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THE LIVING WORLD

Contemporary Perspectives in Life Sciences

Volume I

Editors:

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The Living World: Contemporary Perspectives in Life Sciences Volume I

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PREFACE

Life sciences have witnessed extraordinary growth over the past few decades, transforming our understanding of the diversity, complexity, and interconnectedness of living organisms. From molecular mechanisms to ecosystem dynamics, contemporary research continues to expand the frontiers of knowledge, offering innovative solutions to global challenges in health, agriculture, biodiversity conservation, and environmental sustainability. *The Living World: Contemporary Perspectives in Life Sciences* presents a collection of scholarly contributions that reflect the breadth and significance of modern biological research.

This volume brings together the work of researchers, academicians, and professionals from diverse disciplines within the life sciences. The chapters encompass a wide range of topics, including molecular biology, genetics, microbiology, biotechnology, zoology, botany, ecology, environmental science, physiology, bioinformatics, and emerging interdisciplinary fields. Collectively, these contributions demonstrate how scientific advancements are enhancing our understanding of life while addressing critical issues such as climate change, food security, disease management, and sustainable resource utilization.

The primary objective of this book is to provide readers with an up-to-date overview of recent developments in life sciences and to encourage interdisciplinary thinking that bridges basic research with real-world applications. By integrating theoretical knowledge with contemporary scientific discoveries, this volume serves as a valuable resource for undergraduate and postgraduate students, educators, researchers, healthcare professionals, and policymakers. It is our hope that the ideas presented in these chapters will stimulate scientific inquiry, inspire innovative research, and promote collaborative efforts across disciplines.

We extend our sincere gratitude to all the contributing authors for their valuable research and scholarly dedication. We also express our appreciation to the reviewers for their insightful comments and constructive suggestions, which have greatly improved the quality of this volume. Our heartfelt thanks are due to the editorial team and the publisher for their unwavering commitment and meticulous efforts in bringing this book to fruition.

We trust that *The Living World: Contemporary Perspectives in Life Sciences* will serve as a meaningful academic reference, inspire future discoveries, and contribute to the advancement of biological sciences for the benefit of society and the sustainable stewardship of our planet.

- Editors

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ELEPHANT FOOT YAM: FROM TRADITIONAL FOOD TO FUNCTIONAL INGREDIENT

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Abstract

Elephant foot yam (*Amorphophallus paeoniifolius*), commonly known as Suran or Jimikand, is an important tropical tuber crop widely cultivated in Andhra Pradesh, Tamil Nadu, West Bengal, and Kerala. It is valued as a vegetable, pickle, and traditional Ayurvedic medicine owing to its exceptional nutritional and therapeutic properties. The tuber is rich in carbohydrates, proteins, dietary fiber, healthy fats, vitamins, and essential minerals such as potassium, phosphorus, calcium, and zinc, making it a nutrient-dense functional food. It exhibits a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antifungal, analgesic, gastroprotective, expectorant, and anticarcinogenic effects. Traditionally, it is used in the management of hemorrhoids, asthma, bronchitis, digestive disorders, inflammation, tumors, and other chronic ailments. Increasing consumer awareness of health-promoting foods has enhanced its importance in developing value-added food products. Owing to its rich nutritional profile and medicinal potential, elephant foot yam offers promising opportunities for improving human health, food security, and the functional food industry.

Keywords: *Amorphophallus paeoniifolius*, Elephant Foot Yam, Functional Food, Nutritional Composition, Medicinal Properties, Value-Added Products.

Introduction

Elephant foot yam (*Amorphophallus paeoniifolius*) is popular as a *Jimmikand* or *Suran*. Andhra Pradesh, Tamil Nadu, West Bengal, and Kerala are its major producers. Different cultivars of this tuber crop are usually grown in the north and east regions of India and utilized in the form of vegetable, pickle and ayurvedic formulations for a variety of illnesses. Many people use this tuber in the form of cooked meal and is commonly used in Ayurveda medicines. Elephant foot yam is a vegetable high in carbohydrates, protein, potassium, zinc, phosphorus, vitamin B6, vitamin A and calcium.

It is commonly used for making curry by cooking it with salt, pepper, tamarind and turmeric powder. Recently, elephant foot yam has received attention from health-conscious people as a vegetable in their regular diet. It is an effective antioxidant that slows down aging process and provide protection against cardiovascular disease and stroke (Firdous *et al.*, 2020). Arsa

(hemorrhoids), pliha (splenic disorders), gulma (tumor conditions), svasa (breathing problems), kasa (cough) and asthma are among the therapeutic uses of elephant foot yam. In Indian Ayurveda, it is frequently utilized as a natural medicine and has been applied to the treatment of numerous deadly and chronic illnesses (Behera *et al.*, 2014).

This edible tuber has demonstrated its effectiveness in lowering the risk of a number of diseases because of its gastro-intestinal protective, pain relieving, expectorant, antimicrobial, antioxidant, anticarcinogenic, anti-inflammatory, and cytotoxic properties (Khan *et al.*, 2008, De *et al.*, 2010). Elephantiasis, cancer, inflammation, haemorrhoid, haemorrhages, bronchitis, asthma, anorexia, dyspepsia, flatulence, colic and constipation all are all treated with it (Shilpi *et al.*, 2005). The tuber also possesses antiprotease, central nervous system depressive and cytotoxic activities (Angayarkanni *et al.*, 2005).

Composition of Elephant Foot Yam

Elephant foot yam or *Suran* is high in starches, proteins, potassium, zinc, phosphorus, vitamin B6, vitamin A and calcium. The solid content in elephant foot yam is between 17 and 24%. It has starch content between 14 and 22%, sugar content between 0.50 and 2%, protein content between 1 and 3% and negligible fat < 0.4% (Chattopadhyay *et al.*, 2009, Kandekar *et al.*, 2021). It contains higher calories, fibers, calcium and iron with relative values of 110kcal/100g, 1.83g, 62.42mg and 4.43 mg/100g (Yadav *et al.*, 2018). Proteins, minerals, vitamins, lipids and carbohydrates make it a nutritious food (Harish *et al.*, 2014). Utomo *et al.* (2021) found the color of yam in the range from yellow to orange with its brightness from 70.53 to 81.63. Its physicochemical composition projects 66.58 to 88.36% moisture, 2.49 to 4.91% ash, 1.42 to 7.89% and 23.84 to 31.37amylose. The genotypes of the yam that had the highest levels of dietary fiber are MLGAR-0101 (55%) and MLGAR-0016 (53%) (Utomo *et al.*, 2021).

Nutritional Significance of Elephant Foot Yam

Elephant foot yam is rich in carbohydrates, calcium, iron, potassium and magnesium. Its flour contains 68% carbohydrates (29% amyloses), 14% dietary reflecting 62% in vitro digestibility (Faridah *et al.*, 2005). Yam is rich in dietary fibers, and vitamins and it also contains a large number of necessary minerals such as copper, iron, zinc and magnesium (Koni *et al.*, 2017). According to Koni *et al.* (2021), yam is a carbohydrate-rich food that can be used in place of rice. It is a low-fat food that is high in fiber, potassium, calcium (53mg/100g), phosphorus (21mg/100g), vitamin A (122mg/100g), vitamin B6 (0.2mg/100g) and trace minerals including selenium, zinc and copper (Das *et al.*, 2009). It also has a fair quantity of protein and carbohydrates. 18.0% carbohydrates, 1%–5% protein and up to 2% fat are present in the tubers. It contains vitamins (A, B1, B2), minerals (Ca, K, P, Zn) and starch which serves as a key source of energy (Chattopadhyay *et al.*, 2009). The most common micromineral is potassium with

concentration of 327.83mg per 100g, after that phosphorus (166.91mg/g), calcium (161.08mg/g) and iron (3.43mg/g).

The nutritional illnesses that are brought on by insufficient vitamins A and C and calcium intake can be addressed by consuming enough elephant foot yam tubers (Padmaja *et al.*, 2013). It contains protein (9.81g/100g), carbohydrate (25.54g/100g), fat (1.41g/100g), fiber (5.7g/100g), moisture (66.08g/100g) and ash (4.83g/100g) (Basu *et al.*, 2014). A comparative study on comparison nutritional value of two types of elephant foot yam (NDA-5 and NDA-9) reported that, NDA-5 has 11.20% moisture, 88.90% dry matter, 7.43% crude protein, 0.50% crude fat, 2.69% crude fiber, 4.97% total ash and 73.4% carbohydrate, respectively (Yadav *et al.*, 2016). The values for NDA-9 were 11.00% moisture, 6.49% dry matter, 0.65% crude protein, 3.20% crude fat, 4.87% total ash and 73.79% carbohydrates, respectively.

The 1.16% crude protein, 1.17% crude fat and 3.44% crude fiber are found in elephant foot yam flour. Potassium (1443.33mg/kg), calcium (8535.76mg/kg) and magnesium (1512.39mg/kg) are the minerals found in yam flour. Oxalates (318.51mg/kg), tannins (0.46%), cyanide (35878 ppm) and phytates (0.165%) are the anti-nutrient factors found in yam. It is a common local food that is quite simple to grow and can be used as a substitute for other sources of carbohydrates. The tubers of elephant foot yams contain potassium (34mg/100g), calcium (50mg/100g), vitamin A (434IU/100g), proteins (2.14%), fat (0.46%), and fibers (1.68%). Moreover, it contains phenols, alkaloids and flavonoids, all of which are essential for healthy physical activities. It is water-rich and has very little fat as well (Krishna *et al.*, 2022).

Mitigating Anti-Nutrient Components of Elephant Foot Yam

Oxalate and phytate are two antinutrient components found in elephant foot yam. The oxalic acid content in yam is 1.3% (Paul *et al.*, 2013). In addition to being an irritant, oxalate is thought to be toxic and anti-nutritional. The oxalate from elephant foot yam can be lethal to humans when consumed in greater doses (up to 2g) (Libert and Franceschi, 1987). Chelating minerals including iron, calcium, zinc and magnesium oxalates can make them inaccessible to the body. As a result, eating foods high in oxalate may result in a mineral shortage in the body. To avoid oxalate-related illnesses, oxalate must be reduced in the diet. The recommended daily intake of oxalates for people with kidney stones is 40–50mg (Massey *et al.*, 1993). According to extensively conducted research on the anti-nutrient status of elephant foot yam, removing acidity and oxalate helps Araceae plants be used more effectively as food and animal feed. Boiling could be useful for reducing oxalates during food processing. Kumar *et al.* (2017) reported that after 15min of boiling, the elephant foot yam's irritating effect lessens and after an hour of boiling, it completely disappears. Wanasundera and Ravindran *et al.* (1992) reported yam tubers lose 40–50% of their total oxalates when they are boiled. The boiling reduces ($p < 0.05$) oxalate content of yam (Iwuoha and Kalou, 1995). Boiling of various yam cultivars result

in an oxalate decrease of 31–53% (Bhandari and Kawabatta, 2006). The raphides, which are oxalate crystals that resemble needles are accountable for acidity (Lewu *et al.*, 2010).

The oxalates were reduced by 44.76% overall and by 35.39% in soluble form after 10min of boiling (Kumar *et al.*, 2017). However, a further extension of the boiling time had no discernible impact on the oxalates. Also, throughout the boiling phase, a decrease in the elephant foot yam's sensory acidity, phenolic content and antioxidant activity was also noted. Hence, 10min of boiling was reported to be enough to reduce oxalates to a healthy level of 71mg/100g. The intake of 4–9mg/100g of phytate can double the reduction in human Fe absorption (Koni *et al.*, 2017). The elephant foot yam means phytate content was below the 25mg/100g dietary recommendation. By raising the temperature and length of heating time, the phytate level in vegetables can be decreased. Sun-dried yams have had their phytate content by 51%.

Health Benefits of Elephant Foot Yam

Elephant foot yam is beneficial in protecting stomach health. It possesses active antioxidant, anti-diarrheal and anti-inflammatory properties (Singh *et al.*, 2015). Its extracts have analgesic, cytotoxic, immune-modulating, anthelmintic, anti-inflammatory, hepatoprotective and anxiolytic effects (Shilpi *et al.*, 2005). It is useful in monitoring the digestion system and liver function. The elephant foot yam improves digestion, which relieves constipation. Also, it aids in the treatment of severe gastrointestinal conditions like piles and prolonged diarrhea. It promotes the well-being of our digestive system (Dey *et al.*, 2010). The quercetin in elephant foot yam. is efficient at preserving liver functions (Sharstry *et al.*, 2010). It lowers the chances of hyperglycaemia, heart diseases, tumour and inflammatory disorders. The elephant foot yam methanol extract contains anti-inflammatory effects (Shankhajit *et al.*, 2010). As a result, it is helpful for many medical disorders. It aids in the pain relief of inflammatory disorders including ulcerative colitis and arthritis. These tubers as an important source of providing dietary fiber (Parvathi *et al.*, 2016). Dietary fiber lowers serum cholesterol, prevents colon cancer, promotes intestinal health and acts as a preventative measure for obesity, diabetes and cardiovascular illnesses. Elephant foot yams are beneficial for treating obesity since they are unusually high in dietary fiber and low in calories (Benil *et al.*, 2017). The study also reported rats fed a meal of mung bean and elephant foot yam had their blood cholesterol levels significantly reduced. It projects low glycaemic index thereby maintaining blood sugar levels (Santosa *et al.*, 2017). Research has demonstrated that elephant foot yam has anti-diabetic properties. As a result, incorporating it into your regular diet may help you manage your diabetes. The yam has an amazing antioxidant property. Hence, it guards against oxidative stress, which reduces the cancer risk. The aging process may also be slowed down by these antioxidants. Elephant foot yam reduces LDL or bad cholesterol levels and increase omega-3 fatty acid levels when consumed regularly

(Krishna *et al.*, 2022). Heart patients can frequently take this tuber because it is very low in fat and raises levels of HDL, or good cholesterol.

