in mitochondria, which triggers a process called mitophagy. The PINK1/Parkin pathway was a big part of this process. We also studied silkworms exposed to rotenone and saw motor problems and less expression of the TH gene, which is important for dopamine production. These findings show how rotenone affects mitochondria and lead to PD like symptoms in silkworms. This supports the use of silkworms as a model for studying PD. This research adds to our understanding of how rotenone causes harm and shows that silkworms can be a helpful model for PD research. In short, our studies on silkworm behavior show that rotenone might be a good tool for creating Parkinson's disease (PD) models in this species. The silkworms treated with rotenone showed slower movement, trouble moving around, and lower levels of TH, which are all signs of PD. This suggests that silkworms could be a useful model for studying parts of PD. However, we still need to explore more to see if rotenone can fully recreate a wide range of PD-like symptoms in silkworms. Future research should look at more symptoms to better understand PD. Learning how rotenone affects different PD symptoms can help us see how good silkworms are as a model. Also, we need to study the molecular changes that happen when rotenone causes PD-like symptoms. Looking into things like oxidative stress, mitophagy, and neuro degeneration can help us understand how PD develops and improve the silkworm model. This could lead to a more accurate and realistic PD model in silkworms. While the early results are encouraging, the true value of the silkworm model for PD research depends on understanding the full impact of rotenone. More research is needed to uncover all PD-like symptoms in silkworms and to make this model a strong tool for PD studies.

Advantages of Silkworm in Research:

Silkworms are great for scientific studies because they have several benefits, like being able to eat specially made food, having access to collections of different genetic materials, and allowing the use of advanced techniques such as RNA interference and CRISPR/Cas9.

(i) Availability of artificial feed:

In the early stage of its life, called the larval stage, the silkworm eats only mulberry leaves. Because of this, people have traditionally fed silkworms only mulberry leaves. However, scientists have developed various types of artificial food that can be used to raise silkworms. Now, silkworm larvae can be raised on these artificial diets, which offer a controlled and consistent way to feed them. This makes it easier to conduct experiments that require specific dietary conditions. It also allows scientists to change the nutrients in the diet to study how it affects the silkworms' growth, behavior, and other characteristics. Artificial diets usually mix mulberry leaf powder with plant-based foods like soybean, wheat, or corn meal, along with vitamins, antioxidants, and inorganic salts (Cappellozza *et al.,* 2005). Using artificial diets also reduces the amount of work needed to maintain a silkworm culture. These artificial foods allow silkworms to be raised in a lab year-round, as long as the temperature, humidity, and light conditions are properly controlled. This means research is not limited to certain seasons like it is with traditional methods.

(ii) Availability of Germplasm banks:

Germplasm banks hold different strains and types of the insect, which help scientists study how genetic differences are passed down and how traits are inherited. These genetic materials are stored in germplasm banks so they can be used in the future. Silkworm germplasm banks have been set up to keep and store the many different strains of this insect (Pereira *et al.,* 2013). This makes it easier for scientists to get a wide range of genetic samples for their work, which helps in studying genetic differences and how traits are inherited. The genetic material of silkworms is a key resource for genetic and molecular biology research and for basic studies in silk farming. Many important genetic findings made in silkworms are at the forefront of genetic research. The Silk DB database gives access to genomic and transcriptomic data from various silkworm strains (Lu *et al.,* 2020). Researchers can use these resources to explore the genetic reasons behind different characteristics and study how silkworms have evolved over time. Using germplasm banks also helps in creating genetically modified silkworm strains that can be used to study various biological questions, using tools like RNAi and CRISPR/Cas9.

(iii) Using state of the art molecular techniques:

Research on silkworms has greatly improved because of new molecular tools like RNAi and CRISPR/Cas9. These technologies have also changed the field of genetics. Scientists can find out how specific genes work and what roles they play in different biological processes by using RNAi to turn off certain genes in silkworms (You *et al.,* 2020). CRISPR/Cas9 allows researchers to edit the silkworm genome, creating modified strains that help study various biological questions (Zhang *et al.,* 2015). One big advantage of using silkworms as a model organism in life science research is the ability to study gene functions and change the genome using tools like RNAi and CRISPR/Cas9.

Conclusion:

Using model organisms is essential in life science research because they offer a deep, real, and accurate look into the processes being studied. Different animal models are widely used for this purpose, especially higher-order animals like mice and rats. While these animals offer a better understanding of biological processes due to their similarity to humans in terms of physiology, they also come with challenges such as high costs of care, ethical concerns, and long life spans. For a long time, *Drosophila melanogaster* and *Caenorhabditis elegans* have been used as model organisms and have contributed greatly to life science discoveries. *D. melanogaster* is useful in genetic studies, neurobiology, human behavior, drug development, and regenerative biology, while *C. elegans* is important for developmental biology and has helped in the discovery of caspases, RNA interference, and micro-RNA. These invertebrates have significantly reduced the reliance on higher animal models. However, their small size can limit their use in certain areas like toxicological research, where a larger body is needed for drug injections. Using *Bombyx mori* as an alternative invertebrate model could help overcome these limitations. Silkworms have several advantages, including a short life cycle, larger body size compared to other invertebrates, fewer ethical issues, lower breeding and maintenance costs, a wide range of mutant strains, and a fully sequenced genome with 14,623 genes, of which 8,469 have direct homology with humans. Although its use is still in early stages, silkworms have great potential, especially in toxicological studies. They can be used to test environmental toxicants, such as pesticides that are harmful to non-target organisms, including humans, and to find ways to reduce their harmful effects. Despite its potential, silkworms have some drawbacks. They are not closely related to humans, and some of their genes have no homology with human genes. They are also poikilothermic, meaning they don't regulate their body temperature, and they lack an acquired immune system. These factors limit their use in all branches of life science. Additionally, silkworms lack emotions, so they can't be used in neuronal behavior studies, but they can help study the connections between neurodegenerative diseases like Parkinson's and Alzheimer's with other conditions like gout and diabetes due to their well developed nervous system. Although mammals cannot be completely replaced as model organisms, silkworms can play a supporting role in understanding various biological processes. In conclusion, using silkworms as a model in life science offers many benefits, providing new solutions and opening new research possibilities that go beyond traditional models.

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MOLLUSCS IN ECO TOXICOLOGICAL MONITORING: A REVIEW

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Introduction:

Rapid agricultural expansion, urbanization, and industrial growth have intensified environmental pollution. The disposal of industrial effluents and municipal waste has become a major challenge in developing cities. Ghosh and Singh (2005) reported that uncontrolled disposal of agricultural waste, drainage, spillage, mining, and smelting introduce metalliferous contaminants into uncontaminated sites, thereby disrupting ecosystems. Heavy metals are a major class of pollutants that severely affect invertebrate and vertebrate health (Duruibe *et al.,* 2007) and disrupt terrestrial and aquatic communities in natural ecosystems (Sanchez, 2008).

Bioindicator:

An effective bioindicator should be sensitive, representative, functionally important in the ecosystem, and easy to collect, identify, and analyze. Qunfang *et al.* (2008) reported, microinvertebrate including Algae, Macrophyte, Zooplankton and Protozoa were useful for monitoring the heavy metal pollution. Its wide occurrence, abundantness in species, sensitivity to response, became suitable bioindicator for the bio monitoring of metal pollution in aquatic ecosystem.

Molluscs as Bio indicators of Heavy Metal Pollution

Molluscs particularly bivalves and gastropods—are among the most widely used biological indicators for monitoring heavy metal contamination in aquatic environments. Their ecological distribution, sedentary lifestyle, and capacity to bioaccumulate contaminants make them excellent sentinels for assessing ecosystem health. Heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg), copper (Cu), and zinc (Zn) persist in water bodies and often accumulate within the tissues and shells of mollusks, providing researchers with measurable indicators of environmental exposure. Gupta and Singh (2011) reported that gastropods and bivalves are valuable tools for environmental monitoring due to their ability to accumulate chemicals in different tissues. Molluscs are effective bioindicators because of their abundance, wide geographical distribution, long lifespan, and ease of collection and identification. Gastropod molluscs are known to concentrate metals more efficiently than other invertebrates (Nicolaidou and Nott, 1990).

“Molluscs inhabit diverse aquatic environments, including marine, estuarine, and freshwater habitats, enabling pollution assessment across wide geographic areas. Species such as Mytilus edulis, Perna viridis, and Lymnaea stagnalis are abundant and easy to collect, making them suitable for long-term monitoring. As key components of food webs, molluscs also indicate contamination risks to higher trophic levels, linking ecosystem health with public health.

Bioaccumulation through Feeding and Filtration

Bivalves are efficient filter feeders, processing large volumes of water daily. This enables them to accumulate dissolved heavy metals and particulate-bound contaminants more efficiently than many other organisms.

Tissue-Specific Accumulation

Different tissues—gills, digestive glands, muscles—accumulate heavy metals at varying levels.

- Digestive glands often show the highest concentrations.
- Shells provide long-term, historical records of exposure.

This tissue-specific uptake allows detailed analysis of both recent and chronic pollution.

Heavy Metal Pollution Assessment Tools with Molluscan

Using molluscs, researchers can measure:

- Concentrations of metals in tissues
- Lipid peroxidation and oxidative stress biomarkers
- Enzymatic changes (e.g., catalase, SOD)
- Stable isotope ratios in shells

These tools offer a multi-level understanding—from organismal to ecosystem-scale pollution effects. Gastropods were known as bioindicators of heavy metal pollution in the environment (Dallinger *et al.* 2001). Gastropod species have exhibited different bioaccumulation for various metal serving for potential bioindicators in aquatic metal pollution (Liang *et al.* 2004).