Yam as a Nutritive Food Ingredient

The health benefits potential and dietary importance of elephant foot yam have gained the interest of nutritionists and researchers for the development of innovative food products fortified with elephant foot yam. Yadav *et al.* (2018) developed value-added sweet biscuits made using elephant foot yam flour. Regarding aroma, texture, taste and general acceptability there is no noticeable difference between the treatments. The control group's sweet biscuit had the largest width (cm) and thickness (cm) with corresponding values of 5.58cm and 1.16cm. For protein, carbs, fiber, calcium and iron there was a considerable variation between treatments. He also reported a significant rise in energy, carbohydrates, fiber, calcium and iron in yam flour supplemented in dhokla. Yam flour added to dhokla increased its protein and fat content. Behera *et al.* (2019) conducted a study on preparation of lacto-pickles using elephant foot yam and probiotic *Lactobacillus Plantarum*. The greatest output of fermented lacto-pickles was discovered to be after 22-day fermentation period. Lohan *et al.* (2020) utilized this starch-rich yam tuber in making chips and other foods. The cake prepared from composite flour by blending wheat flour and elephant foot yam flour presented 15.20% moisture, fat-0.80%, protein- 4.50% and fiber-5.30% (Saklani *et al.*, 2020).

Conclusion

Elephant foot yam is a nutritionally rich tuber containing protein, minerals, vitamins, fats, fibers and carbohydrates. Its medicinal properties (anti-inflammatory, gastroprotective, analgesic, antibacterial, antioxidant and antifungal) have gained the attention of nutritionists, food scientists and medical researchers. The utilization of such a nutrient-dense powerhouse, elephant food yam in development of new food products will leave no stone unturned in evolution of value-added food product sector.

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SYNTHESIS, STRUCTURAL CHARACTERIZATION, AND EVALUATION OF ANTIFUNGAL POTENTIAL OF NOVEL AMINOPHENAZONE-DERIVED SCHIFF BASE COMPOUNDS

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Abstract

Schiff bases are an important class of bioactive compounds with diverse pharmacological properties, including antimicrobial and antifungal activities. In the present study, five novel aminophenazone-based Schiff base derivatives (C1–C5) were synthesized through the condensation of 4-aminophenazone with different substituted aromatic aldehydes using a simple and eco-friendly solvent-free grinding method. The synthesized compounds were purified by recrystallization from ethanol and obtained in moderate to good yields (65–76%). Preliminary physicochemical characterization by melting point determination and thin-layer chromatography (TLC) confirmed the purity of the synthesized compounds. Structural characterization was carried out using Fourier Transform Infrared (FT-IR) spectroscopy, Proton Nuclear Magnetic Resonance (^1H NMR) spectroscopy, and Liquid Chromatography–Mass Spectrometry (LC-MS). The presence of the characteristic azomethine ($\text{C}=\text{N}$) stretching band in the FT-IR spectra, the azomethine proton signal in the ^1H NMR spectra, and the expected molecular ion peaks in the LC-MS analysis confirmed the successful formation of the Schiff base derivatives. The antifungal activity of the synthesized compounds was evaluated against *Candida albicans* and *Aspergillus niger* using the agar well diffusion and broth microdilution methods. All compounds exhibited appreciable antifungal activity, with Compound C4 (3,4,5-trimethoxy derivative) showing the highest activity, followed by Compound C1 (4-bromo derivative). The enhanced activity of methoxy- and bromo-substituted derivatives suggests that aromatic substitution significantly influences antifungal efficacy. These findings indicate that aminophenazone-derived Schiff bases possess promising antifungal potential and may serve as valuable lead compounds for the development of novel antifungal agents.

Keywords: Schiff bases, Aminophenazone, 4-Aminoantipyrine, Solvent-free synthesis, FT-IR, ^1H NMR, LC-MS, Antifungal activity, *Candida albicans*, *Aspergillus niger*.

1. Introduction

Schiff bases are an important class of organic compounds containing an azomethine or imine functional group ($>\text{C}=\text{N}-$), formed by the condensation of a primary amine with an aldehyde or ketone. They were first reported by the German chemist Hugo Schiff in 1864 and have since

attracted considerable attention because of their simple synthesis, structural diversity, and wide range of biological and industrial applications. The presence of the imine linkage imparts unique electronic and coordination properties, making Schiff bases valuable intermediates in organic synthesis, medicinal chemistry, coordination chemistry, and material science.

Structurally, Schiff bases are represented by the general formula $R_1R_2C=NR'$, where R' is an aryl or alkyl group. Depending on the nature of the substituents, they may exist as aromatic or aliphatic derivatives and can function as mono-, bi-, or multidentate ligands. Their strong affinity for transition metal ions enables the formation of stable coordination complexes with metals such as copper, nickel, zinc, cobalt, and iron, which have important applications in catalysis and bioinorganic chemistry.

Schiff bases exhibit diverse pharmacological properties, including antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, anticancer, and antidiabetic activities. The biological activity of these compounds is largely attributed to the azomethine ($>C=N-$) group, which can interact with various biological targets and enhance membrane permeability. In addition to their therapeutic potential, Schiff bases play important roles as intermediates in enzymatic reactions. For example, pyridoxal phosphate (PLP)-dependent enzymes and retinal in rhodopsin form Schiff base intermediates that are essential for amino acid metabolism and visual phototransduction, respectively.

Owing to their ease of synthesis, structural flexibility, and broad spectrum of biological activities, Schiff bases continue to be extensively investigated for the development of novel bioactive molecules. In recent years, Schiff base derivatives synthesized from heterocyclic compounds such as aminophenazone have gained significant interest because of their enhanced antimicrobial and antifungal activities, making them promising candidates for the discovery of new therapeutic agents.

2. Materials and Methods

2.2.1 Synthesis of Schiff Base Derivatives

Schiff base derivatives were synthesized by a solvent-free grinding method. 4-Aminophenazone (0.01 mol) was mixed with the respective aromatic aldehyde (0.02 mol) in a mortar and ground thoroughly at room temperature until a yellow solid formed. The reaction mixture was allowed to stand overnight, and reaction completion was monitored by Thin Layer Chromatography (TLC). The crude products were purified by recrystallization from ethanol, dried, and designated as Compounds C1–C5.

2.2.2 Purification and Yield

The purified products were washed, recrystallized from ethanol, dried in a desiccator, and weighed. Percentage yield was calculated using the theoretical and practical yields of each compound.

2.2.3 Physical Characterization

The synthesized compounds were characterized by determining melting point and TLC. Melting point was used to assess purity, while TLC (silica gel G; chloroform: methanol, 9:1) confirmed reaction completion and purity through R_f values.

2.2.4 Spectral Characterization

Structural confirmation of the synthesized compounds was performed using FT-IR, ¹H NMR, ¹³C NMR, and LC-MS. FT-IR confirmed the formation of the azomethine (C=N) group, NMR established the chemical structure and proton/carbon environments, and LC-MS verified the molecular weight of the synthesized Schiff base derivatives.

2.2.5 Antifungal Activity

The antifungal activity of Compounds C1–C5 was evaluated against *Candida albicans* and *Aspergillus niger*. Preliminary screening was performed using the agar well diffusion method by measuring the zone of inhibition. Minimum Inhibitory Concentration (MIC) was determined by the broth microdilution method according to CLSI guidelines using serial dilutions of the compounds. Fluconazole or ketoconazole served as the positive control, while DMSO was used as the negative control.

3. Results

3.1.1 Synthesis of Schiff Base Compounds

Five aminophenazone-based Schiff base derivatives (C1–C5) were successfully synthesized by a solvent-free grinding method through condensation of 4-aminophenazone with different substituted aromatic aldehydes. Formation of yellow crystalline solids indicated successful Schiff base formation. The reactions proceeded smoothly at room temperature, and completion was confirmed by TLC, which showed disappearance of the starting materials and formation of single new spots for each compound.

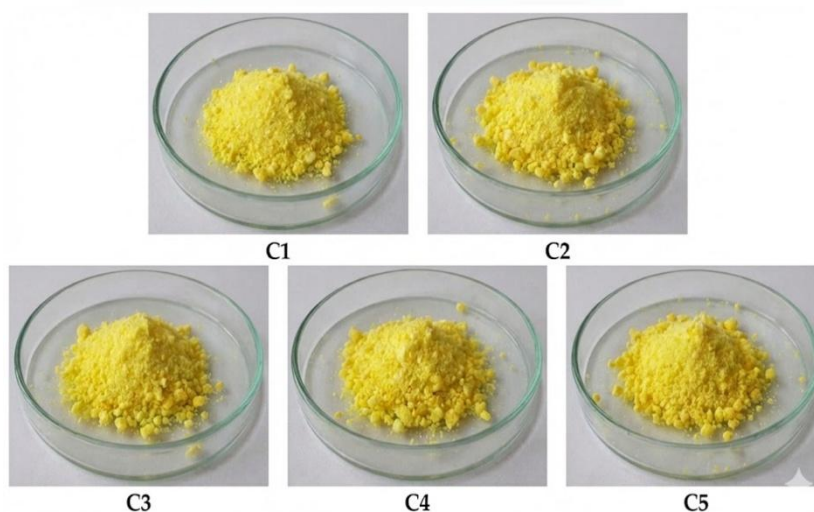


Figure 1: Derivative Compounds of Aminophenazone

3.1.2 Purification and Yield

The synthesized compounds were purified by recrystallization from ethanol to obtain pure yellow crystalline products. The percentage yields ranged from 65–76%, with Compound C5 showing the highest yield (76%) and Compound C3 the lowest (65%), demonstrating the efficiency of the adopted synthetic procedure.

Table 1: Percentage Yield of Synthesized Schiff Base Compounds

Compound Code	Compound Name	Percentage Yield (%)
C1	4-(4-Bromobenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one	68%
C2	4-(4-Hydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one	70%
C3	4-(4-Nitrobenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one	65%
C4	4-(3,4,5-Trimethoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one	72%
C5	4-(3,4-Dimethoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one	76%

3.1.3 Physical Characterization

Melting point and TLC analysis confirmed the purity of the synthesized compounds. All derivatives exhibited sharp melting points (181–185°C) and a single TLC spot with R_f values ranging from 0.55 to 0.66, indicating successful synthesis and absence of significant impurities.

Table 2: Melting Point and Thin Layer Chromatography (TLC) Analysis of Synthesized Schiff Base Compounds

Compound Code	Melting Point Range (°C)	Nature of Melting	Solvent System (TLC)	R _f Value	Number of Spots	Purity Indication
C1	182 °C	Sharp	Chloroform: Methanol (9:1)	0.62	One	Pure
C2	185°C	Sharp	Chloroform: Methanol (9:1)	0.58	One	Pure
C3	184°C	Sharp	Chloroform: Methanol (9:1)	0.55	One	Pure
C4	181°C	Sharp	Chloroform: Methanol (9:1)	0.66	One	Pure
C5	182°C	Sharp	Chloroform: Methanol (9:1)	0.64	One	Pure

3.1.4 Spectral Characterization

FT-IR spectra confirmed Schiff base formation by the appearance of the characteristic azomethine (C=N) stretching band at 1612–1629 cm^{-1} , while the pyrazolone carbonyl (C=O) absorption was observed at 1671–1688 cm^{-1} . Additional peaks corresponding to hydroxyl, bromo, nitro, and methoxy substituents further supported the proposed structures.

^1H NMR spectra showed characteristic signals for methyl, aromatic, and azomethine (N=CH) protons, confirming successful condensation. LC-MS analysis exhibited molecular ion peaks corresponding to the expected molecular weights of all compounds, providing further evidence of structural confirmation.