Advantages of Using Molluscs as Bioindicators

- High sensitivity to heavy metals
- Easy sampling, handling, and laboratory analysis
- Cost-effective monitoring compared to chemical-only assessments
- Ability to reflect bioavailable metal concentrations, not just total metals
- Integration of exposure over time, unlike water samples that show only snapshots

Conclusion:

Molluscan species are among the most effective and reliable bioindicators of heavy metal pollution due to their ecological characteristics, physiological adaptations, and capacity for metal accumulation. Their use provides valuable insights into environmental quality, pollution sources, and ecological risks. As heavy metal contamination continues to threaten aquatic ecosystems worldwide, mollusks offer indispensable tools for monitoring, protecting, and managing environmental health.

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EFFECT OF SEASONAL CHANGES ON MIGRATION IN BIRDS: A REVIEW FROM THE MAHARASHTRA REGION

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Abstract:

Seasonal migration in birds is a complex ecological phenomenon influenced by climatic variables, food availability, habitat conditions, and breeding requirements. Maharashtra, owing to its diverse physiographic zones ranging from the Western Ghats to the Deccan Plateau and extensive coastal ecosystems, supports a wide diversity of resident and migratory avifauna. Earlier studies from the region documented well-defined seasonal arrival and departure patterns of migratory birds, particularly waterfowl and waders (Rahmani *et al.,* 2021; Kasambe *et al.,* 2019). Recent studies (2022–2024) further indicate measurable shifts in migratory timing, distribution, and abundance, largely attributed to changing monsoon patterns, rising temperatures, and anthropogenic pressures on wetlands. This review synthesizes both earlier and recent literature from Maharashtra to evaluate the effects of seasonal changes on bird migration and to highlight emerging conservation concerns.

Keywords: Bird Migration, Seasonal Variation, Maharashtra, Avifauna, Climate Variability, Wetlands.

Introduction:

Bird migration is an adaptive strategy allowing species to track favorable environmental conditions across seasons (Rahmani *et al.,* 2021). Maharashtra lies along important migratory flyways and hosts a large number of winter migrants from the Palearctic and Central Asian regions. Early studies from the state highlighted the role of seasonal temperature decline and photoperiod in triggering southward migration, with peak winter abundance recorded between October and February (Pande *et al.,* 2019; Kasambe *et al.,* 2019). Recent regional studies have emphasized that climate variability is altering these traditional cues, resulting in changes in arrival and departure schedules of migratory birds (Kasambe *et al.,* 2023; Jathar *et al.,* 2024). Understanding both historical and recent trends is critical for effective avian conservation in Maharashtra.

Geographic and Ecological Context of Maharashtra

Maharashtra encompasses heterogeneous habitats including the Western Ghats forests, Deccan Plateau grasslands, agricultural landscapes, inland wetlands, and the Konkan coast. Wetlands such as Jayakwadi, Nandur Madhmeshwar, and Ujani, along with coastal mudflats like Thane Creek, function as crucial wintering and stopover sites. Earlier avifaunal surveys documented high seasonal concentrations of migratory waterbirds at these sites (Kasambe *et al.,* 2019; Patil *et* *al.,* 2020). Long-term monitoring and recent assessments indicate that habitat condition and water availability vary seasonally and significantly influence migratory bird assemblages (BNHS, 2023; Wadatkar *et al.,* 2024).

Seasonal Drivers of Bird Migration

1. Temperature and Photoperiod

Decreasing temperatures and shortening photoperiods in northern latitudes initiate southward migration (Rahmani *et al.,* 2021). In Maharashtra, peak arrival of migratory waterbirds generally occurs between October and November, as documented in earlier wetland studies (Kasambe *et al.,* 2019; Pande *et al.,* 2019). Recent observations from central Indian wetlands reveal delayed arrivals in some duck and wader species, potentially linked to warmer winters (Kasambe *et al.,* 2023).

2. Rainfall and Monsoon Dynamics

The southwest monsoon governs wetland hydrology and primary productivity. Earlier studies emphasized the importance of post-monsoon flooding in attracting large congregations of migratory birds (Patil *et al.,* 2020; Rahmani *et al.,* 2021). More recent research from the Western Ghats shows that irregular monsoon onset and uneven rainfall distribution can negatively affect winter bird assemblages by altering food availability and habitat structure (Jathar *et al.,* 2024).

3. Food Availability

Seasonal peaks in aquatic invertebrates, fish, and vegetation strongly influence habitat selection by migratory birds. Previous studies from inland wetlands and agricultural landscapes reported strong correlations between food abundance and migratory bird density (Mahabal & Bastawade, 2022). Coastal studies along the Konkan region further document associations between seasonal tidal cycles, prey availability, and shorebird presence (Patil *et al.,* 2022).

Migration Patterns Observed in Maharashtra

Table 1: Major Migratory Bird Groups and Seasonal Occurrence in Maharashtra

Bird Group	Common Species	Season of Occurrence	Key Habitats	Source
Waterfowl	Northern Pintail (*Anas acuta*), Bar-headed Goose (*Anser indicus*)	Winter (Oct–Feb)	Reservoirs, wetlands	Rahmani *et al.,* 2021; Kasambe *et al.,* 2019; BNHS, 2023
Waders	Ruff (*Calidris pugnax*), Common Sandpiper (*Actitis hypoleucos*)	Winter	Mudflats, wetlands	Kasambe & Wadatkar, 2020; Patil *et al.,* 2022
Raptors	Steppe Eagle (*Aquila nipalensis*)	Winter	Open landscapes	Pande *et al.,* 2019; Pande *et al.,* 2022
Passerines	Rosy Starling (*Pastor roseus*)	Winter	Agricultural fields	Mahabal & Bastawade, 2022

Effects of Seasonal Changes on Migratory Behavior

1. Timing of Arrival and Departure

Earlier studies reported relatively stable arrival and departure periods for migratory birds in Maharashtra wetlands (Kasambe *et al.,* 2019; Patil *et al.,* 2020). Recent long-term datasets, however, indicate statistically significant shifts in arrival timing of migratory waterbirds, with some species arriving later and departing earlier than previously recorded (Kasambe *et al.,* 2023).

2. Changes in Distribution and Abundance

Changes in land use, urbanization, and seasonal drying of wetlands have caused redistribution of migratory birds to alternative habitats. Earlier observations noted declining abundance at some traditional sites (Pande *et al.,* 2019), while recent studies report increased use of peri-urban wetlands and coastal areas during winter (Chavan & Kulkarni, 2023; Patil *et al.,* 2022).

3. Influence of Climate Change

Climate change has emerged as a key driver modifying seasonal cues for migration. While earlier literature primarily attributed variation to natural seasonal cycles (Rahmani *et al.,* 2021), recent studies demonstrate that rising temperatures and erratic rainfall patterns are reshaping migratory bird communities in Maharashtra (Wadatkar *et al.,* 2024).

Important Migratory Sites in Maharashtra

Table 2: Key Migratory Bird Habitats and Seasonal Importance

Site	District	Habitat Type	Peak Migratory Season	References
Nandur Madhmeshwar	Nashik	Wetland	Winter	Wadatkar *et al.,* 2021; Wadatkar *et al.,* 2024
Jayakwadi Reservoir	Aurangabad	Reservoir	Winter	Patil *et al.,* 2020; BNHS, 2023
Ujani Wetland	Solapur	Wetland	Winter	Kasambe *et al.,* 2019
Thane Creek	Thane	Coastal mudflat	Winter	BNHS, 2022; Patil *et al.,* 2022

Conservation Implications

Synthesis of earlier and recent studies highlights the need for adaptive conservation strategies that account for altered seasonal dynamics. Protection of wetlands during critical winter months, maintenance of post-monsoon water levels, and reduction of anthropogenic disturbance were emphasized in earlier works (Rahmani *et al.,* 2021) and are further reinforced by recent findings (BNHS, 2023; Wadatkar *et al.,* 2024).

Conclusion:

Seasonal changes significantly influence bird migration in Maharashtra through their effects on climate, food resources, and habitat availability. Earlier studies established baseline migratory patterns, while recent research reveals noticeable shifts in timing and distribution, likely driven by climate variability and habitat alteration. Integrating historical data with recent long-term monitoring is essential for understanding ongoing changes and ensuring effective conservation of migratory birds in Maharashtra.