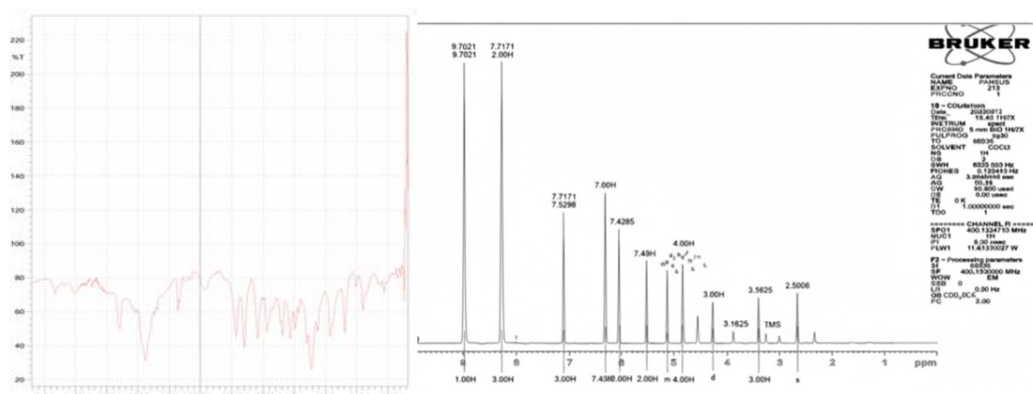


Figure 2 (a): FTIR Graph for Derivative 4-(4-Bromobenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (Compound 1) and ^1H NMR Graph for 4-(4-Bromobenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (Compound 1)

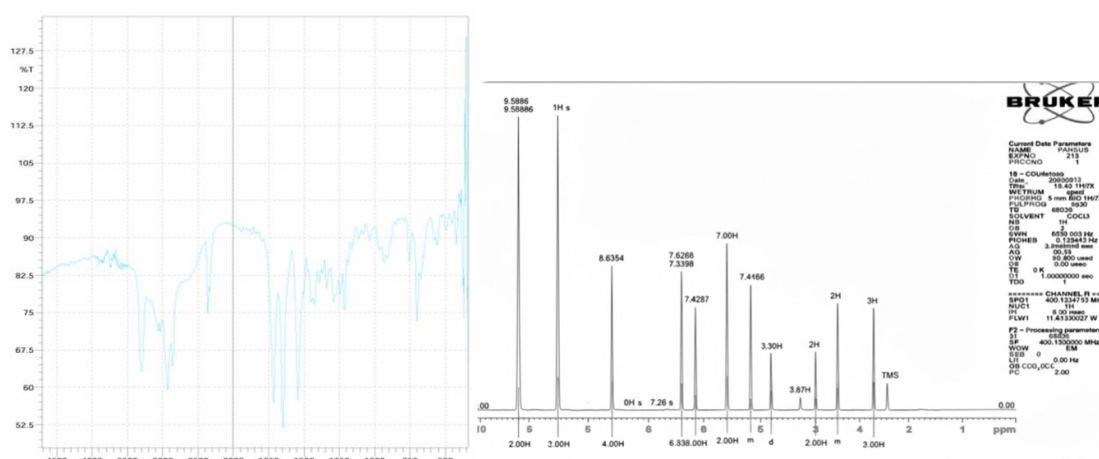


Figure 2(b): FTIR Graph for 4-(4-Hydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (Compound 2) and ^1H NMR Graph for 4-(4-Hydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (Compound 2)

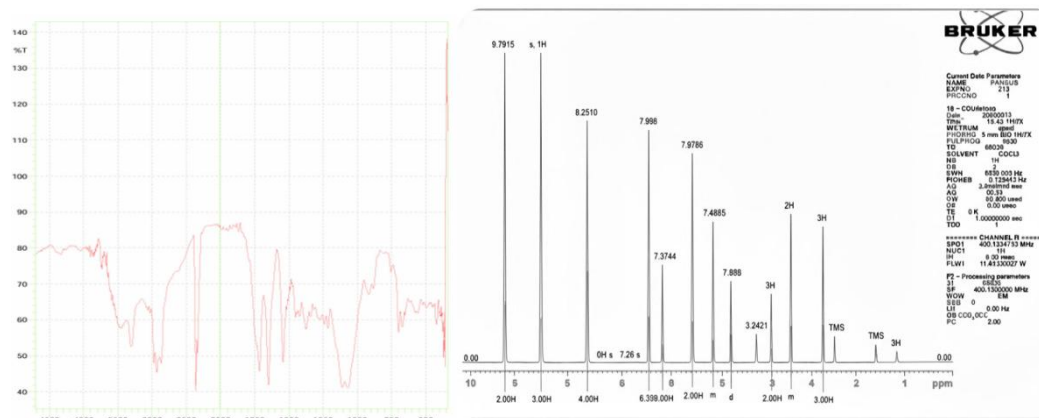


Figure 2(c): FTIR Graph for 4-(4-Nitrobenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (Compound 3) and H1 NMR Graph for 4-(4-Nitrobenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (Compound 3)

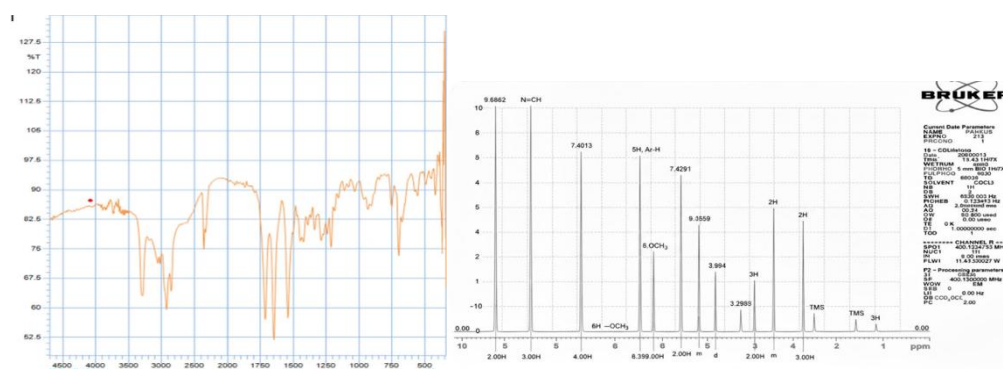


Figure 2(d): FTIR Graph for 4-(3,4,5-Trimethoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (Compound 4) and H1 NMR Graph for 4-(3,4,5-Trimethoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (Compound-4)

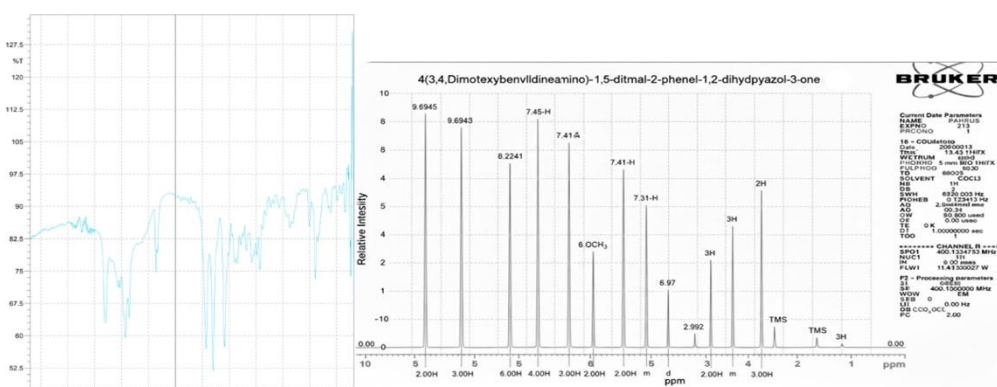


Figure 2(e): FTIR Graph for 4-(3,4-Dimethoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (Compound 5) and H1 NMR for 4-(3,4-Dimethoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (Compound 5)

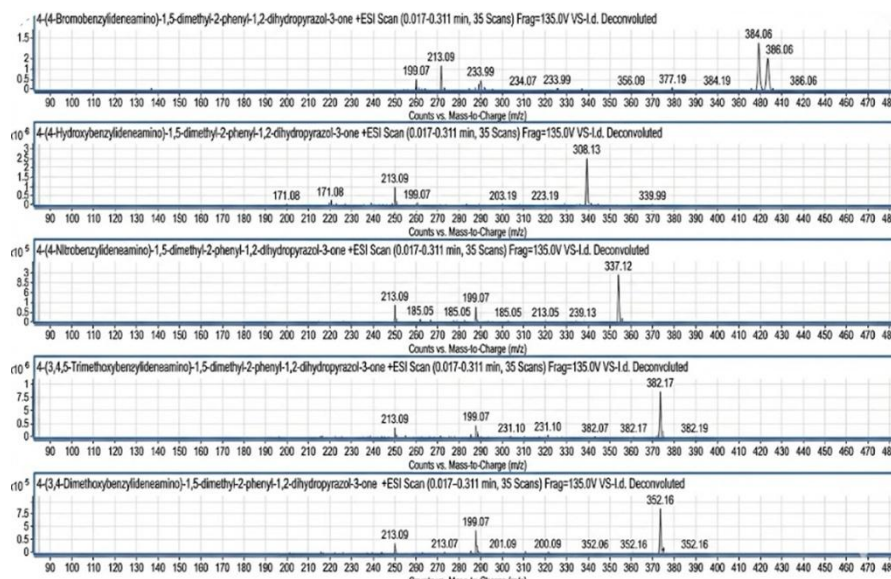


Figure 2(f): Mass Spectrum (LC-MS) for Aminophenazone Derivative Compounds (C1-C5)

3.1.5 Antifungal Activity

All synthesized compounds exhibited antifungal activity against *Candida albicans* and *Aspergillus niger*. Compound C4 (trimethoxy derivative) showed the highest activity with the largest inhibition zones (20 and 18 mm) and the lowest MIC values (31.25 and 62.5 $\mu\text{g/mL}$), followed by C1 (bromo derivative). Compound C3 (nitro derivative) showed comparatively lower activity. Overall, methoxy- and bromo-substituted derivatives demonstrated superior antifungal potential.

Table 3: Antifungal Activity of Synthesized Schiff Base Compounds by Agar Well Diffusion Method

Compound Code	Zone of Inhibition (mm) <i>Candida albicans</i>	Zone of Inhibition (mm) <i>Aspergillus niger</i>
C1 (Bromo)	18 $\pm$ 0.6	16 $\pm$ 0.5
C2 (Hydroxy)	15 $\pm$ 0.5	13 $\pm$ 0.4
C3 (Nitro)	14 $\pm$ 0.4	12 $\pm$ 0.3
C4 (Trimethoxy)	20 $\pm$ 0.7	18 $\pm$ 0.6
C5 (Dimethoxy)	17 $\pm$ 0.6	15 $\pm$ 0.5
Standard (Fluconazole)	24 $\pm$ 0.8	22 $\pm$ 0.7
Negative Control (DMSO)	—	—

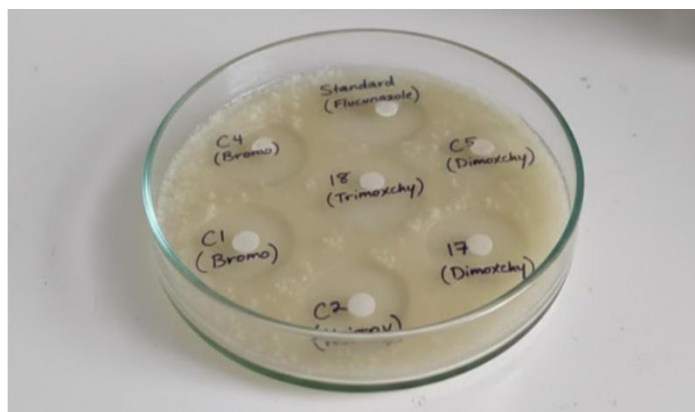


Figure 3: Zone Inhibition of Standards and Aminophenazone Derivative Compounds

Table 4: Minimum Inhibitory Concentration (MIC) of Synthesized Schiff Base Compounds

Compound Code	MIC ($\mu\text{g/mL}$) <i>Candida albicans</i>	MIC ($\mu\text{g/mL}$) <i>Aspergillus niger</i>
C1	62.5	125
C2	125	250
C3	250	250
C4	31.25	62.5
C5	62.5	125
Standard (Fluconazole)	16	32

Conclusions

In the present study, five novel aminophenazone-based Schiff base derivatives (C1–C5) were successfully synthesized using a simple, eco-friendly, and solvent-free grinding method. The synthesized compounds were obtained in moderate to good yields (65–76%) and purified by recrystallization. Their purity and identity were confirmed by melting point determination and TLC analysis, which showed sharp melting points and single spots, indicating successful synthesis and high purity.

The chemical structures of the synthesized derivatives were successfully established using FT-IR, ^1H NMR, and LC-MS analyses. The characteristic azomethine ($\text{C}=\text{N}$) stretching band observed in the FT-IR spectra, along with the corresponding $\text{N}=\text{CH}$ proton signals in the ^1H NMR spectra and the expected molecular ion peaks in the mass spectra, confirmed the successful formation of the Schiff base derivatives.

Biological evaluation demonstrated that all synthesized compounds possessed promising antifungal activity against *Candida albicans* and *Aspergillus niger*. Among the synthesized derivatives, Compound C4 (3,4,5-trimethoxy derivative) exhibited the highest antifungal activity, showing the largest zones of inhibition and the lowest MIC values, followed by Compound C1 (4-bromo derivative). The enhanced activity of methoxy- and bromo-substituted

derivatives suggests that the nature of aromatic substituents plays a significant role in influencing antifungal potency.

Overall, the findings indicate that aminophenazone-derived Schiff bases represent promising lead molecules for the development of new antifungal agents. Further studies involving molecular docking, toxicity evaluation, and in vivo pharmacological investigations are warranted to establish their mechanism of action and therapeutic potential.

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ANIMAL COMMUNICATION: DIVERSITY OF SIGNALS AND COMMUNICATION SYSTEMS

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Abstract

The chapter reviews the foundations and diversity of animal communication, emphasizing communication as an adaptive process involving the transmission of information that influences the behaviour and physiology of receivers. It examines the roles of senders, receivers, and environmental conditions in shaping signalling systems and discusses major communication modalities, including chemical, visual, auditory, tactile, electrical, and bioluminescent signals. The text highlights the ecological and evolutionary significance of pheromones, gestures, facial expressions, colouration, birdsong, and alarm calls, illustrating how different signal types are adapted to specific environmental and social contexts. Particular attention is given to signal specificity, honesty, and complexity, as well as to communication networks and audience effects in social species. The communication systems of primates are explored as important models for understanding the evolutionary origins of human language, revealing both shared cognitive foundations and the unique features of human linguistic abilities. Finally, contemporary theoretical perspectives, including the handicap principle and receiver psychology, are discussed, underscoring the dynamic interplay between signal production, perception, and evolution. Overall, animal communication emerges as a multifaceted and indispensable component of survival, reproduction, and social organization across the animal kingdom.

Keywords: Animal Communication, Chemical Signalling, Visual Communication, Auditory Communication, Signal Specificity, Primate Communication, Language Evolution.

Introduction

Foundations and Major Modes of Communication

Communication is one of the most fundamental biological processes that sustain life across the animal kingdom. From the simplest invertebrates to highly complex mammals, animals rely on communication systems to interact with their environment and with one another. Animal communication can be broadly defined as the transfer of information from a sender to a receiver in such a way that it influences the behaviour, physiology, or internal state of the receiver, either immediately or in the future (Bradbury & Vehrencamp, 2011). This process is not merely

incidental but is deeply embedded in the evolutionary fabric of life, contributing significantly to survival, reproduction, and social organization.