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FISH WASTE GENERATION AND UTILISATION IN INDIA

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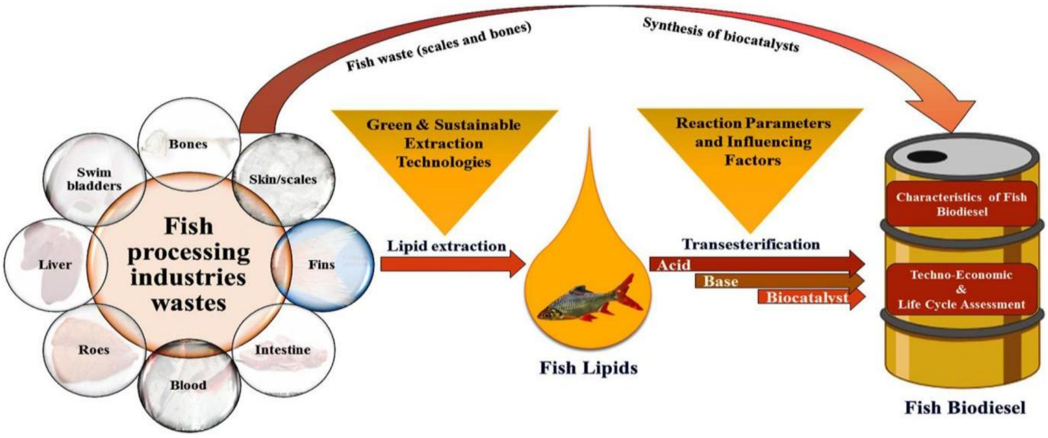
Introduction:

The seafood industry generates a significant amount of waste. These include processing discards such as shell, head, bones, gut, fin, and skin and large amounts of wastewater discharged as effluents and low-value underutilized fish obtained as by-catch during commercial fishing operations. Discards, effluents, and by-catch are high in nutrients such as proteins, amino acids, lipids with high polyunsaturated fatty acid (PUFA), carotenoids, and minerals. As a result, seafood waste is also responsible for both the depletion of essential nutrients and the emergence of substantial environmental hazards. During the 2015–16 year, the waste produced by the fish processing companies in India alone was estimated to be 567,474 tonnes, with the bulk coming from the processing of prawns, followed by finfish and cephalopods (Rajeswari, Padmavathy, Srinivasan, Ahilan, & Aanand, 2018). Certain parts of India are severely affected by the problem of environmental contamination caused by waste generated from the fish processing sector. The states of Gujarat (30.51%), Maharashtra (23%), and Kerala (17.5%) produced the most waste from the processing of fish and shellfish (Wangkheirakpam *et al.,* 2019).

According to Sachindra and Mahendrakar. (2014), 35–40% of the meat produced during fish processing is edible. The remaining non-edible parts include bones, skin/scales, swim bladders, intestines, roes, liver, and blood, accounting for 10–50% of the overall weight. According to Suresh and Kumar (2012), up to 75% of the raw material produced by shellfish, particularly crustaceans, comprises non-edible elements such as the head, shell (carapace), viscera, and appendages are also rich sources of beneficial components such as proteins, lipids, enzymes, bioactive peptides, colors, flavors, vitamins, and minerals. It is essential to recycle these wastes into commercial products to provide them value and reduce the environmental pollution hazard (Bruno, Ekorong, Karkal, Cathrine, & Kudre, 2019). Unfortunately, these wastes are frequently thrown away and released into the ocean or dumped there without first treatment, often exceeding the legal limits. As a result, a large proportion of bioactive-rich materials are lost, and they may have a harmful impact on marine and coastal ecosystems. Furthermore, 36% of the entire weight of fish is wasted during the processing of fish, which results in a huge economic loss. Disposing of these materials is more challenging because of their high organic content (Sasidharan, Baiju, & Mathew, 2013). According to Wangkheirakpam *et al.* 2019, one efficient method for managing solid waste is to recycle these by-products into marketable goods.

Fish waste by-products are used for a variety of things, including human consumption (such as mince, roe, fish heads, and nutraceuticals), agricultural or related purposes (such as fertilizer and compost), and non-nutritional uses (such as biodiesel and fuel, chitin and chitosan, carotenoid pigments, leather, and gelatin), cosmetics (such as collagen), and enzyme isolation (Marsh & Bechtel, 2012). Notably, these waste products have high levels of nitrogen (6%), phosphorus (5%), and potassium (4%), which can support the growth of living things like plants, animals, and microorganisms (Ramalingam, Thirunavukkarasu, Chandy, & Rajaram, 2014) or be used to create products with added value that are relevant to the market (Simpson, 2007). The crude protein content of fish by-products varies from 8% to 35% (Sila & Bougatef, 2016).

Fisheries is an important economic activity in India and presently, the country occupies the second position globally in whole fish production with annual production of about 11.41 million tonnes during 2016-17. Fishery sector funds about 0.9% to National GDP and 5.17% to agriculture GDP (DADF, 2016). The impact might have been much improved if fish wastes and byproducts had been effectively utilized (Jayathilakan *et al.,* 2012). Fish markets and fish processing industries of India generates enormous quantities of discarded fish waste which are presently considered as loss and discarded without recovery of any useful product (Nurdiyana and Mazlina, 2009). It is estimated that India generates >2 metric million tons of waste from fish processing plants only (Mahendrakar, 2000). Among various maritime states, the largest amount of fish wastes was found to be produced from Gujarat (30.51%) followed by Maharashtra (23%) and Kerala (17.5%) (Zynudheen, 2008). As these wastes are rich in nutrients, unless efficiently managed, are likely to be dumped in the environment causing pollution and health related complications (Selvi *et al.,* 2014). Adverse environmental effects associated with fish waste include, accumulations of waste sludge and whole fish parts in near-shore locations, generation of toxic hydrogen sulfide, ammonia and greenhouse gases such as carbon dioxide and methane (Tchoukanova *et al.,* 2012), increased gathering of scavengers in discharge locations and noxious conditions caused by odours, bacteria and waste decomposition (U.S. Environmental Protection Agency Report, 2010). Similarly, if fish wastes are discarded in the ocean itself, aerobic bacteria of water decompose this organic matter using oxygen so that a considerable reduction of oxygen in water occurs. Apart from the release of toxic gases, the waste also overloads nitrogen, phosphorus and ammonia, leading to pH variation and increased turbidity of water resulting in low productivity. Apart from increased phytoplankton production, eutrophication can cause many other effects such as changes in energy and nutrient fluxes, pelagic and benthic biomass and community structure, fish stocks, sedimentation and nutrient cycling (Fang *et al.,* 2004). Besides, inadequate handling of fish waste can have serious consequences to human health such as contamination of water supply, increased incidences and spread of many infectious diseases, deterioration groundwater and other local ecosystems etc.



Oil and Mince Extraction:

The oil from head, bone and viscera was extracted separately by solvent extraction method as described earlier using chloroform: methanol; ratio of 2:1.. Mince was extracted from the bones and spines using a deboning machine. The protein content in the mince was analysed by the Kjehldahl method.

Fish Oil:

Fish oil, a fish powder by-product, was pre-treated by filtration, placed during a reactor with two catalysts (iron oxide and phosphate monobasic) and mixed with ozone bubbling (about 8000 ppm) for one hour temperature which is named primary ozone treatment. Some scientists evaluated the ozone-treated fish waste oil as a way of transportation diesel oil. Then the sample was filtered again and treated with ozone at the same conditions for 30 min, but without the presence of catalysts called secondary ozone treatment. The oil manufactured from fish waste was tested for its density, flash point, pour point, heating value, distillation test, and sulphur content. The yield of the produced fuel was 95–96%, after filtration, primary and secondary treatments. The method of production of bio-diesel from fish source is also reported by Arvanitoyannis and Kassaveti (2007)). The obtained oil was found to have suitable properties for use in diesel engines, such as almost identical higher heating value compared with commercial diesel fuel, no production of sulphur oxides, lowered or any soot, poly-aromatic and carbon dioxide emissions. These properties suggested that the obtained oil had better properties than methyl-esterified oil waste and was suitable for diesel engines, especially at oldness

Encapsulation of Oil:

Biodegradable Film:

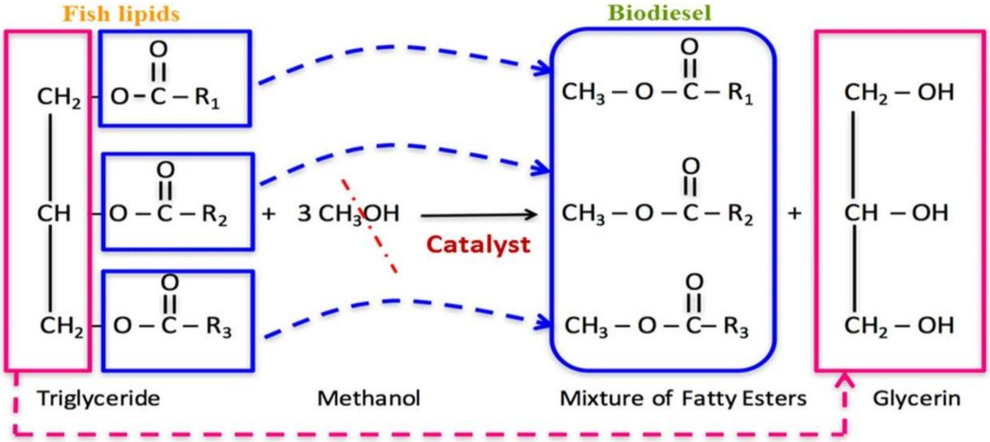
Biodegradable packaging material from mince dispersion of fish waste was synthesized to serve as “Wealth from Waste”- an eco-friendly initiative. Film dispersions were gamma-irradiated at a dose of 10 and 25 kGy before casting. A significant decrease in viscosity and increase in yellowness was observed with irradiation which can be linked to higher oxidative fragmentation of proteins at 25 kGy as compared to 10 kGy. The appearance of different films is shownThe physical properties of prepared films were tested, where film prepared after irradiation at 10 kGy showed better Tensile strength, Young's Modulus with lower water solubility and elongation at break as compared to the control, which had minimum tensile strength and maximum elongation at break. The 25 kGy irradiated film had the highest opacity with yellowness and water vapour permeability. Gamma-irradiation affects the physical properties of the film, hence, irradiation of protein dispersion from waste is an effective tool to develop films with improved quality.