In natural ecosystems, organisms constantly exchange information. A bird singing at dawn, an ant laying a chemical trail, or a dog baring its teeth are all examples of communication. These signals may be intentional, such as courtship displays designed to attract mates, or unintentional, such as odours emitted by prey that predators exploit. Thus, communication encompasses both signals, which have evolved specifically to convey information, and cues, which are incidental pieces of information that other organisms can use.

The process of communication is influenced by three fundamental components: the sender, the receiver, and the environment. The sender must produce a signal that can be effectively transmitted, the receiver must possess the sensory mechanisms to detect and interpret the signal, and the environment must allow for the signal's transmission without excessive degradation. These components are interconnected, and the effectiveness of communication depends on their interaction. For instance, a visual signal may be highly effective in open daylight conditions but may fail in dense forests or nocturnal environments.

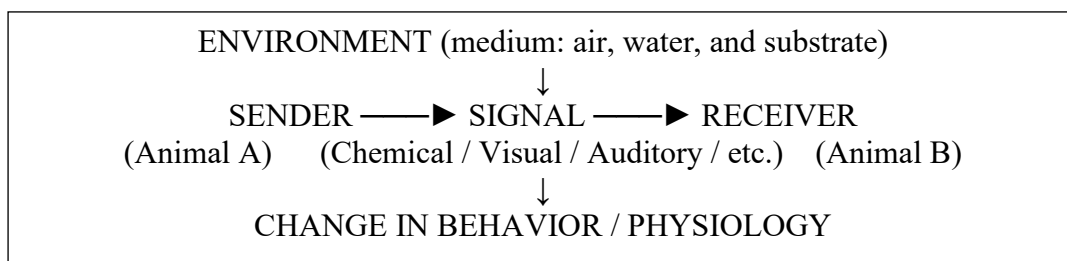


Figure 1: Basic Model of Animal communication

Animal signals can take multiple forms depending on the sensory modalities involved. These include chemical, visual, auditory, tactile, and electrical signals. Each type of communication has its own advantages and limitations, shaped by evolutionary pressures and ecological contexts. For example, chemical signals can persist over time and travel around obstacles, whereas visual signals can convey complex information rapidly but are limited by light availability and line-of-sight constraints.

The study of animal communication is inherently interdisciplinary. It draws from ethology, ecology, neurobiology, evolutionary biology, and even psychology. Researchers must consider not only how signals are produced and perceived but also why they have evolved and how they function in natural settings. Communication systems are shaped by natural selection to maximize efficiency and reliability while minimizing costs such as energy expenditure and risk of predation.

Furthermore, communication is not limited to individual interactions. In many species, particularly social animals, communication occurs within complex networks involving multiple

individuals. These networks may include dominance hierarchies, cooperative groups, and even interspecific interactions where signals are understood across species boundaries. For example, alarm calls of certain bird species can be recognized and responded to by other species, highlighting the ecological significance of shared communication systems.

Another important concept in animal communication is the idea of “audience effects,” where the presence of additional individuals influences the signalling behaviour of the sender. Animals may modify their signals depending on who is watching or listening, indicating a level of social awareness and cognitive complexity. Such phenomena are especially evident in primates and other socially sophisticated animals.

Overall, animal communication represents a dynamic and adaptive system that reflects the interplay between biological constraints and environmental challenges. It provides a window into understanding not only animal behaviour but also the evolutionary origins of human language and cognition.

Chemical Communication

Among the various modes of communication, chemical signalling is considered the most ancient and widespread. Chemical communication involves the release and detection of chemical substances known as pheromones, which influence the behaviour or physiology of other individuals of the same species (Wyatt, 2014). This form of communication is particularly dominant in invertebrates, especially insects, but is also present in vertebrates, including mammals.

One of the defining features of chemical communication is its independence from light and visual contact. Unlike visual signals, chemical signals can be effective in darkness and can travel around obstacles. This makes them particularly useful in environments where visibility is limited, such as underground burrows, dense vegetation, or nocturnal habitats. Additionally, chemical signals can persist in the environment for extended periods, allowing the sender to leave a “message” that can be detected long after it has departed.

However, this persistence can also be a disadvantage. Because chemical signals linger, they may interfere with newer signals, potentially leading to confusion or misinterpretation. Furthermore, the transmission of chemical signals is relatively slow compared to visual or auditory signals. The speed at which a chemical signal affects a receiver depends on factors such as diffusion rate, environmental conditions, and the sensitivity of the receiver’s sensory organs.

Chemical signals can be broadly categorized into two types: releaser pheromones and primer pheromones. Releaser pheromones trigger immediate behavioural responses. For example, alarm pheromones released by injured insects can cause nearby individuals to flee or become aggressive. Similarly, sex pheromones attract potential mates, facilitating reproduction. Primer pheromones, on the other hand, induce longer-term physiological changes. These are particularly

important in social insects such as bees, ants, and termites, where they regulate colony structure and function.

[SENDER] → Releases pheromone → diffuses in environment → detected by receptors → [RECEIVER]

Example:

Ant → Trail pheromone → Other ants follow → Food source reached

In termite colonies, for instance, reproductive individuals secrete pheromones that inhibit the development of reproductive capabilities in other members. This ensures that only specific individuals reproduce, maintaining social organization. These chemical signals are distributed throughout the colony via grooming and food exchange processes known as trophallaxis.

Chemical communication also plays a crucial role in territorial marking and individual recognition. Many mammals use scent marking to delineate their territories and communicate their presence to others. These chemical markers often contain complex mixtures of compounds that convey information about the individual's identity, sex, reproductive status, and even health condition.

In addition to intraspecific communication, chemical signals can also function in interspecific interactions. For example, predators may use chemical cues to locate prey, while prey species may detect predator odours and respond with avoidance behaviours. This highlights the dual role of chemical signals as both signals and cues, depending on the context.

Overall, chemical communication is a versatile and highly effective mode of signalling that has been conserved throughout evolutionary history. Its persistence, ability to function in diverse environments, and capacity to convey complex information make it a cornerstone of animal communication systems.

Visual Communication

Visual communication is one of the most diverse and complex forms of signalling in the animal kingdom. It involves the use of body movements, postures, coloration, and spatial orientation to convey information. Unlike chemical signals, visual signals can transmit information rapidly and with high precision. However, they are dependent on environmental conditions such as light availability and unobstructed lines of sight.

One of the most prominent forms of visual communication is the use of gestures. Gestures involve specific movements of body parts that convey particular meanings. These movements often highlight distinctive morphological features, enhancing the effectiveness of the signal. A classic example is observed in herring gulls, where chicks peck at a red spot on the parent's bill to stimulate feeding behaviour. This interaction demonstrates how a combination of physical traits and behavioural actions can form an effective communication system.

Research by Frans de Waal has shown that primates, particularly apes, use intentional gestures that may represent a precursor to human language. These gestures are not merely reflexive but are used deliberately to achieve specific outcomes, indicating a level of cognitive sophistication. Facial expressions represent another important aspect of visual communication. Many animals possess the ability to convey emotional states through changes in facial features. For example, dogs express aggression by baring their teeth and snarling, while fear may be indicated by flattened ears and widened eyes. Studies on rodents have revealed that even mice exhibit distinct facial expressions in response to pain, suggesting that emotional communication is more widespread than previously thought.

Gaze direction also plays a critical role in visual communication, particularly in social animals. The ability to follow the gaze of another individual allows animals to coordinate their actions and share information about their environment. This behaviour, known as gaze following, has been observed in a variety of species, including primates, birds, and dogs. In some cases, animals can even follow gaze around obstacles, indicating an advanced level of cognitive processing.

Coloration is another powerful visual signal. Animals may use colour to communicate information about their identity, reproductive status, or emotional state. Colour change can occur either as a long-term morphological adaptation or as a rapid physiological response. Cephalopods such as octopuses and cuttlefish are particularly notable for their ability to change colour almost instantaneously using specialized cells called chromatophores. These changes can be used for camouflage, communication, and deception.

In some species, visual signals are passive rather than active. For example, sexual dimorphism—differences in size or coloration between males and females—can convey information about sex and reproductive status without any behavioural change. Similarly, cyclical changes in coloration, such as the swelling and reddening of the genital area in certain primates during ovulation, serve as signals of fertility.

Despite its advantages, visual communication has limitations. It is ineffective in low-light conditions and can be obstructed by physical barriers such as vegetation or terrain. As a result, many species rely on multiple modes of communication, combining visual signals with auditory or chemical cues to enhance effectiveness.

Advanced Modes and Signal Complexity

Light Communication (Bioluminescence)

While visual communication typically depends on ambient light, some organisms have evolved the remarkable ability to produce their own light through biochemical processes known as **bioluminescence**. This phenomenon is especially widespread in marine environments, where sunlight penetration is limited, but it is also found in terrestrial organisms such as fireflies.

Bioluminescent communication represents a fascinating adaptation that allows organisms to overcome environmental constraints and enhance signal visibility in darkness.

Bioluminescence results from a chemical reaction involving luciferin (a light-emitting molecule), luciferase (an enzyme), oxygen, and ATP. The energy released during this reaction is emitted as visible light. In communication contexts, this light is not random but highly regulated and often species-specific in pattern, intensity, and timing.

One of the well-studied examples of bioluminescent communication is found in fireflies (family Lampyridae). Male fireflies emit flashes of light in characteristic patterns while flying, which serve as mating signals. Females, typically perched on vegetation, respond with their own flashes, allowing males to locate them. The timing and pattern of these flashes are highly specific to each species, thereby preventing interspecific mating (Lloyd, 1966).

Interestingly, bioluminescent signalling can also be exploited for predation. In certain species of the genus *Photuris*, females mimic the flash patterns of other firefly species to lure unsuspecting males. Once the males approach, they are captured and consumed. This phenomenon, known as aggressive mimicry, illustrates how communication systems can be co-opted for deceptive purposes.

Another advantage of bioluminescence is its reduced detectability by predators. Many bioluminescent organisms produce wavelengths of light that are less visible to predators or combine their signals with chemical defences that make them unpalatable. Thus, the evolution of light-based communication reflects a balance between attracting mates and avoiding predation.

Auditory Communication

Auditory communication is one of the most versatile and extensively studied forms of animal signalling. Sound waves can travel long distances, penetrate obstacles, and function effectively in both light and dark environments. As a result, many animals rely heavily on acoustic signals for communication.

Sound production in animals involves specialized anatomical structures. In birds, the syrinx allows for the production of complex vocalizations, while in mammals, the larynx serves a similar function. Insects such as crickets produce sound through stridulation, a process involving the rubbing of body parts together.

Bird vocalizations have been a major focus of research in animal communication. These vocalizations are broadly classified into calls and songs. Calls are typically short, simple, and used in a variety of contexts such as alarm signalling, food begging, and maintaining group cohesion. Songs, on the other hand, are longer, more complex, and often associated with reproductive functions such as mate attraction and territorial defences (Catchpole & Slater, 2008).

The pioneering observations of Gilbert White demonstrated that closely related bird species could be distinguished by their songs even when their physical appearances were nearly identical. This discovery underscored the importance of acoustic signals in species recognition and taxonomy.

Alarm calls are particularly interesting because they often transcend species boundaries. Many bird species respond to the alarm calls of other species, indicating that these signals carry generalized information about danger. Research on vervet monkeys has shown that they produce distinct alarm calls for different types of predators, such as leopards, eagles, and snakes. Each call elicits a specific response, demonstrating a form of semantic communication (Seyfarth *et al.*, 1980).

Birdsong also exhibits remarkable diversity and complexity. In many species, males possess a repertoire of songs that they use in different contexts. Some species engage in duetting, where males and females coordinate their vocalizations in precisely timed sequences. This behaviour is thought to strengthen pair bonds and coordinate reproductive activities.

Another fascinating aspect of birdsong is the presence of dialects. Populations of the same species in different geographic regions may develop distinct song patterns. These dialects can play a role in mate selection, as females often prefer males with familiar local songs, thereby reinforcing reproductive isolation and local adaptation.

Auditory communication is not limited to birds. Insects, amphibians, and mammals also rely on sound for communication. Male frogs, for example, produce calls to attract females, often inflating vocal sacs to amplify the sound. In crickets, species-specific temporal patterns of sound pulses allow females to identify conspecific males.

Despite its advantages, auditory communication is subject to environmental constraints such as background noise and signal attenuation. Animals must therefore adapt their signals to maximize transmission efficiency, often by adjusting frequency, amplitude, or timing.

Specificity of Signals and Information Content

One of the most important aspects of animal communication is the level of specificity conveyed by signals. Signals can vary in the amount and type of information they provide, ranging from general warnings to highly detailed individual identification.

Species-specific signals are particularly important in reproductive contexts. By ensuring that mating occurs only between individuals of the same species, these signals prevent the production of unfit or sterile hybrids. Acoustic signals, such as frog calls and bird songs, are often highly species-specific.

In addition to species identity, some signals convey information about individual identity. For example, bird songs can be individually distinctive, allowing neighbours to recognize each other.

This recognition reduces the need for repeated aggressive encounters, thereby conserving energy and minimizing the risk of injury (Tibbetts & Dale, 2007).

Individual-specific signals are also common in mammals, particularly in the form of scent marking. Chemical signatures can encode information about an individual's identity, sex, age, and reproductive status. These signals play a crucial role in maintaining social structure and territorial boundaries.

In contrast, some signals are anonymous, conveying information without identifying the sender. Alarm calls are a prime example. By not revealing the caller's identity, these signals reduce the risk of attracting predators while still providing valuable information to others.