Enzyme:

The enzyme market size was around $ 6.3 billion in 2017 and will see growth of around 6.8% in the CAGR through 2024. The expansion of the food and beverage industry due to the growing needs of the population, including the need to improve the flavor, quality and texture of food is leading to continued growth of the enzymes market. Furthermore, the growth of this sector is also attributable to the enormous applications that enzymes can have in the detergent industry, increasing the effectiveness of detergents by aiding in stain removal (257). The internal organs, including stomach, pancreas, and intestines, are the most important fish byproducts in terms of the number of enzymes found, many of which are cold active, present high catalytic activities also at relatively low concentrations, and stability in a wide range of pH. The complete list of isolated and characterized enzymes from fish have been reviewed by (10,43). Proteases represent the largest group of enzymes naturally found in fish. These enzymes catalyse the hydrolysis of peptide bonds, through different mechanisms of action.

Bio Diesel:

The non-edible part of fish includes head, dorsal fins, tail, skin, viscera and liver. These parts are generally considered as worthless and dumped on land or water bodies without recovering the valuable products from them (Thazeem *et al.,* 2022). Depending on the nature of processing, including gutting, scaling, and filleting, 20–80 % of the total catch volume translates to the fish waste generated. According to the size, shape and composition of each species, the amount of waste generated will vary with an average range like viscera (12–18 %), bones (9–15 %), muscle-trimmings (15–20 %), scales (5 %) and skin and fins (1–3 %) (Afreen *et al.,* 2024). Fish processing is important to improve the quality and stability of the products (as the viscera may contain microbes and enzymes, which can lead to spoilage) and to lessen the costs incurred by transport of inedible parts of the fish (Singh and Benjakul, 2018). The high concentration and ease of extraction of oil from fish waste makes them an important feedstock for biodiesel production. The crude form of fish oil can be used to produce large quantities of stable biodiesel with a very low cost of production (Saravanan *et al.,* 2023). Fish oils were reported to have similar calorific value to that of petroleum oil. The transesterification step for the production of biodiesel, the fish oil will react with triglycerides along with methanol to produce fatty acid methyl esters (FAME). The presence of fatty acid compounds like palmitic, palmitoleic, oleic, stearic acids in FAME in-creases the potential of biodiesel production (Shanmugam *et al.,* 2020). Fish waste can be used in fuel engines after a sustainable fish waste management (Sathish *et al.,* 2024). Hence fish oil can be a renewable fuel source with less pollution load and will reduce the dependence of the world on petroleum fuels. Apart from the high potential of replacing petroleum diesel, biodiesel has a few limitations, the quality of the biodiesel depends on the properties of FAME of the fish oil like branching of the chain, degree of saturation and chain length (Al Azad *et al.,* 2019). Moreover, this approach is sustainable as they are cheaper than the biodiesel produced from vegetable oil. It has additional advantages like lowering the emission and having similar calorific value as that of petroleum-based biodiesel (Dey *et al.,* 2021). In some studies, on fish-based biodiesel the specifications of kinematic viscosity, specific mass, water content and acidity level, flash point and oxidation stability have been found to be in accordance with the specifications required



FW is suitable for agricultural use owing to high contents of nutrients, such as N, P, and Ca). Several fertiliz-ers made of fishmeal are now commercially available and some are authorized for use in organic agriculture. Even fish effluent can be used to irrigate cherry tomato plants. Composting initiatives using FW have been carried out in various parts of the world in search of alternative and viable techniques for transforming fish waste into useful agricultural product. Considering the benefits of composting, the composting itself is biotransformation process of organic materials into stable and complex macromolecules under the action on microorganisms including fungi, bacteria, and corresponding enzyme(s).

It includes four phases:

(i) an initial decomposition phase;

(ii) a thermophilic phase of intense microbial decomposition;

(iii) the second thermophilic phase; and

(iv), a maturation phase.

Rapidly multiplying thermophilic bacterial species dominate during the thermophilic phase till the moment when the bulk of the easily decomposable substrate is exhausted. The majority of the remaining material is woody with lignin as the dominating part, which is also stabilized by humic acids and fungi. The final product, compost, can be used as a soil amendment that improves soil texture and fertility and thus reduces the use of synthetic fertilizers applied to the soil. In this case, the conversion should be performed by implementation of novel technologies for the recycling of waste in the form of compost for their use in agriculture. In addition to the usage of compost as a fertilizer, applying compost to the soil may increase the carbon storage capacity within the soil, which reduces greenhouse gas (GHG) emissions into the atmosphere The above-mentioned material is of great potential use in agriculture. However, its stabilization is recommended prior to its use to prevent problems associated with the appearance of phytotoxic substances). When compost is land applied, finished compost with no phytotoxin can be regarded as a source of nutrients to improve soil structural properties and accelerate germination. Therefore, assessing the phytotoxicity degree of the compost products is essential to achieve high quality compost. The aim of the present paper was to assess the fertilizing power of compost consisting of FW and pine bark. The phy-totoxicity degree of the compost and effects of the compost on seed germination and primary root growth were determined with white mustard (*Sinapis alba* L.). Additionally, the effect of the compost application on ice lettuce (Lactuca sativa L.) was determined. The authors hypothesized that FW compost might be a suitable for agricultural use.

High-Added Value Biomaterials:

Fish processing involves various operations, resulting in the generatio of by-products including offal, heads, roe, and shells. These surplus materials, whether edible or non-edible, are considered by-products. In recent years, numerous studies have focused on utilizing compounds derived from fishery industry by-products. These biomaterials have found applications in diverse sectors such as functional foods, pharmaceuticals, nutraceuticals, biomedicals, livestock, aquaculture feed, agriculture, biodiesel, and other chemicals. The literature emphasises that significant investments have been made in producing items for human consumption, particularly in the extraction and purification of bioactive peptides, enzymes, and biopolymers for biotechnological or pharmaceutical purposes, as they offer high profitability. This approach aligns with the principles of the circular economy by not only providing marketable products but also promoting sustainability within the aquaculture and fishing industries. By reducing the impact of human exploitation on marine resources and preserving coastal environments where these activities are concentrated, this approach contributes to a more sustainable future. Consequently, it is crucial for the fishing and food processing industry to establish comprehensive strategies for utilizing captured and processed waste tdevelop new products).

Recent research has focused on exploring various biomaterials from seafood by-products, including proteins, lipids, chitin/chitosan, derivatives, minerals, enzymes, pigments, and aromatic compounds, with the aim of harnessing their potential for producing valuable products.

Hydroxyapatite:

Hydroxyapatite, having the chemical formula Ca10(PO4)6(OH)2, is highly regarded as a material for biomedical implants used in bone filling. This is not only due to its chemical similarity to bone tissue but also because of its bioactivity, biocompatibility, high osteoconductivity, and non-toxic osteoactivity. Synthetic hydroxyapatite, with its chemical formula and properties resembling the main inorganic component of bones and teeth, has found wide application as a biomaterial in orthopedic and dental fields. It serves to repair or replace hard tissues and deliver drugs. Hydroxyapatite-based biomaterials have been extensively studied for creating artificial bone grafts, either solely composed of hydroxyapatite or as surface coatings (28). Fish bones, containing approximately 70% inorganic matter consisting mostly of minerals and hydroxyapatite, are a significant source of calcium. The extraction of hydroxyapatite from fish bone waste typically involves thermal extraction, which includes removing residual proteins, drying, and high-temperature calcination. The proportions of hydroxyapatite and b-tricalcium phosphate (b-TCP) produced depend on the specific fish bone characteristics and process temperature. Collagen and Gelatin: Collagen, which constitutes a significant proportion of the body's dry weight, is the primary protein present in the extracellular matrix of tissues. Tropocollagen serves as the fundamental unit of collagen and possesses a helical structure. It consists of three α chains that repeat a characteristic chain motif (Gly-X-Y)n, where X and Y are commonly occupied by proline and hydroxyproline, respectively. The self-assembly of these triple helices leads to the formation of collagen fibrils. Marine organisms offer a secure and convenient source of high-quality collagen, especially when compared to collagen derived from land animals. Various marine species such as crustaceans, mollusks, annelids, and different parts of fish such as their skin, scales, bones, and fins have been extensively studied as alternative collagen sources. Consequently, researchers have explored the utilisation of bio-waste, particularly from the organic fraction of fish waste, to discover additional raw materials for collagen production. Gelatin, which is derived from collagen, a fibrous protein, is a heterogeneous mixture of water-soluble proteins with a high molecular weight. The global demand for gelatin has been on the rise in the past decade. Similar to collagen, gelatin sourced from marine animals is considered a viable substitute for mammalian-derived gelatin due to its ability to address concerns related to religious perspectives, safety, and stability of use.