The level of specificity in a signal is closely related to its function. Signals used in mating and territorial defence tend to be highly specific, while those used in general warnings or group coordination are often less so. This variation reflects the adaptive pressures shaping communication systems.

Primate Communication and Evolution of Language

Communication in Primates

Primates represent some of the most socially complex animals, and their communication systems reflect this complexity. Living in tightly knit social groups, primates rely on a combination of visual, auditory, and chemical signals to maintain social bonds, coordinate activities, and resolve conflicts.

Olfactory communication is particularly important in prosimians such as lemurs. These animals possess well-developed scent glands and use chemical signals to mark territories and communicate reproductive status. For example, ring-tailed lemurs mark their environment using secretions from wrist glands, creating scent trails that convey individual identity and territorial ownership.

In contrast, higher primates such as monkeys and apes rely more heavily on visual and auditory signals. Facial expressions, gestures, and body postures play a central role in conveying emotional states and intentions. Threat displays, known as agonistic behaviours, are used to establish dominance and avoid physical conflict. These displays may include staring, baring teeth, or adopting aggressive postures.

Affiliative behaviors, such as grooming, are equally important in primate communication. Grooming not only serves hygienic purposes but also strengthens social bonds and reduces tension within the group. Studies have shown that grooming can lead to the release of endorphins, producing a calming effect and reinforcing social cohesion (Dunbar, 2010).

Vocal communication in primates is generally less complex than in birds but still serves important functions. Calls may signal alarm, maintain group cohesion, or coordinate movement.

However, unlike human language, primate vocalizations are largely limited to expressing immediate emotional states and do not exhibit the same level of flexibility or abstraction.

Evolution of Language

The evolution of human language is one of the most intriguing questions in science. While many animals possess sophisticated communication systems, human language is unique in several key respects. It is symbolic, meaning that words represent concepts arbitrarily; open-ended, allowing for the creation of an infinite number of expressions; and displaced, enabling communication about events that are not immediately present.

Comparative studies of animal communication have historically played a limited role in understanding language evolution due to the perceived gap between human language and animal signalling systems. However, research on primates has begun to bridge this gap.

Experiments with chimpanzees, bonobos, and gorillas have demonstrated that these animals can learn elements of human language, particularly through sign language. Although their abilities are limited compared to humans, they can use symbols to communicate basic needs and concepts, suggesting that the cognitive foundations of language may have deep evolutionary roots.

Research by Frans de Waal emphasizes that primate communication includes intentional gestures, social awareness, and emotional expression, all of which are important precursors to language. However, non-human primates lack key features such as syntax and displacement, which are essential components of human language.

Another important distinction is the discreteness of human language. Words are composed of distinct units of sound that can be combined in systematic ways. While some primates exhibit limited discreteness in their vocalizations, they do not use these sounds to construct complex, rule-based systems of communication.

The evolution of language likely involved a gradual accumulation of cognitive and social adaptations, including increased brain size, enhanced memory, and the development of symbolic thinking. Social complexity may have played a crucial role, as the need to maintain relationships and coordinate group activities would have favoured more sophisticated communication systems.

Theoretical Perspectives in Animal Communication

Modern theories of animal communication focus on the evolutionary dynamics of signalling systems. One influential concept is the handicap principle, proposed by Zahavi, which suggests that signals must be costly to be reliable. For example, elaborate displays or loud calls may indicate the fitness of the sender, as only high-quality individuals can afford such costs.

Another important concept is signal honesty. Signals must convey accurate information to be effective; otherwise, they lose their value. However, deception can also evolve under certain conditions, as seen in aggressive mimicry.

The study of communication also involves understanding the role of the receiver. Signals evolve not only based on the sender's characteristics but also on the sensory and cognitive abilities of the receiver. This interaction, known as receiver psychology, plays a critical role in shaping communication systems.

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ENVIRONMENTAL DNA FOR BIODIVERSITY ASSESSMENT AND CONSERVATION

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Abstract

Biodiversity monitoring is fundamental to understanding ecosystem health and developing effective conservation strategies. Conventional survey methods, although widely used, are often labour-intensive, invasive, and limited in detecting rare, cryptic, or low-abundance species. Environmental DNA (eDNA), which involves the analysis of genetic material released by organisms into environmental matrices such as water, soil, sediment, and air, has emerged as a transformative molecular approach for biodiversity assessment. This chapter reviews the principles, analytical workflow, applications, advantages, challenges, and future prospects of eDNA in biodiversity monitoring and conservation. It outlines the major steps involved in eDNA analysis, including environmental sampling, DNA extraction, polymerase chain reaction (PCR)-based amplification, metabarcoding, next-generation sequencing, and bioinformatic analysis. The chapter further examines the broad applications of eDNA across freshwater, marine, wetland, and terrestrial ecosystems for species detection, invasive species surveillance, endangered species monitoring, fisheries management, habitat assessment, ecosystem restoration, and microbial diversity studies. The advantages of eDNA over conventional survey methods are discussed alongside key limitations, including DNA degradation, contamination, primer bias, incomplete reference databases, and the need for standardized protocols. Emerging developments such as artificial intelligence-assisted bioinformatics, portable and long-read sequencing technologies, environmental RNA (eRNA), and the integration of eDNA with remote sensing and geographic information systems are also highlighted. Overall, eDNA is presented as a powerful complement to traditional ecological surveys, offering sensitive, non-invasive, and scalable approaches for biodiversity assessment. Continued technological innovation, methodological standardization, and interdisciplinary collaboration will further strengthen its role in evidence-based conservation and sustainable ecosystem management.

Keywords: Environmental DNA (eDNA), Biodiversity Assessment, Biodiversity Conservation, Metabarcoding, Next-Generation Sequencing, Molecular Ecology, Environmental Genomics, Bioinformatics, Ecosystem Monitoring, Conservation Biology.

1. Introduction

Biodiversity underpins ecosystem structure, function, and resilience by sustaining essential ecological processes such as nutrient cycling, pollination, water purification, climate regulation, and food-web stability. These ecosystem services are fundamental to environmental sustainability and human well-being. However, biodiversity is declining at an unprecedented rate due to habitat loss, climate change, pollution, invasive species, and overexploitation of natural resources. The *Global Assessment Report on Biodiversity and Ecosystem Services* estimates that nearly one million species are threatened with extinction, highlighting the urgent need for effective biodiversity monitoring and conservation strategies (IPBES, 2019, Bohmann *et al.*, 2014, Thomsen & Willerslev, 2015).

Reliable biodiversity assessment is essential for conservation planning, ecosystem management, and environmental policy. Conventional monitoring methods—including direct observation, trapping, electrofishing, camera trapping, acoustic surveys, and morphological identification—have contributed significantly to ecological research but are often labour-intensive, time-consuming, and dependent on specialist taxonomic expertise. Moreover, these approaches frequently fail to detect rare, cryptic, or low-abundance species, particularly in inaccessible ecosystems such as wetlands, tropical forests, and deep-water habitats (Ficetola *et al.*, 2008; Jerde *et al.*, 2011; Thomsen & Willerslev, 2015; Goldberg *et al.*, 2016).

Advances in molecular ecology have transformed biodiversity monitoring through the development of environmental DNA (eDNA). Environmental DNA comprises genetic material released by organisms through skin cells, mucus, scales, hair, feathers, saliva, urine, feces, reproductive materials, and decomposing tissues. These DNA fragments accumulate in environmental matrices such as water, soil, sediment, snow, and air, enabling species detection without direct observation or capture (Taberlet *et al.*, 2012; Barnes & Turner, 2016). As a rapid, highly sensitive, and non-invasive approach, eDNA has become an important complement to conventional biodiversity surveys (Bohmann *et al.*, 2014; Thomsen & Willerslev, 2015).

The feasibility of eDNA for species detection was first demonstrated by Ficetola *et al.* (2008), who identified the invasive American bullfrog (*Lithobates catesbeianus*) from pond water samples. Shortly thereafter, Jerde *et al.* (2011) successfully detected invasive Asian carp using eDNA before they were captured by conventional methods, highlighting its exceptional sensitivity for early detection. Building on these pioneering studies, advances in polymerase chain reaction (PCR), quantitative PCR (qPCR), droplet digital PCR (ddPCR), DNA metabarcoding, next-generation sequencing (NGS), and bioinformatics have greatly improved the accuracy, efficiency, and taxonomic breadth of eDNA-based biodiversity assessments (Goldberg *et al.*, 2016; Deiner *et al.*, 2017; Ruppert *et al.*, 2019).

Today, eDNA is widely applied across freshwater, marine, terrestrial, and wetland ecosystems to monitor fishes, amphibians, reptiles, birds, mammals, plants, fungi, and microbial communities. It supports biodiversity inventories, invasive species surveillance, endangered species monitoring, fisheries management, habitat assessment, and ecosystem restoration (Hänfling *et al.*, 2016; Valentini *et al.*, 2016; Deiner *et al.*, 2017; Thomsen & Willerslev, 2015). Although challenges such as DNA degradation, contamination, primer bias, and incomplete reference databases remain, continued improvements in molecular techniques, standardized protocols, and bioinformatics are enhancing the reliability of eDNA-based monitoring (Goldberg *et al.*, 2016; Tsuji *et al.*, 2019).

This chapter reviews the fundamental principles, analytical workflow, applications, conservation significance, advantages, limitations, and future prospects of environmental DNA in biodiversity assessment. By synthesizing recent advances in molecular ecology and conservation biology, it highlights the growing role of eDNA as a complementary tool for evidence-based conservation and sustainable ecosystem management.

2. Fundamentals of Environmental DNA

2.1 What is Environmental DNA (eDNA)?

Environmental DNA (eDNA) refers to genetic material released by organisms into their surrounding environment that can be recovered from environmental samples without directly collecting or observing the organisms themselves. DNA enters environmental matrices through skin cells, mucus, hair, feathers, scales, saliva, urine, feces, reproductive products, pollen, spores, root exudates, and decomposing tissues. These DNA fragments accumulate in water, soil, sediment, snow, ice, and even air, where they persist temporarily before degrading under the influence of biological, chemical, and physical processes (Taberlet *et al.*, 2012; Thomsen & Willerslev, 2015).

The development of eDNA analysis has revolutionized biodiversity assessment by providing a rapid, non-invasive, and highly sensitive alternative to conventional survey methods. Following the pioneering work of Ficetola *et al.* (2008), eDNA has become an indispensable tool in molecular ecology, conservation biology, fisheries, and wildlife monitoring. Advances in polymerase chain reaction (PCR), quantitative PCR (qPCR), droplet digital PCR (ddPCR), DNA metabarcoding, and next-generation sequencing (NGS) have substantially improved the sensitivity, accuracy, and taxonomic coverage of eDNA analyses, enabling simultaneous detection of multiple species from a single environmental sample (Deiner *et al.*, 2017; Goldberg *et al.*, 2016; Ruppert *et al.*, 2019).

Unlike conventional biodiversity surveys, eDNA sampling requires minimal disturbance to organisms and habitats, making it particularly suitable for detecting rare, endangered, cryptic, or elusive species. Consequently, eDNA has become an important complementary tool for

biodiversity assessment, invasive species surveillance, ecosystem monitoring, and conservation planning (Barnes & Turner, 2016; Thomsen & Willerslev, 2015).

2.2 Types of Environmental DNA

Environmental DNA is generally classified into two categories according to its origin: **organismal DNA** and **extra-organismal DNA**.

2.2.1 Organismal DNA

Organismal DNA originates from intact cells or whole organisms present in environmental samples, including microorganisms, plankton, algae, fungal spores, pollen, and microscopic invertebrates. Because the DNA remains enclosed within living or recently living cells, it is relatively protected from degradation and usually provides high-quality genetic material for molecular analyses. Organismal DNA is widely used in microbial ecology and environmental microbiology to characterize microbial communities, investigate ecosystem functions, and analyse species interactions using high-throughput sequencing technologies (Taberlet *et al.*, 2012; Deiner *et al.*, 2017).

2.2.2 Extra-organismal DNA

Extra-organismal DNA consists of extracellular genetic material released through natural biological processes such as skin shedding, mucus secretion, excretion, reproduction, and tissue decomposition. This DNA is no longer associated with intact organisms and forms the basis of most eDNA-based biodiversity surveys. However, because extracellular DNA is directly exposed to environmental conditions, it is more susceptible to degradation caused by ultraviolet radiation, microbial activity, enzymatic digestion, oxidation, and temperature fluctuations. Understanding the behaviour of extra-organismal DNA is therefore essential for accurate interpretation of eDNA monitoring results (Barnes & Turner, 2016; Tsuji *et al.*, 2019).

2.3 Sources of Environmental DNA

Environmental DNA is continuously introduced into ecosystems through biological activities of animals, plants, fungi, and microorganisms. Animals release DNA through epithelial cells, mucus, feces, urine, saliva, blood, reproductive materials, feathers, scales, and carcass decomposition, whereas plants contribute DNA via pollen, seeds, roots, leaf litter, and decaying tissues. Microorganisms release DNA through cell division, lysis, biofilm formation, and decomposition, resulting in environmental samples that often contain genetic material from multiple taxa simultaneously (Taberlet *et al.*, 2012; Bohmann *et al.*, 2014).

The principal substrates used for eDNA studies include water, soil, sediment, air, and snow or ice. Water remains the most widely used matrix because DNA disperses efficiently and can be sampled relatively easily for aquatic biodiversity assessments. Soil and sediment are commonly used for terrestrial organisms and microbial communities, whereas airborne eDNA has recently emerged as a promising approach for monitoring terrestrial vertebrates and plant diversity (Clare

et al., 2022; Lynggaard *et al.*, 2022). The choice of sampling substrate depends on the target organisms, habitat characteristics, and study objectives.

2.4 Persistence and Degradation of Environmental DNA

Environmental DNA is inherently transient and begins degrading soon after its release into the environment. The persistence of eDNA determines the temporal window during which species can be detected and is therefore critical for interpreting biodiversity surveys. DNA degradation is influenced by several environmental factors, including temperature, ultraviolet radiation, microbial activity, enzymatic degradation, pH, salinity, dissolved oxygen, and hydrological conditions such as water flow and sediment transport (Barnes & Turner, 2016; Tsuji *et al.*, 2019).

Conversely, low temperatures, limited sunlight, reduced microbial activity, and adsorption of DNA onto sediments or clay particles can prolong DNA persistence. In most freshwater ecosystems, detectable eDNA generally persists from several days to a few weeks, although persistence varies among habitats and species (Barnes & Turner, 2016; Thomsen & Willerslev, 2015). Consequently, eDNA detection usually reflects recent biological activity rather than permanent species occupancy. Standardized sampling strategies that account for environmental conditions, seasonal variation, and DNA transport processes are therefore essential for minimizing false-negative detections and improving the reliability of biodiversity assessments (Goldberg *et al.*, 2016; Ruppert *et al.*, 2019).

3. Workflow of Environmental DNA (eDNA) Analysis

Environmental DNA (eDNA) analysis follows a standardized workflow that begins with environmental sample collection and ends with taxonomic identification and ecological interpretation. The accuracy and sensitivity of eDNA-based biodiversity assessment depend on each stage of the workflow, including sampling design, DNA capture, extraction efficiency, molecular amplification, sequencing, and bioinformatic processing. Consequently, standardized protocols and rigorous quality control are essential to minimize contamination, reduce analytical bias, and ensure reproducibility among studies (Goldberg *et al.*, 2016; Deiner *et al.*, 2017; Tsuji *et al.*, 2019). The general workflow comprises environmental sampling, filtration, DNA extraction, PCR-based amplification, sequencing, and bioinformatic analysis (Figure 1).

3.1 Environmental Sampling

Environmental sampling is the foundation of eDNA analysis because the quality of collected samples directly influences downstream detection success. Depending on the study objectives, samples may include water, soil, sediment, air, snow, or biofilms. Water is the most frequently sampled matrix owing to the continuous release of DNA by aquatic organisms through mucus, epithelial cells, feces, urine, gametes, and decomposing tissues (Thomsen & Willerslev, 2015; Goldberg *et al.*, 2016). In terrestrial ecosystems, soil is widely used for studying plants, fungi,

and microbial communities, whereas airborne eDNA has recently emerged as a promising approach for monitoring vertebrates and plant diversity (Clare *et al.*, 2022; Lynggaard *et al.*, 2022). To prevent contamination, sterile sampling equipment, disposable gloves, field blanks, and immediate sample preservation by cooling or preservation buffers are routinely employed (Goldberg *et al.*, 2016).

3.2 Filtration

Following collection, environmental samples are concentrated to maximize DNA recovery. For aquatic samples, filtration is the most widely adopted method, where water is passed through membrane filters (typically 0.22–1.2 μm) to capture cells, extracellular DNA, and other biological particles (Tsuji *et al.*, 2019). Filter type and pore size are selected according to sample turbidity and target organisms, with glass fibre, cellulose nitrate, polyethersulfone, and polycarbonate membranes commonly used. Efficient filtration is critical because inadequate DNA capture reduces amplification success and overall detection sensitivity (Goldberg *et al.*, 2016).

3.3 DNA Extraction

DNA extraction isolates genetic material while removing substances that inhibit downstream molecular analyses. Commercial silica membrane and magnetic bead-based extraction kits are widely used because they provide consistent DNA recovery and effectively remove inhibitors such as humic acids, polysaccharides, and proteins (Goldberg *et al.*, 2016; McKee *et al.*, 2015; Ruppert *et al.*, 2019). DNA quantity and purity are subsequently evaluated using spectrophotometry, fluorometry, or gel electrophoresis before molecular analysis. High-quality DNA is essential for reliable amplification, sequencing, and species identification (Sales *et al.*, 2020).

3.4 PCR and Quantitative PCR (qPCR)

Polymerase chain reaction (PCR) is the primary technique for amplifying target DNA fragments from environmental samples. Conventional PCR is mainly used to confirm species presence or absence through species-specific primers, whereas quantitative PCR (qPCR) employs fluorescent dyes or probes to monitor DNA amplification in real time and estimate relative DNA concentrations (Ficetola *et al.*, 2008; Goldberg *et al.*, 2016). Although DNA concentration does not always correlate directly with organism abundance because of environmental influences on DNA production and degradation, qPCR has become an indispensable tool for monitoring invasive species, endangered organisms, and aquatic pathogens (Thomsen & Willerslev, 2015).

3.5 DNA Metabarcoding

DNA metabarcoding combines universal genetic markers with high-throughput sequencing to identify multiple species simultaneously from a single environmental sample. Conserved barcode regions, including mitochondrial 12S and 16S rRNA genes, cytochrome *c* oxidase I (COI),

internal transcribed spacer (ITS), *rbcL*, and *matK*, enable taxonomic discrimination across diverse groups of vertebrates, invertebrates, fungi, and plants (Taberlet *et al.*, 2012; Deiner *et al.*, 2017). Because metabarcoding provides community-level biodiversity information, it has become the preferred approach for ecological monitoring of rivers, lakes, wetlands, forests, and marine ecosystems (Caporaso *et al.*, 2012; Valentini *et al.*, 2016).

3.6 Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS) technologies have transformed eDNA research by enabling millions of DNA fragments to be sequenced simultaneously. Platforms such as Illumina MiSeq, NovaSeq, Ion Torrent, and Oxford Nanopore Technologies provide high sequencing throughput, allowing comprehensive biodiversity inventories from a single sample (Garalde *et al.*, 2018; Deiner *et al.*, 2017; Ruppert *et al.*, 2019). Recent developments in long-read and portable nanopore sequencing have further improved taxonomic resolution and enabled near real-time biodiversity monitoring in remote environments (Clare *et al.*, 2022; Lynggaard *et al.*, 2022).

3.7 Bioinformatics and Data Analysis

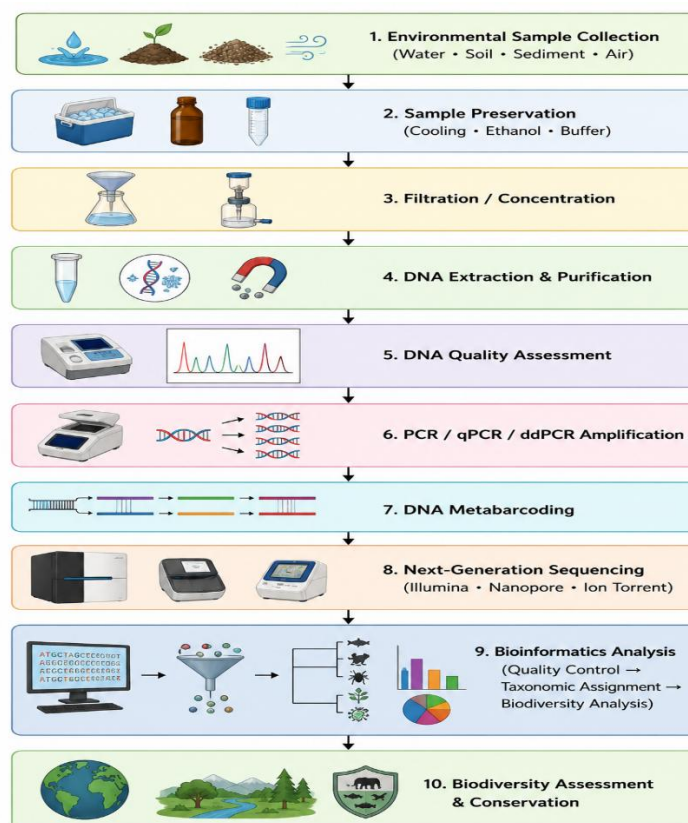


Figure 1: Complete workflow of environmental DNA (eDNA) analysis from environmental sampling to bioinformatic interpretation

Following sequencing, raw DNA reads undergo bioinformatic processing, including quality filtering, chimera removal, sequence clustering or Amplicon Sequence Variant (ASV) inference, taxonomic assignment, and ecological analysis (Deiner *et al.*, 2017). Taxonomic identification is

performed by comparing sequences with curated reference databases such as GenBank, BOLD Systems, SILVA (Quast *et al.*, 2013), PR², and UNITE (Nilsson *et al.*, 2019). Subsequent ecological analyses estimate species richness, alpha and beta diversity, community composition, and temporal or spatial biodiversity patterns (Taberlet *et al.*, 2018; Ruppert *et al.*, 2019). Emerging applications of artificial intelligence, machine learning, and cloud-based bioinformatics are further improving the speed, scalability, and accuracy of eDNA-based biodiversity assessments.

4. Applications of Environmental DNA (eDNA)

Environmental DNA (eDNA) has transformed biodiversity monitoring by providing a rapid, sensitive, and non-invasive approach for detecting organisms across aquatic and terrestrial ecosystems. Initially developed for freshwater environments, eDNA is now widely applied in marine, wetland, terrestrial, and atmospheric ecosystems, enabling the detection of organisms ranging from microorganisms to large vertebrates. Advances in metabarcoding, high-throughput sequencing, and bioinformatics have further expanded its application to community-level biodiversity assessment, ecological monitoring, and conservation planning (Deiner *et al.*, 2017; Thomsen & Willerslev, 2015).

4.1 Freshwater Biodiversity

Freshwater ecosystems support exceptionally high biodiversity despite occupying a small proportion of the Earth's surface. However, these ecosystems are increasingly threatened by habitat degradation, pollution, invasive species, and climate change. Environmental DNA has become one of the most effective tools for freshwater biodiversity assessment because aquatic organisms continuously release DNA into surrounding water through mucus, epithelial cells, feces, urine, and reproductive materials (Thomsen *et al.*, 2012; Barnes & Turner, 2016). Numerous studies have demonstrated that eDNA metabarcoding can accurately characterize fish and amphibian communities from a single water sample, often detecting more species than conventional methods such as electrofishing or netting (Hänfling *et al.*, 2016; Valentini *et al.*, 2016; Pont *et al.*, 2018). Consequently, eDNA has become an indispensable tool for freshwater biodiversity inventories and long-term ecological monitoring.

4.2 Marine Ecosystems

Marine biodiversity monitoring is challenged by the vast extent and inaccessibility of oceanic habitats. Conventional approaches, including trawling and underwater visual surveys, are often labour-intensive and taxonomically limited. Environmental DNA has substantially improved marine monitoring by enabling the detection of fishes, sharks, marine mammals, plankton, and coral-associated organisms from seawater samples (Kelly *et al.*, 2014; Port *et al.*, 2016; Thomsen & Willerslev, 2015). Metabarcoding studies have shown that eDNA frequently detects species overlooked by conventional surveys, making it an effective tool for monitoring marine protected

areas, coastal ecosystems, and ecosystem responses to climate change (Deiner *et al.*, 2017; Valentini *et al.*, 2016).

4.3 Wetland Monitoring

Wetlands are biodiversity hotspots that provide essential ecosystem services such as flood regulation, carbon sequestration, nutrient cycling, and habitat provision. Owing to their ecological complexity and seasonal variability, biodiversity assessments using conventional surveys are often difficult. Environmental DNA enables simultaneous detection of fish, amphibians, aquatic plants, birds, and microorganisms from water and sediment samples, facilitating efficient ecosystem monitoring (Harper *et al.*, 2019; Buxton *et al.*, 2018). Recent studies have also demonstrated the utility of eDNA for evaluating wetland restoration success and detecting biodiversity changes associated with habitat disturbance (Sales *et al.*, 2020).

4.4 Rare and Endangered Species Detection

One of the greatest strengths of eDNA is its exceptional sensitivity for detecting species present at very low population densities. Rare, endangered, nocturnal, and cryptic organisms that are frequently overlooked by conventional surveys can often be identified from trace DNA in environmental samples. Landmark studies demonstrated early detection of invasive Asian carp before physical capture and successful monitoring of endangered freshwater fishes using water samples alone (Jerde *et al.*, 2011; Thomsen *et al.*, 2012). Consequently, eDNA has become an important tool for species recovery programmes, biodiversity monitoring, and conservation management.

4.5 Invasive Species Monitoring

Early detection of invasive alien species is essential for preventing ecological and economic damage. Environmental DNA enables rapid surveillance of invasive organisms before populations become established, substantially improving early warning systems. Numerous invasive fishes, amphibians, molluscs, and crustaceans have been successfully detected using eDNA, allowing timely management interventions and biosecurity responses (Ficetola *et al.*, 2008; Goldberg *et al.* 2016). Because only trace amounts of DNA are required, eDNA has become an integral component of invasive species monitoring worldwide.

4.6 Fisheries Management

Environmental DNA provides fisheries managers with a non-invasive approach for monitoring species distribution, spawning activity, and seasonal migration. Water samples can reveal the presence of commercially important fish species while reducing sampling effort and habitat disturbance. Although DNA concentration does not always directly reflect biomass, quantitative PCR and digital PCR approaches have improved abundance estimation, increasing the value of eDNA for stock assessment and fisheries management (Lacoursière-Roussel *et al.*, 2016; Pont *et al.*, 2018).

Table 1: Major applications of environmental DNA (eDNA) in biodiversity assessment and conservation, including representative target organisms, sample types, landmark studies, and key contributions.