Other Bio Polymer from Fish Waste:

Apart from PHA and chitosan, fish waste is used as a substrate for extraction of biopolymers like gelatin and collagen. Also fish waste is reported to be an excellent substrate for the fermentative production of lactic acid (LA) which can be further polymerized to form poly lactic acid (PLA) films that have extensive application in the biomaterial field. Furthermore, fish scale powder along with commercial starch is used as an additive for the fabrication of biopolymer sheets with enhanced material properties. Collagen is a fibrous insoluble protein majorly composed of amino acids like proline (Pro), glycine (Gly), and hy-droxyproline (Hyp) where Gly serves as the major amino acid in collagen. Collagen basically has a right handed triple helical structure composed of 3 layers of proteins supercoiled together. It is one of the most abundant proteins found in the animal kingdom and is distributed widely in the connective tissues and extracellular matrix (Rajabimashhadi *et al.,* 2023). Based on the super structural organization there are about 28 different types of collagen present in the animal kingdom among which 80–90 % is constituted by types I, II and III. Extracted collagen and its derivatives like gelatin have widespread applications like biomedical applications, cosmetics, nutritional supplements in feeds, biosensors and other applications (Gaikwad and Kim, 2024). 1n 2020 the global estimated value in terms of usage of collagen for biomedical applications was estimated to be USD 870 million and which is expected to increase at a rate of 8 % per annum in the next 6 years. This created an economic and ecological stress on the production of collagen and its derivatives for diverse applications (Lin *et al.,* 2017). Extraction of collagen from fish waste has gained momentum in the last few decades due to the low molecular weight, biocompatibility, ease in extraction, abundance of collagen, problems associated with the pres-ence of pathogens in animal collagens, no major ethical or religious constraints and negligible content of toxins or other biological contaminants (Jafari *et al.,* 2020). Fish skin was reported to have a high amount of type 1 collagen (about 70 %) and its concentration varies from species to species. Although there are multiple procedures reported for the extraction of collagen from fish waste the major steps can be summarized as (a) preparation that involves washing, cleaning and size reduction (b) alkaline or acid pretreatment for removing the non collagenous material and to enhance collagen recovery (c). Extraction of collagen using acids like hydrochloric acid or acetic acid, deep eutectic solvent (DES), supercritical fluid (SCF) and ultrasound assisted methods (d) precipitation and freeze drying. Some Gelatin is a linear biopolymer derived from collagen and has repeating units of Gly-Pro or Gly-Hyp. Gelatin production from collagen involves denaturing the collagen by breaking the hydrogen bonds. Gelatin is classified as type A and type B based on the processing con-ditions. Gelatin extraction from fish waste basically involves three major steps (a) raw material preparation, (b) conversion of collagen to gelatin, and (c) gelatin purification and drying.

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SUSTAINABLE APPROACHES TO FRESHWATER PRAWN CULTURE

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Introduction:

A significant portion of the human diet consists of aquatic animals including fish, crabs, and mollusca. The shrimp is one such crustacean that has a lot of health-promoting ingredients, such as vitamins A and D. Prawns also have very little fat and are abundant in free amino acids and glycogen. Prawn is commercial important animal it fulfills the requirement of food and give economic benefit to the human. It is a beneficial to farmer. The rearing of prawn is called shellfish culture. The area of the pond for shrimp culture should be 0.1 to 0.2 hectares and the depth should be 1.5 to 2.0 meters.

Water should be available in the pond for about 8 to 10 months. Shrimp can be cultured in small and large ponds, village ponds, natural water bodies, artificial reservoirs, as well as farm ponds. There are about 100 varieties of freshwater shrimp. Among them, the giant shrimp (*Macrobrachium rosenbergii)* is larger than all other varieties in size and is called jumbo shrimp. These shrimps have a wide variety of food intake, natural immunity, good growth in fresh as well as low salinity water, tolerance to environmental changes, rapid growth, high nutritional value, large demand in local and foreign markets and good market price. As the technology of seed production of this shrimp has been developed, jumbo shrimp farming is becoming profitable.

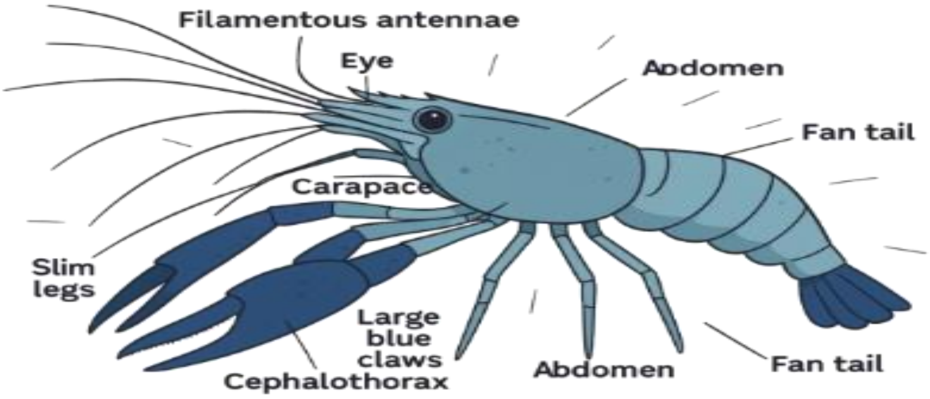


Figure 1: Freshwater Prawn

Essential factors for shrimp culture

1. Availability of seeds
2. Availability of ponds

3. Rearing Pond
4. Conservation pond
5. Fertilizer and feed scheme

Prawn Seed Availability:

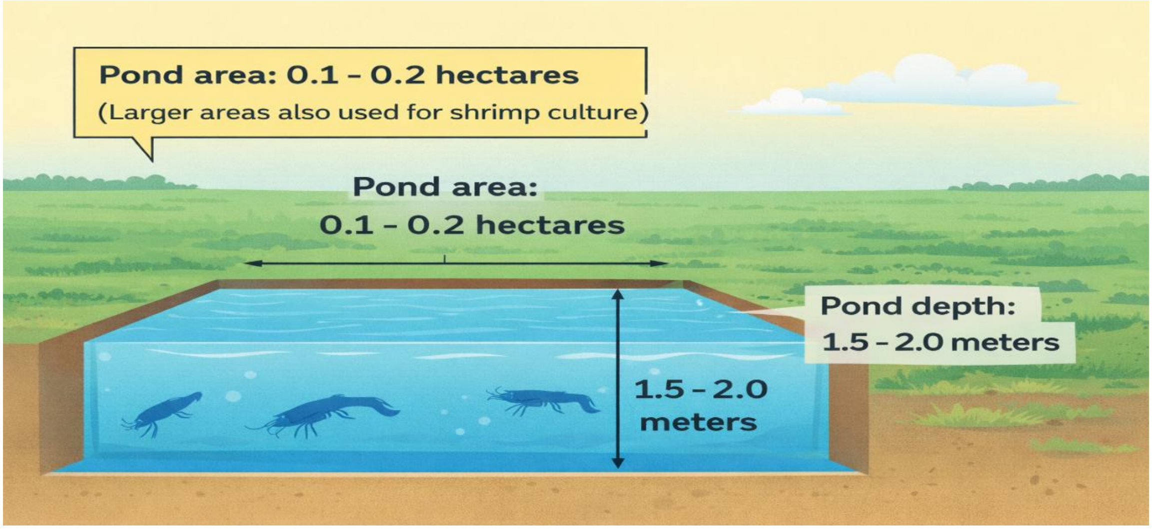
Jumbo shrimp seed can be obtained in two ways. One is by collecting naturally produced seed from the creek and the other is by obtaining it from a seed production center. The availability of natural seed is limited. During the period from September to November, the young shrimp are caught in nets near the mouth of the river while migrating from brackish water to fresh water. There is no guarantee that the seed caught in this way will be only jumbo shrimp. The seeds found contain seeds of more than one type of shrimp. They are difficult to identify because they are very small. In addition, such seeds are diseased and of different sizes, and a large number of these seeds die during transportation.

Natural seed of shrimp is available in the Maharashtra state in the Wada area near Palghar and in some places in Rayugadh, Thane and Ratnagiri districts. There is a market for natural seed in Bhadoch in Gujarat state. Some of the above Hatchery-produced seed should be used for shrimp culture rather than natural seed, as this seed is of one size and type. Due to the strict care taken during seed production, this seed is disease-free.

Signs of identifying healthy and strong fry agility swimming vigorously. Not staying together, evenly distributed on the surface of the tank, being very cautious when hitting the tank with your hand, swimming direction against the water current. The colour of the fry should be dark brown. Both sides of the body Parallel black stripes should be visible on the sides. After the seed grows a little, the tip of the stem-like part on the head of the seed turns pink. These are the signs of healthy seeds. If there are white spots on the seed body, they are the signs of diseased seeds. Such seeds should be destroyed immediately. Eating the food given quickly is the sign of healthy seeds. If the seeds do not eat the food given quickly, then there is a high possibility that such seeds are diseased.

Selection of Pond for Shrimp Culture

The area of the pond should be 0.1 to 0.2 hectares and the depth should be 1.5 to 2.0 meters. Ponds with larger areas are also used for shrimp culture, but in such ponds, food cannot be provided properly to all the pond fish. Since the water quality cannot be controlled, it is difficult to get the desired production. Water should be available in shrimp culture ponds for about 8 to 10 months. Shrimp culture can be done in small and large ponds, village ponds, natural water bodies, artificial reservoirs, and farm ponds.



Ponds for Shrimp Culture

Rearing Pond

The seeds obtained naturally or from seed production centers are very small (1.0 cm). Such seeds are called post larvae. These small seeds are very fragile. These seeds need to be carefully nurtured and grown to the size of 2-3 cm baby shrimps, after which they should be released into a culture pond. For this, small earthen ponds of 50 to 200 square meters in area, cement ponds or plastic tanks can be used. If these facilities are not available, small rectangular cages (hapas) made of mesh can be constructed and such cages can be erected in one corner of the rearing pond and the seeds can be reared in them. For this, approximately 2000 to 3000 seeds per square meter can be stored in the rearing pond.

During this time, to provide these seeds with support and adhesion, items such as tree branches, pieces of cauldron or pot, pieces of cement or plastic pipes, and pieces of old, useless bicycle/scooter tires of appropriate size should be placed at the bottom of the rearing pond or tank.

These seeds should be fed with earthworms or fine pieces of mussels, rice bran, groundnut meal, soybean meal, etc., at a rate of 10 percent of the weight of the chick, 3 to 4 times a day. Depending on the management of the nursery pond, 60 to 90 percent of the young seeds can survive. The seeds that develop in this way are suitable for release into the nursery pond. 40,000 to 50,000 seeds per hectare should be stocked for single shrimp farming. If carp species are to be released in shrimp ponds, 15,000 to 25,000 shrimp seeds (2.5 to 4.0 cm size) should be stocked per hectare in such mixed farming.

Conservation Pond

Depending on the type of pond, it is necessary to prepare it beforehand. During preparation, perennial leaf weed in the pond should be completely eradicated. To eliminate predatory fish and other animals, a mixture of 175 kg of bleaching powder and 100 kg of urea should be released into the pond water. Urea should be released into the water 6 to 7 hours before adding bleaching powder to the pond. This will eliminate predatory fish and animals in the pond. Nets should be installed on the path where water enters and exits the pond. Shrimps grow by shedding their shells from time to time. After shedding their shells, the body of the shrimp becomes very soft. At that time, there is a risk of other shrimps eating the shelled shrimp. Therefore, it is necessary to provide some shelter in the pond to keep them safe during such a situation. Shrimps also have the habit of hiding in safe places like burrows. For this, before storing shrimp seeds in the culture pond, tree branches, nylon nets, pieces of cement pipes, and worn truck tires should be placed in the pond to hang the shrimp.

Types of Shrimp Farming

Individual shrimp culture and mixed shrimp culture with carp can be done. If shrimp seed is available in abundance and cheaply, 40,000 to 50,000 shrimp seed per hectare should be released into the pond to establish a monoculture of independent shrimp. If polyculture of carp with shrimp is to be done, the shrimp seed rate should be 15,000 to 25,000 per hectare. The seed size should be 2.5 to 4.0 cm. In mixed culture, all water levels are used. Since shrimp live at the bottom of the pond, there is no competition between the mid-water feeders and the top-water feeders. Therefore, in mixed shrimp farming, surface-dwelling fish such as carp or cyprinids should not be released into the pond.

The rate of release of seeds of Catla and Rohu fish. The amount of fish should be 1500 per hectare for Catla and 1500 per hectare for Rohu. Farmers who are cultivating shrimp for the first time should move towards 'single shrimp farming' only after experiencing the combined cultivation of shrimp and fish. The method of cultivating shrimp with carp fish has become popular in India. This method makes good use of natural as well as artificial food. The biological cycle of the pond also functions well.

Physical and Chemical Properties of Water in A Conservation Pond

Water temperature - 25 to 32 ℃
Water transparency - approximately 40 cm.
Water pH - 7.0 to 8.5
Amount of dissolved oxygen in water - 5 to 8 mg. per liter.
Water salinity - 50 to 100 ppm.

Supply of Supplementary Food During the Cultivation Period

During the shrimp culture period, relying solely on the natural productivity or natural food of the pond is very dangerous because the shrimp cannot survive on a hungry stomach. They should have ample food available. If food is not available, the shrimp eat their own species. Due to this, the large shrimp in the pond eat other small shrimp. They are harmed. This reduces the number of shrimp in the pond and leads to a significant decrease in production. Shrimp food requires a high amount of protein. Along with this, animal proteins should be used. Animal proteins are supplemented by earthworms, conch, mussels, shrimp, fish waste, animal intestines thrown away from slaughter, and useless parts. All of these are used as supplementary food. This helps shrimp grow rapidly.

Other important ingredients in shrimp food are rice bran, rice polish, peanut meal, soybeans, etc., which should be mixed in the right proportions with vegetable ingredients and used as feed. Food prepared using various food ingredients should be placed in large baskets or trays near the edge of the pond in the morning and evening. The place and time of placing the food should be the same. The amount of food can generally be determined by the amount of food remaining in the tray. The amount of feed per hectare should be divided into three equal portions of 2 kg. Feeding rate should be started at 2 kg per hectare and increased to 10 kg per day per hectare at the end of the 6-12 month rearing period, which would require about 1200 kg of supplementary feed. If fish are cultured along with shrimp, the fish or shrimp food becomes useful to others, and the food is utilized better. Along with this, the amount of food given needs to be increased by 8 to 10 percent.

Shrimp Production

Although shrimp seeds are of the same size and released at the same time in a culture pond, growth is never uniform. This results in differences in the weight and size of the shrimp. To monitor the growth and health of shrimp in the culture pond, a net should be placed in the pond every 15 days and tested. When the shrimp weighs 50 grams or more, the shrimp should be sold. This will give other smaller shrimps more space to eat. They will grow at a proper rate. Generally, after 6 to 8 months of rearing, there is no problem in getting a yield of 1000 to 1500 kg of shrimp per hectare in monoculture of shrimp. In mixed culture of fish and shrimp (polyculture), the expected yield is 500-600 kg of shrimp and 1000-1200 kg of fish per hectare.

Sale of Shrimp

Freshwater shrimp are generally ready for sale when they weigh 50 grams. Shrimp are ready to be sold after 6-8 months. A cast net should be used to catch shrimp. If all the shrimp are to be sold at once, the water level in the pond should be lowered and the shrimp should be caught using a drag net. Store the caught shrimp in a plastic crate and wash it under running water. After that, put ice and shrimp in layers in clean crushed ice. Use one kilogram of crushed ice to store one kilogram of shrimp. Shrimp should be sold immediately without storing for long periods of time. If shrimp and fish are cultured together, gill nets should be used to catch the fish. Cast nets should be used to catch shrimp.

Factors Affecting Shrimp Production Selection of Culture Pond

If you want to build a pond in your farm and cultivate shrimp, the land should be of loamy and black clay soil. The soil should have high water retention capacity so that water does not drain away. This will prevent the essential elements from leaching out along with the water. There will be no need for an electric or diesel pump to maintain the water level continuously. The chosen site should usually be deep and should be lower than the surrounding area. If shrimp are to be cultured in a pond, water should be available in the pond for twelve months. The dams of the pond should be strong.

Elimination of Fish Predators and Fish

Before releasing baby shrimp into the seed culture pond, ensure that the pond is free of unwanted predatory fish. For this, bleaching powder and urea solution should be used. Shrimp seed should not be released into the pond if the water temperature is high.

Shrimp Seed Storage

After bringing the seeds, do not release them into the pond immediately. First, let the seed bags soak in the pond water for 5 to 10 minutes. Then, slowly open the mouth of each bag and add a little water from the tank. Generally, this process should be given 7-10 minutes. After this, slowly release the seeds into the pond water. All the seeds should be released in the pond in equal quantities, not in one place. Do not release all the seeds in the pond in equal quantities, not in one place. Do not store more seeds than the required amount in the pond.

Fertilizer Method

When applying fertilizer to the pond, make sure that the fertilizer is spread throughout all areas of the pond. The manure should be stored in a pile along the edge of the pond. The pond should be completely filled with water before adding fertilizers. Fertilizers should be applied after the pond water stops flowing, or the pond water should not be changed immediately after fertilization. If algae start growing on the pond water, urea and other fertilizers should not be applied. Monitor the movement of shrimp while applying fertilizers.

Use of Complementary Foods

Make sure there is enough natural food in the pond. If shrimp or fish start to come to the surface of the pond or if they start to behave strangely, add new water to the pond or release the water from the pond in the form of a spray into the pond using an electric pump or diesel pump. The water in the pond should not be changed during the day. If natural food is found to be insufficient, the amount of supplementary food should be increased. If too much food is put into the pond, the water in the pond gets polluted. Therefore, do not use excessive food. Supplementary feeding should be given in the morning or evening after sunset. The feeding place and time should be followed regularly.

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METALS AS A CAUSE OF OXIDATIVE STRESS IN FISH: A REVIEW

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Abstract:

This review summarizes the current knowledge on the contribution of metals to the development of oxidative stress in fish. Metals are important inducers of oxidative stress in aquatic organisms, promoting formation of reactive oxygen species through two mechanisms. Redox active metals generate reactive oxygen species through redox cycling, while metals without redox potential impair antioxidant defences, especially that of thiol-containing antioxidants and enzymes. Elevated levels of reactive oxygen species lead to oxidative damage including lipid peroxidation, protein and DNA oxidation, and enzyme inactivation. Antioxidant defences include the enzyme system and low molecular weight antioxidants. Metal-binding proteins, such as ferritin, ceruloplasmin and metallothioneins, have special functions in the detoxification of toxic metals and also play a role in the metabolism and homeostasis of essential metals. Recent studies of metallothioneins as biomarkers indicate that quantitative analysis of mRNA expression of metallothionein genes can be appropriate in cases with elevated levels of metals and no evidence of oxidative damage in fish tissue. Components of the antioxidant defence are used as biochemical markers of oxidative stress. These markers may be manifested differently in the field than in results found in laboratory studies. A complex approach should be taken in field studies of metal contamination of the aquatic environment.