Application	Target Organisms	Sample Type	Representative Study	Key Contribution
Freshwater biodiversity	Fish, macroinvertebrates, amphibians	Water	Thomsen <i>et al.</i> (2012)	Detection of endangered freshwater species
Marine ecosystems	Fish, sharks, marine mammals	Seawater	Valentini <i>et al.</i> (2016)	Community-level biodiversity assessment
Wetland monitoring	Fish, amphibians, plants	Water, sediment	Harper <i>et al.</i> (2019)	Biodiversity assessment in isolated wetlands
Rare species detection	Endangered vertebrates	Water	Jerde <i>et al.</i> (2011)	Early detection of rare species
Invasive species	Fish, molluscs, amphibians	Water	Ficetola <i>et al.</i> (2008)	Early invasion monitoring
Fisheries management	Commercial fishes	Water	Lacoursière-Roussel <i>et al.</i> (2016)	Fish abundance estimation
Amphibian monitoring	Frogs, salamanders, newts	Water	Goldberg <i>et al.</i> (2011)	Non-invasive amphibian detection
Mammal monitoring	Terrestrial mammals	Water, soil, air	Lynggaard <i>et al.</i> (2022)	Airborne eDNA for mammal detection
Bird monitoring	Wetland and terrestrial birds	Air, water	Clare <i>et al.</i> (2022)	Multi-species avian detection
Microbial diversity	Bacteria, fungi, protists	Soil, water, sediment	Caporaso <i>et al.</i> (2012)	Community profiling using metabarcoding

4.7 Amphibian Monitoring

Amphibians are among the most threatened vertebrates globally because of habitat loss, disease, and climate change. Environmental DNA has proven highly effective for detecting frogs, salamanders, and newts from breeding ponds, wetlands, and streams, frequently outperforming traditional visual surveys (Goldberg *et al.*, 2011; Thomsen *et al.* (2012). This approach has

become increasingly important for monitoring breeding populations and evaluating habitat restoration.

4.8 Mammal Monitoring

Although initially developed for aquatic systems, eDNA is increasingly applied to terrestrial mammals. Mammalian DNA can be recovered from water, soil, snow, cave sediments, and airborne particles. Recent airborne eDNA studies have successfully detected mammals in forests and zoological parks, demonstrating considerable potential for monitoring elusive and endangered wildlife without capture or camera trapping (Lynggaard *et al.*, 2022).

4.9 Bird Monitoring

Birds continuously shed feathers, skin cells, feces, and respiratory particles that contribute DNA to water, soil, and air. Recent airborne eDNA studies have demonstrated successful detection of multiple bird species simultaneously, highlighting the potential of this technique for avian biodiversity assessment, migratory bird monitoring, and conservation planning (Clare *et al.*, 2022; Lynggaard *et al.*, 2022). When integrated with conventional bird surveys, eDNA can improve estimates of species richness and community composition.

4.10 Microbial Diversity

Environmental DNA has revolutionized microbial ecology by enabling cultivation-independent characterization of bacterial, archaeal, fungal, and protist communities. High-throughput sequencing has revealed extensive microbial diversity across aquatic, terrestrial, and extreme environments, improving our understanding of nutrient cycling, ecosystem functioning, and host-microbe interactions (Caporaso *et al.*, 2012; Amaral-Zettler, 2012; Thompson *et al.*, 2017). Consequently, microbial eDNA has become an essential component of ecosystem monitoring and environmental assessment.

5. Role of Environmental DNA (eDNA) in Biodiversity Conservation

Environmental DNA (eDNA) has become an important tool in biodiversity conservation by providing rapid, non-invasive, and highly sensitive detection of species across aquatic and terrestrial ecosystems. Unlike conventional monitoring methods, which often require extensive field effort and taxonomic expertise, eDNA enables early species detection, community-level biodiversity assessment, and long-term ecological monitoring using environmental samples. These capabilities have made eDNA increasingly valuable for protected area management, ecosystem restoration, endangered species conservation, habitat assessment, and evidence-based environmental decision-making (Thomsen & Willerslev, 2015; Deiner *et al.*, 2017).

5.1 Protected Area Monitoring

Protected areas play a central role in conserving biodiversity, yet effective management requires continuous monitoring of species diversity and ecological change. Conventional surveys are often constrained by difficult terrain, dense vegetation, and the presence of elusive wildlife.

Environmental DNA enables comprehensive biodiversity assessment from water, soil, sediment, or air samples, allowing simultaneous detection of multiple taxonomic groups with minimal habitat disturbance (Bohmann *et al.*, 2014; Valentini *et al.*, 2016). Repeated eDNA surveys can detect temporal changes in community composition, evaluate conservation interventions, and identify newly established or recolonizing species, making eDNA an increasingly valuable component of long-term monitoring in protected areas (Deiner *et al.*, 2017; Harper *et al.*, 2019).

5.2 Ecosystem Restoration

Restoration of degraded ecosystems requires reliable methods to evaluate biological recovery and ecosystem function. Environmental DNA enables rapid assessment of species recolonization and community development by comparing biodiversity before, during, and after restoration activities. Because eDNA can detect species at low abundance, it frequently identifies early colonizers before they become detectable using conventional methods (Buxton *et al.*, 2018; Sales *et al.*, 2020). Applications in wetlands, rivers, mangroves, floodplains, and coral reefs demonstrate that eDNA provides an efficient means of tracking restoration trajectories and evaluating restoration success across multiple taxonomic groups.

5.3 Monitoring Endangered Species

Monitoring threatened species is fundamental to conservation planning, yet many endangered organisms occur at low densities or occupy inaccessible habitats. Environmental DNA offers a highly sensitive, non-invasive approach for detecting species from trace genetic material present in environmental samples. Studies have successfully monitored endangered fishes, amphibians, reptiles, and mammals using water, soil, and sediment samples, often detecting species missed by conventional surveys (Thomsen *et al.*, 2012; Goldberg *et al.*, 2011). Consequently, eDNA has become an important tool for identifying critical habitats, monitoring reintroduction programmes, and supporting species recovery initiatives (Jerde *et al.*, 2011).

5.4 Habitat Assessment and Ecosystem Health

Changes in biodiversity often provide early indications of habitat degradation caused by pollution, land-use change, invasive species, or climate change. Environmental DNA metabarcoding enables simultaneous assessment of diverse biological communities, including fishes, amphibians, plants, fungi, macroinvertebrates, and microorganisms, providing a comprehensive measure of ecosystem health (Taberlet *et al.*, 2012; Deiner *et al.*, 2017). When integrated with physicochemical measurements, remote sensing, and geographic information systems (GIS), eDNA supports identification of biodiversity hotspots, detection of ecological disturbances, and adaptive ecosystem management (Bohmann *et al.*, 2014).

5.5 Conservation Planning and Decision-Making

Reliable biodiversity data are essential for identifying priority conservation areas and allocating limited conservation resources. Environmental DNA improves conservation planning by

generating standardized biodiversity datasets across multiple ecosystems with relatively low sampling effort. These data are increasingly incorporated into species distribution models to predict habitat suitability, identify ecological corridors, prioritize restoration sites, and assess landscape connectivity (Barnes & Turner, 2016; Ruppert *et al.*, 2019). Integration of eDNA with GIS, ecological modelling, and remote sensing further strengthens evidence-based conservation planning at regional and landscape scales.

5.6 Policy Implications

Environmental DNA is increasingly influencing biodiversity policy and environmental governance. International initiatives, including the Kunming–Montreal Global Biodiversity Framework, emphasize standardized biodiversity monitoring to support conservation targets (Convention on Biological Diversity, 2022). Several countries have incorporated eDNA into freshwater monitoring, invasive species surveillance, fisheries management, and ecological restoration programmes (Thomsen & Willerslev, 2015; Deiner *et al.*, 2017). However, wider policy adoption requires standardized sampling protocols, quality assurance procedures, validated reference databases, and harmonized data interpretation frameworks. Continued international collaboration and methodological standardization will be essential for integrating eDNA into national and global biodiversity monitoring programmes (Goldberg *et al.*, 2016; Tsuji *et al.*, 2019).

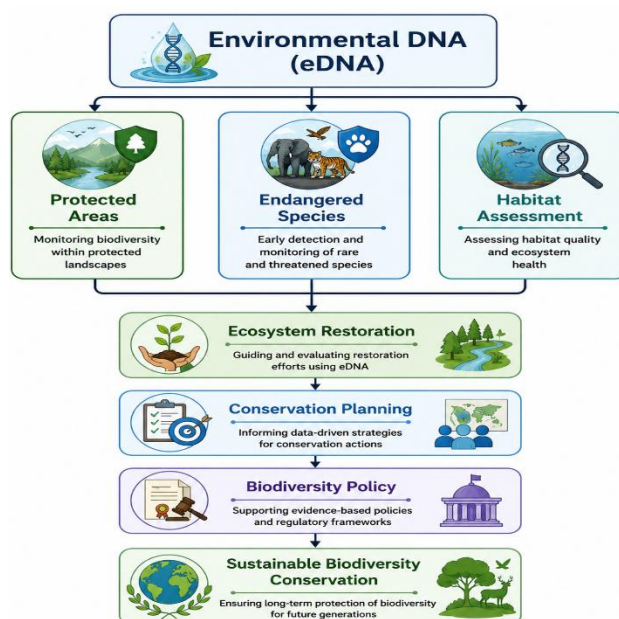


Figure 2: Role of Environmental DNA (eDNA) in Biodiversity Conservation

6. Advantages of Environmental DNA Over Traditional Survey Methods

Accurate biodiversity assessment is essential for ecological research, wildlife management, and conservation planning. Conventional monitoring methods, including visual surveys, trapping, electrofishing, netting, camera trapping, and acoustic monitoring, have provided valuable

ecological information but are often labour-intensive, time-consuming, and dependent on taxonomic expertise. Moreover, these approaches may underestimate biodiversity by failing to detect rare, cryptic, nocturnal, or low-abundance species (Thomsen & Willerslev, 2015; Deiner *et al.*, 2017). Environmental DNA (eDNA) has emerged as a powerful complement to traditional survey methods by enabling species detection from genetic material present in environmental samples such as water, soil, sediment, and air. Because organisms continuously release DNA into their surroundings, eDNA provides a rapid, highly sensitive, and non-invasive approach for biodiversity assessment without requiring direct observation or capture (Taberlet *et al.*, 2012; Barnes & Turner, 2016).

One of the principal advantages of eDNA is its exceptional detection sensitivity. Trace amounts of DNA can reveal the presence of rare, endangered, invasive, or cryptic species that may be overlooked by conventional field surveys, making eDNA particularly valuable for early detection and conservation monitoring (Jerde *et al.*, 2011; Thomsen *et al.*, 2012). Its non-invasive sampling strategy also minimizes disturbance to wildlife and sensitive habitats, making it suitable for monitoring protected species and fragile ecosystems (Goldberg *et al.*, 2016).

Environmental DNA further improves sampling efficiency by enabling simultaneous detection of multiple taxonomic groups through metabarcoding. A single environmental sample may contain DNA from numerous organisms, reducing the need for separate surveys targeting different taxa. This capability makes eDNA particularly useful for large-scale biodiversity inventories, ecosystem assessments, and long-term ecological monitoring (Valentini *et al.*, 2016; Deiner *et al.*, 2017; Hänfling *et al.*, 2016).

Although laboratory analysis requires specialized molecular facilities, eDNA can reduce the overall cost of large-scale monitoring by decreasing field effort, personnel requirements, and sampling time. Standardized molecular workflows also improve reproducibility and reduce observer bias associated with morphological identification (Ruppert *et al.*, 2019). In addition, advances in portable sequencing technologies, automated sampling, and cloud-based bioinformatics are facilitating near real-time biodiversity monitoring in remote environments (Clare *et al.*, 2022; Lynggaard *et al.*, 2022).

Despite these advantages, eDNA should be regarded as a complementary rather than replacement tool for conventional ecological surveys. While highly effective for determining species presence and community composition, eDNA provides limited information on population size, age structure, reproductive status, behaviour, and individual health. Detection success may also be influenced by DNA degradation, environmental transport, contamination, primer bias, and incomplete reference databases (Goldberg *et al.*, 2016; Tsuji *et al.*, 2019). Consequently, integrating eDNA with conventional monitoring approaches provides the most comprehensive framework for biodiversity assessment and conservation planning.

Table 2: Comparison of traditional biodiversity survey methods and environmental DNA (eDNA)-based monitoring approaches

Parameter	Traditional Survey Methods	Environmental DNA (eDNA)
Sampling approach	Direct observation or specimen collection	Collection of environmental samples (water, soil, sediment, air)
Species detection	Requires physical observation or capture	Detects DNA released by organisms without direct observation
Detection sensitivity	Moderate; low for rare or cryptic species	High; capable of detecting low-abundance species
Sampling disturbance	May disturb organisms and habitats	Non-invasive with minimal habitat disturbance
Taxonomic coverage	Often limited to specific groups	Multiple taxonomic groups can be assessed simultaneously
Field effort	High labour and extensive fieldwork	Reduced field effort with simplified sampling
Time requirement	Time-consuming surveys	Rapid sampling and processing
Cost (large-scale monitoring)	High due to repeated surveys and personnel	Cost-effective for large-scale monitoring after initial laboratory setup
Requirement for taxonomic expertise	High dependence on experienced taxonomists	Relies primarily on molecular identification and reference databases
Detection of rare/endangered species	Often difficult	Highly sensitive and effective
Invasive species monitoring	Detection may occur after establishment	Enables early detection at low abundance
Community-level assessment	Usually requires multiple survey methods	Single sample can reveal entire communities via metabarcoding
Quantitative population estimates	Direct counts often possible	Limited; DNA concentration may correlate with abundance but is influenced by environmental factors
Behavioural and demographic information	Provides behavioural, age, sex, and health data	Cannot directly assess behaviour or demographics

Environmental limitations	Weather, accessibility, observer bias	DNA degradation, transport, contamination, incomplete reference databases
Reproducibility	May vary among observers	High when standardized protocols are followed
Best application	Population ecology, behavioural studies, demographic assessments	Biodiversity inventories, conservation monitoring, invasive species surveillance, ecosystem assessment

Note: *Traditional ecological surveys and eDNA-based methods are complementary approaches. Integrating both methods often provides the most comprehensive assessment of biodiversity, species distribution, and ecosystem health.*

7. Challenges and Limitations of Environmental DNA (eDNA)

Despite its considerable advantages, environmental DNA (eDNA) has several biological, technical, and analytical limitations that can influence species detection and data interpretation. Factors such as DNA degradation, contamination, false-positive and false-negative detections, primer bias, incomplete reference databases, and lack of standardized protocols may affect the reliability of biodiversity assessments. Understanding these challenges is essential for designing robust eDNA studies and ensuring accurate ecological interpretation (Goldberg *et al.*, 2016; Deiner *et al.*, 2017).