Keywords: ROS; Metallothioneins; Glutathion; Superoxide Dismutase; Antioxidant Defence

Introduction:

Metals, especially heavy metals, are important contaminants of aquatic environments worldwide. Metal pollution has increased with the technological progress of human society. Industry, mining, advanced agriculture, household waste, and motor traffic are all among the activities considered to be major sources of metal pollution. Metals can accumulate in aquatic organisms, including fish, and persist in water and sediments (Luoma and Rainbow, 2008a). Fish are an important component of human nutrition, and those from contaminated sites present a potential risk to human health. Since fish occupy the top of the aquatic food chain, they are suitable bioindicators of metal contamination. Metals are well-known inducers of oxidative stress, and assessment of oxidative damage and antioxidant defences in fish can reflect metal contamination of the aquatic environment (Livingstone, 2003).

Speciation of metals, their solubility and complexation, are important factors that influence the toxicity of metals in the aquatic environment. The amount of dissolved metal strongly depends on water pH. The interaction of metals can alter their toxic effects on aquatic organisms both positively and negatively (Jezierska and Witeska, 2001). Different modes of exposure to metals also play a role in metal toxicity. Fish take up metals through the gills, digestive tract and body surface (Tao *et al.,* 2001; Kamunde *et al.,* 2002).

Various metal ions are involved in oxidative stress in fish. This review concentrates on the most important and most studied metals (Fe, Cu, Cr, Hg and Pb) and metalloids (As, Se).

Oxidative stress and Antioxidant Defences

Oxidative stress is an unavoidable aspect of aerobic life. It is the result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defences in living organisms (Nishida, 2011). Reactive oxygen species are induced by substances such as transitional metal ions, pesticides, and petroleum pollutants (Slaninova *et al.,* 2009; Lushchak, 2011). Free radicals are also produced by endogenous cellular sources during normal cell metabolism. Mitochondrial respiration is the main endogenous source of ROS. Elevated production of ROS can cause oxidation of proteins and lipids, alterations in gene expression, and changes in cell redox status (Livingstone, 2003).

Mechanisms of antioxidant defences in fish include the enzyme system and low molecular weight antioxidants, similar to those in mammals, although the specific isoforms of enzymes in various fish species have not been well identified (Di Giulio and Meyer, 2008). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-s-transferase (GST) are the main antioxidant enzymes and important indicators of oxidative stress. Reduced glutathione (GSH) and oxidized glutathione disulphide (GSSG) play a key role in nonenzymatic antioxidant defence. Metalbinding proteins such as ferritin, ceruloplasmin, and metallothioneins (MTs) have special functions in the detoxification of toxic metals, and also play a role in the metabolism and homeostasis of essential metals (Kelly *et al.,* 1998).

Metallothioneins are low molecular weight proteins rich in cysteine residues that can bind various metals, including mercury, silver, copper, cadmium, lead, zinc, and cobalt, with varying affinities (Hamer, 1986). It has been reported that different fish species possess different isoforms of MTs (Smirnov *et al.,* 2005). Metallothioneins are involved in the regulation of the essential metals copper and zinc and in the detoxification of non-essential metals (Amiard *et al.,* 2006). Zinc has an essential function in the activation of metal-regulated transcription factors which initiate expression of the MT genes (Roesijadi, 1996).

Mechanisms of Metal-Induced Oxidative Damage

The involvement of metals in oxidative damage is multi-faceted. In general, metals produce free radicals in two ways. Redox active metals such as iron, copper, chromium, and vanadium generate ROS through redox cycling. Metals without redox potential, such as mercury, nickel, lead, and cadmium, impair antioxidant defences, especially those involving thiol-containing antioxidants and enzymes (Stohs and Bagchi, 1995). A third important mechanism of free radical production is the Fenton reaction, by which ferrous iron (II) is oxidized by hydrogen peroxide to ferric iron (III), a hydroxyl radical, and a hydroxyl anion (Valko *et al.,* 2005). The superoxide radical can reduce iron to its ferrous form. Copper, chromium, vanadium, titanium, cobalt, and their complexes can also be involved in the Fenton reaction (Lushchak, 2011).

Metals Involved in Oxidative Stress in Fish

Iron

Iron is an essential element required for many physiological functions, and its homeostasis is strictly regulated by various mechanisms. In biological systems iron exists in three oxidation states (II, III, and IV). The majority of iron in the organism is bound to haemoglobin, transferrin, ferritin, and iron-containing enzymes. Therefore, only a trace amount of free iron is present (Valko *et al.,* 2005). Excessive uptake of iron or disturbances in its regulation can be toxic which is related to its ability to catalyze ROS formation via the Fenton reaction. Iron may also potentiate the toxicity of chemicals such as paraquat or 2,3,7,8-tetrachlorodibenzo-pdioxine. Xenobiotics release bound iron and enable it to produce free radicals (Stohs and Bagchi, 1995). Various substances capable of producing superoxide radicals can induce the oxidative potential of iron, since the metabolism of iron and the superoxide are interconnected. Elevated production of superoxide anions increases the release of free iron (Emerit *et al.,* 2001). The deleterious effects of iron include DNA damage, lipid peroxidation (LPO), and oxidation of proteins (Valko *et al.,* 2005).

Lipid peroxidation and alterations in antioxidant enzyme activity in embryonic and adult medaka Oryzias latipes exposed to nano-iron was reported by Li *et al.* (2009). Dose-dependent inhibition of SOD activity and increased production of malondialdehyde (MDA) was observed in medaka embryos. Activity of hepatic and cerebral SOD in adult medaka was initially reduced following nano-iron exposure but subsequently increased with exposure time. There was no evidence of oxidative damage in adult fish; therefore, this study suggested that medaka embryos are more sensitive to nano-iron exposure than are adults. Also, according to Baker *et al.* (1997), an iron-enriched diet in the African catfish Clarias gariepinus induced LPO in the liver and heart.

Copper

Copper plays an essential function in a variety of metabolic processes. It is a component of many enzymatic and structural proteins, including Cu-Zn SOD, cytochrome oxidase, and ceruloplasmin. Copper occurs naturally in soil and water. Mining, industrial discharges, and copper-based pesticides, especially algaecides, are sources of water contamination (WHO, 1998). Copper toxicity to fish and its bioavailability in water vary with physicochemical properties of water, i.e., pH, alkalinity, suspended solids, organic compound content, and hardness (Di Giulio and Meyer, 2008). The concentration of free copper, cupric ion (II), increases with water acidity. Copper hydroxide predominates in water of pH 8.0 and higher (Tao *et al.,* 2001). Calcium, as a contributor of water hardness, was shown to reduce the harmful effects of copper on the growth of Nile tilapia (Abdel-Tawwab *et al.,* 2007).

The cellular toxicity of copper can be explained through its participation in the Fenton reaction. Cuprous (I) ion can catalyse the formation of hydroxyl radicals. Copper-induced oxidative damage can be augmented by various substances. Gravato *et al.* (2006) observed an increase in copper-associated LPO and DNA damage in the European eel Anguilla anguilla pre-exposed to β-naphthoflavone (BNF), a polynuclear aromatic hydrocarbon-like compound. This study suggests a synergistic relationship between copper and BNF. β-naphthoflavone was shown to increase the activity of ethoxyresorufinO-deethylase in liver, causing a reduction in copper. This mechanism facilitates copper redox cycling, leading to enhanced levels of ROS.

Copper binds thiol-containing molecules such as glutathione. The inhibition of total GSH was observed in the livers of three-spined sticklebacks Gasterosteus aculeatus exposed to copper sulphate. Concurrent with the depletion of GSH, enzymatic biomarkers such as CAT, SOD, and GPx increased within the first week of exposure and then recovered, concomitant with copper accumulation in the liver. The recovery of GSH and a return of antioxidant enzymes to basal levels suggest that metallothioneins play a role in detoxification (Sanchez *et al.,* 2005). Gravato *et al.* (2006) attribute the depletion of GSH to direct copper interference with GSH synthesis, inhibition of glutathione reductase, and the participation of GSH as a substrate in detoxification reactions. The depletion of GSH in fish muscle after copper sulphate exposure has also been reported (Jena *et al.,* 2009).

Chromium

Chromium compounds are used in ferrochrome production, electroplating, pigment production, and tanning. These industries, together with the burning of fossil fuels, and waste incineration are sources of chromium in air and water and chromium is ubiquitous in nature (WHO, 1988).

The most biologically important oxidative states of chromium are trivalent (Cr III) and hexavalent (Cr VI). The trivalent and hexavalent forms of chromium are involved in redox cycling (Stohs and Bagchi, 1995). Cell reducing agents such as GSH and nicotinamideadenine dinucleotidephosphate (NADPH) reduce Cr (VI) to the pentavalent state (V), which can participate in the Fenton reaction to produce hydroxyl radicals. The hexavalent form can be reduced to the trivalent form. This transformation is considered to be a major means of detoxification of Cr (VI) in biological systems. Chromium (III) plays a stimulatory role in physiological glucose metabolism. Chromium (VI) actively enters cells through an anion (phosphate) transport mechanism. Chromium (III), meanwhile, is not able to use this mechanism

(Valko *et al.,* 2005). Fish mucus can reduce the oxidative state of Cr (VI) and decrease its penetration, providing fish protection against chromium pollution (Arillo and Melodia, 1990). Kubrak *et al.* (2010) compared the effects of hexavalent and trivalent ions in the goldfish; both ions were found to induce oxidative stress.

Chromium (IV) is known to be carcinogenic in humans (WHO, 1988), and harmful effects of chromium on DNA have been described in fish. Ahmad *et al.* (2006) described genotoxicity of chromium in the gill and kidney of the European eel *Anguilla anguilla*.

DNA damage and an elevation of LPO were observed in the tissues of Chinook salmon Oncorhynchus tshawytscha during chronic exposure to hexavalent chromium in water. According to the authors, the accumulation of Cr (VI) in the kidney led to macroscopic and microscopic abnormalities and negatively affected fish growth and survival (Farag *et al.,* 2006).

A study conducted by Kuykendall *et al.* (2006) also reported DNA damage following chromium exposure. The formation of DNA-protein crosslinks was observed in erythrocytes in the fathead minnow *Pimephales promelas* and largemouth bass *Micropterus salmoides* exposed to hexavalent chromium in water and in the diet. These reports suggest that oxidative-induced alterations of DNA are the main effect of chromium in the studied fish species.

Cadmium

Cadmium is a non-essential metal with no known biological function. The source of cadmium in the aquatic environment is industrial activity (Stohs and Bagchi, 1995). Cadmium does not generate ROS directly, but can alter GSH levels and influence cell thiol status, inducing the expression of metallothioneins in the liver. Changes in GSH and MTs can lead to LPO of the cell membrane. Cadmium enters the electron transport chain in mitochondria, leading to accumulation of unstable semiubiquinones which donate electrons and create superoxide radicals. Cadmium also affects antioxidant enzymes, especially SOD and CAT, and is able to displace copper and iron in various proteins, freeing these metals to then participate in the Fenton reaction (Ercal *et al.,* 2001). Reduced CAT activity following Cd exposure has been reported by Romeo *et al.* (2000) in the kidney of the sea bass Dicentrarchus labrax. This decreased activity was explained by the authors as the direct binding of cadmium to CAT.

Metallothioneins play a major role in the detoxification of cadmium, and this process is clearly organspecific (De Smet *et al.,* 2001). The induction of de novo synthesis of MTs following cadmium exposure has been described in several studies (Jebali *et al.,* 2006; Ghedira *et al.,* 2010). According to De Smet *et al.* (2001), MT induction following intraperitoneal injection of cadmium, as described by Ghedira *et al.* (2010), is evidence of a genetic ability to synthesise MTs. Contradictory results were reported from a field study conducted by Kovarova *et al.* (2009), where no significant correlation between cadmium liver content and MT concentration was observed.

Mercury

Mercury is an important pollutant of water worldwide. A variety of human activities are connected with mercury pollution (silver and gold mining, coal combustion, dental amalgams) (Luoma and Rainbow, 2008b). Organic methylmercury and inorganic (mercurous, mercuric) forms exist in nature. Organic forms are the result of methylation of inorganic mercury by microorganisms in sediments and water. Methylmercury is generally more toxic to fish than the inorganic forms (Houserova *et al.,* 2006). Mercury reacts with the thiol groups of GSH, which can induce GSH depletion and oxidative stress in tissue (Stohs and Bagchi, 1995).

Monteiro *et al.* (2010) described changes in biomarkers of oxidative stress following exposure to inorganic mercury. Methylmercury was shown to induce oxidative stress in several field studies (Larose *et al.,* 2008; Mieiro *et al.,* 2010). The data presented in these studies suggest that both organic and inorganic forms of mercury participate in the formation of ROS.

Metallothioneins also play a protective role in response to mercury exposure. The mRNA expression of two MT genes was noted by Navarro *et al.* (2009) in the liver of feral carp Cyprinus carpio from a mercury-contaminated river. No biochemical evidence of oxidative damage associated with these changes was found in the tissue. This suggests that quantitative analysis of the mRNA expression of MT genes can be a suitable biomarker of subtoxic metal exposure in cases of elevated levels of metals and no evidence of oxidative damage in fish tissue. No significant correlations between total mercury content and MT levels were described by Mieiro *et al.* (2011) in different fish tissues from a mercurycontaminated area.

The induction of MTs in the liver, gill, and heart of the tropical freshwater fish Brycon amazonicus was measured by Monteiro *et al.* (2010) following a 96 h exposure to inorganic mercury. Significant alterations in the expression of the antioxidant enzymes SOD, CAT, GST, GPx, and GR were observed, leading to oxidation of lipids and proteins. Induction of the SODCAT systems represents a rapid adaptive response to mercury exposure. As mentioned, mercury influences GSH concentration. In this study, an increase in GSH content was observed without changes in GSSG levels in the liver and gill. The authors explained this as enhanced hepatic uptake of amino acid substrates and activity of biosynthetic enzymes leading to the protection of the fish from oxidative damage. Other authors have also observed increases in GSH levels following mercury exposure (Rana et al.,1995; Elia *et al.,* 2000). Depletion of GSH was reported by Elia *et al.* (2003) and Mieiro *et al.* (2010). Metal-induced decreases in GSH levels could be the result of direct binding of the metal to GSH through its SH group (formation of metal-SG complexes) or of enhanced oxidation of this thiol (Elia *et al.,* 2003).

Lead

Lead is a major environmental pollutant. Paint, cosmetics, human medicines, food supplements, and petroleum-based fuels are sources of lead pollution (Stohs and Bagchi, 1995).

Lead accumulation in sediment is of significance for aquatic organisms. Lead is not a transition metal and cannot readily undergo valence changes. Lead can induce oxidative damage through direct effects on the cell membrane, interactions between lead and haemoglobin, which increase the auto-oxidation of haemoglobin, auto-oxidized δ-aminolevulinic acid, interactions with GR, or through the formation of complexes with selenium, which decrease GPx activity (Ercal *et al.,* 2001).

An intraperitoneal injection of lead was administered to the toadfish Halobatrachus didactylus and its effects on aminolevulenic acid dehydratase (ALA-D) activity, MT levels, and LPO in the liver, kidney, and blood were investigated over seven days (Campana *et al.,* 2003). The results showed an increase in MT levels, suggesting that lead can induce the synthesis of MTs, although to a lesser degree than some other metals. The authors proposed that lead is not a good inducer of LPO, because a decrease in MDA levels was measured in the liver, and the induction of LPO observed in the kidney was ambiguous. No significant variations in ALA-D as a result of lead exposure were reported. Maiti *et al.* (2010) described elevated MDA levels in the brains of walking catfish Clarias batrachus following a 60-day exposure to waterborne lead.

Arsenic

Arsenic is a known carcinogen in human. Arsenic forms inorganic and organic complexes in the environment. Arsenite (III) and arsenate (V) are inorganic forms that can be methylated. The trivalent arsenite is biologically more active than pentavalent arsenate. Glutathione plays a key role in the cell redox status induced by arsenic. Glutathione is an electron donor in the reduction of arsenate to arsenite. Arsenic cell metabolism generates ROS, although the mechanisms are not clear. Reactive nitrogen species are also involved in oxidative damage associated with arsenic (Bhattacharya and Bhattacharya, 2007).

The central role of GSH in arsenic toxicity was described in several studies. Allen *et al.* (2004) described the biochemical toxicity of arsenite in Channa punctatus. Levels of GSH, GSSG, and LPO in the liver and kidney were measured during 90 days of exposure. The authors reported durationdependent changes in GSH levels, with positive peaks at seven, 30, and 90 days of exposure, as an adaptive response of fish to arsenic. The progression of LPO showed a similar pattern.

The induction of LPO, an increased GSSG/GSH ratio, and excess production of hydrogen peroxide were observed in the Indian catfish Clarias batrachus exposed to nonlethal doses of arsenic for 10 days (Bhattacharya and Bhattacharya, 2007). The authors explained the elevated concentration of hydrogen peroxide as arsenic-induced alterations of peroxisome.

Oxidative stress-induced apoptosis was suggested by Seok *et al.* (2007) as a possible mechanism of arsenic toxicity in a zebrafish Danio rerio liver cell line.

Arsenobetaine and arsenocholine are non-toxic organic forms of arsenic present in fish. According to Ciardullo *et al.* (2010), the majority of total arsenic in fish tissue is present as arsenobetaine. This is similar to the conclusions of Harkabusova *et al.* (2009). The data presented suggest that an assessment of arsenic speciation needs to be included in studies dealing with arsenic pollution, especially in field conditions, taking into consideration the risk to humans of fish consumption.

Conclusions:

The above-mentioned studies document that oxidative stress induced by metals is an important issue in aquatic ecosystems. The response of fish to oxidative damage after acute and also chronic metal exposure is evident under laboratory conditions as well as in field studies.

The components of antioxidant defences are diversely influenced by metals. Both increases and decreases in enzyme activities and also enhanced and reduced levels of non-enzymatic components have been described after metal exposure. A specific biomarker of oxidative stress caused by metals does not exist, and for that reason a complex approach should be taken. Metallothioneins seem to be a suitable biomarker of metal exposure, especially under laboratory conditions. In field studies the applicability of MT content in fish tissues as a biomarker is questionable following chronic metal exposure. In several field studies there were no significant correlations found between MT content and cadmium as well as between MT content and mercury in fish tissue.

Frequently, aquatic contamination involves various chemicals that interact with one another. For that reason, studies on metal-metal interactions are required. According to the above-mentioned studies selenium pre-exposure reduces oxidative damage caused by lead, copper, cadmium and chromium. On the other hand, metal-induced oxidative damage can be augmented by various substances. A synergistic relationship has been described between βnaphthoflavone and copper and also BNF and chromium. Copper reduces the toxicity of delthametrin and endosulfan and calcium has a protective role against copper toxicity. Laboratory studies dealing with multiple metal interactions should be performed to enable a better understanding of mechanisms of metal toxicity in the aquatic environment.

Fish can be used as bioindicators of metals in the environment by studying the induction of oxidative stress; however, the specific forms of biomarkers and mechanisms of their action still need to be investigated.

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