7.1 DNA Degradation

Environmental DNA is inherently unstable and begins degrading soon after its release. DNA persistence is influenced by temperature, ultraviolet (UV) radiation, microbial activity, pH, salinity, dissolved oxygen, and hydrological conditions. Elevated temperatures and intense UV exposure accelerate DNA degradation, whereas adsorption onto sediments or clay particles may prolong DNA persistence (Barnes & Turner, 2016; Tsuji *et al.*, 2019). Because eDNA generally reflects recent biological activity, delayed sampling or inappropriate preservation can reduce detection success. Consequently, standardized sampling protocols and rapid sample preservation are essential for reliable biodiversity assessment.

7.2 False-Positive Detection

False-positive detections occur when DNA from a species is detected despite its absence from the sampling site. Such errors may result from field or laboratory contamination, downstream transport of DNA, contaminated equipment, or amplification of non-target DNA (Goldberg *et al.*, 2016). Strict contamination-control measures, including sterile sampling equipment, field blanks, extraction blanks, negative controls, and physically separated laboratory workspaces, are therefore fundamental components of eDNA studies.

7.3 False-Negative Detection

False negatives arise when target species are present but remain undetected because DNA concentrations fall below analytical detection limits. Contributing factors include insufficient sampling effort, inefficient DNA extraction, PCR inhibition, inappropriate primer selection, seasonal variation in DNA shedding, and rapid environmental degradation (Ficetola *et al.*, 2008; Goldberg *et al.*, 2016; Tsuji *et al.*, 2019). Replicate sampling, optimized extraction protocols, and sensitive molecular assays substantially improve detection probability.

7.4 Contamination

Contamination remains one of the most important technical challenges in eDNA research because even minute quantities of foreign DNA can lead to misleading results. Potential contamination sources include field collection, sample transport, laboratory processing, PCR amplification, sequencing, and operator error. Dedicated clean laboratories, ultraviolet sterilization, disposable consumables, and comprehensive quality-control procedures are widely recommended to minimize contamination risk (Goldberg *et al.*, 2016).

7.5 Primer Bias and Taxonomic Resolution

Primer selection strongly influences species detection because universal primers rarely amplify all taxa with equal efficiency. Sequence mismatches may reduce amplification of certain organisms while preferentially amplifying others, resulting in biased estimates of community composition (Taberlet *et al.*, 2012; Deiner *et al.*, 2017). In addition, short barcode fragments and closely related species may limit species-level identification. The use of multiple primer sets, multi-marker metabarcoding, and long-read sequencing technologies is improving taxonomic resolution and reducing amplification bias (Ruppert *et al.*, 2019).

7.6 Reference Database Limitations

Accurate taxonomic identification depends on comprehensive and well-curated genetic reference databases such as GenBank, BOLD Systems, SILVA, UNITE, and PR². However, many species—particularly from tropical and understudied regions—remain poorly represented, resulting in ambiguous or incorrect taxonomic assignments (Taberlet *et al.*, 2018; Ruppert *et al.*, 2019). Expanding regional DNA barcode libraries and improving database quality remain priorities for strengthening eDNA-based biodiversity assessments.

7.7 Cost, Technical Infrastructure, and Standardization

Although eDNA often reduces field survey costs, laboratory analyses require molecular facilities, sequencing platforms, bioinformatics infrastructure, and trained personnel. These requirements can limit implementation in biodiversity-rich regions with limited resources (Ruppert *et al.*, 2019). Furthermore, differences in sampling methods, filtration, DNA preservation, primer selection, sequencing platforms, and analytical pipelines reduce comparability among studies (Goldberg *et al.*, 2016). International efforts to standardize

sampling protocols, laboratory procedures, quality assurance, and reporting guidelines are therefore essential for improving reproducibility and facilitating global biodiversity monitoring (Tsuji *et al.*, 2019).

Table 3: Major challenges associated with environmental DNA (eDNA) analysis and potential mitigation strategies

Challenge	Cause	Potential Impact	Possible Mitigation
DNA degradation	Temperature, UV radiation, microbial activity	Reduced detection probability	Rapid preservation, cold storage, optimized sampling
False positives	Contamination, downstream DNA transport	Incorrect species detection	Field blanks, negative controls, sterile protocols
False negatives	Low DNA concentration, PCR inhibition, poor sampling	Failure to detect target species	Replicate sampling, optimized extraction, sensitive assays
Contamination	Field or laboratory contamination	Misleading biodiversity estimates	Dedicated clean laboratories, contamination controls
Primer bias	Unequal amplification efficiency	Underrepresentation of certain taxa	Multi-marker metabarcoding, improved primer design
Limited taxonomic resolution	Conserved genetic markers, short DNA fragments	Reduced species-level identification accuracy	Long-read sequencing, multiple barcode regions
Incomplete reference databases	Missing or misidentified sequences	Incorrect taxonomic assignment	Expansion and continuous curation of regional and global DNA reference databases
High laboratory costs	Molecular equipment, sequencing, bioinformatics	Limited accessibility	Portable sequencing technologies, shared laboratory facilities, and declining sequencing costs
Lack of protocol standardization	Different sampling and analytical workflows	Reduced reproducibility	International standardization and reporting guidelines

Note: Most limitations associated with eDNA can be minimized through standardized sampling protocols, rigorous quality-control procedures, appropriate molecular workflows, and comprehensive reference databases.

8. Future Perspectives of Environmental DNA (eDNA)

Environmental DNA (eDNA) has evolved from a molecular tool for species detection into a comprehensive platform for biodiversity assessment, ecosystem monitoring, and conservation planning. Continued advances in sequencing technologies, bioinformatics, artificial intelligence (AI), and environmental genomics are expected to further improve the accuracy, efficiency, and scalability of eDNA-based monitoring. Emerging developments such as long-read sequencing, portable sequencing platforms, environmental RNA (eRNA), and integration with remote sensing are likely to expand the role of eDNA in ecological research and evidence-based conservation (Deiner *et al.*, 2017; Ruppert *et al.*, 2019).

8.1 Artificial Intelligence-Assisted Biodiversity Monitoring

Artificial intelligence and machine learning are increasingly being incorporated into eDNA workflows to improve sequence processing, taxonomic assignment, ecological modelling, and biodiversity prediction. AI-assisted bioinformatics can automate quality control, classify unknown sequences, and integrate molecular data with environmental variables to predict species distributions and identify biodiversity hotspots. These developments have considerable potential to strengthen conservation planning and adaptive ecosystem management as genomic datasets continue to expand (Deiner *et al.*, 2017; Ruppert *et al.*, 2019).

8.2 Long-Read and Portable Sequencing

Conventional metabarcoding relies primarily on short-read sequencing platforms, which may have limited ability to distinguish closely related species. Long-read technologies such as Pacific Biosciences (PacBio) and Oxford Nanopore sequencing generate longer DNA fragments that improve taxonomic resolution and phylogenetic analyses (Garalde *et al.*, 2018; Ruppert *et al.*, 2019). Portable devices, including the Oxford Nanopore MinION, further enable DNA sequencing directly in the field, reducing sample-processing time and supporting near real-time biodiversity assessment in remote ecosystems (Clare *et al.*, 2022; Lynggaard *et al.*, 2022). These advances are expected to enhance rapid detection of invasive species, endangered organisms, and emerging pathogens.

8.3 Real-Time Biodiversity Monitoring

Future eDNA monitoring is expected to integrate automated environmental sampling, portable sequencing, cloud computing, and AI-driven analysis into continuous monitoring networks. Such systems could provide near real-time information on biodiversity change, invasive species spread, ecosystem degradation, and disease outbreaks, thereby improving early warning systems and supporting adaptive conservation management (Goldberg *et al.*, 2016; Deiner *et al.*, 2017).

8.4 Citizen Science and Community Participation

Environmental DNA sampling is well suited to citizen science because environmental samples can often be collected using standardized protocols without advanced taxonomic expertise.

Participation by local communities, students, and conservation organizations can substantially increase the spatial coverage of biodiversity monitoring while promoting environmental awareness. Combined with centralized laboratories and digital reporting platforms, citizen science programmes have the potential to generate large-scale biodiversity datasets that complement professional ecological surveys (Bohmann *et al.*, 2014).

8.5 Integration with Remote Sensing and GIS

The integration of eDNA with remote sensing, unmanned aerial vehicles (UAVs), and geographic information systems (GIS) is expected to improve landscape-scale biodiversity assessment. While eDNA provides information on species occurrence and community composition, remote sensing contributes spatial data on habitat structure, vegetation cover, hydrology, and land-use change. Together, these approaches enhance species distribution modelling, habitat suitability assessment, biodiversity hotspot identification, and conservation planning (Bohmann *et al.*, 2014; Deiner *et al.*, 2017; Ruppert *et al.*, 2019).

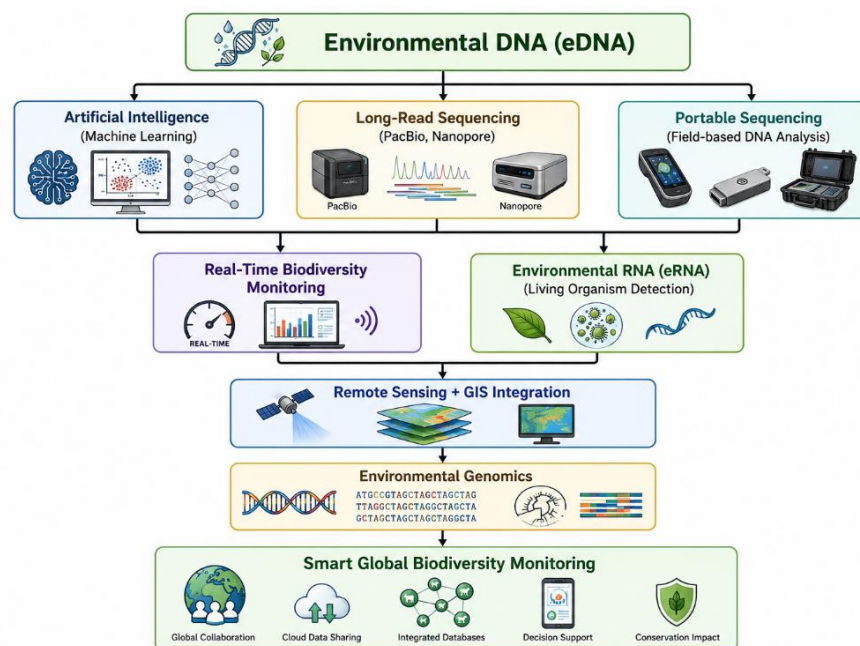


Figure 2: Emerging technologies shaping the future of environmental DNA research and biodiversity monitoring

8.6 Environmental RNA (eRNA) and Environmental Genomics

Environmental RNA (eRNA) is an emerging molecular tool that may complement eDNA by providing evidence of recent biological activity because RNA degrades more rapidly than DNA. Beyond species detection, environmental genomics—including metagenomics, metatranscriptomics, metaproteomics, and metabolomics—offers opportunities to investigate ecosystem functioning, microbial interactions, nutrient cycling, and responses to environmental change. As sequencing technologies become more affordable and computational tools continue to improve, these approaches are expected to play an increasingly important role in next-

generation biodiversity monitoring and ecosystem management (Cristescu, 2019; Taberlet *et al.*, 2018; Ruppert *et al.*, 2019).

Conclusion

Environmental DNA (eDNA) has emerged as a transformative tool for biodiversity assessment and conservation by enabling rapid, sensitive, and non-invasive detection of organisms from environmental samples. Advances in molecular biology, high-throughput sequencing, and bioinformatics have expanded its application from species detection to community-level biodiversity assessment across freshwater, marine, terrestrial, and wetland ecosystems (Thomsen & Willerslev, 2015; Deiner *et al.*, 2017). Throughout this chapter, the principles, analytical workflow, major applications, conservation significance, advantages, challenges, and emerging developments of eDNA have been discussed, highlighting its growing role in modern ecological research and environmental management.

Compared with conventional survey methods, eDNA offers several important advantages, including high detection sensitivity, minimal habitat disturbance, broad taxonomic coverage, and the ability to monitor multiple species simultaneously. These characteristics make it particularly valuable for biodiversity inventories, invasive species surveillance, endangered species monitoring, ecosystem restoration, and conservation planning. Nevertheless, challenges such as DNA degradation, contamination, primer bias, incomplete reference databases, and methodological variation continue to influence data reliability. Continued efforts to standardize sampling protocols, improve molecular techniques, expand curated reference databases, and develop robust bioinformatic pipelines will be essential for maximizing the accuracy and reproducibility of eDNA-based studies (Goldberg *et al.*, 2016; Tsuji *et al.*, 2019).

Emerging technologies, including artificial intelligence-assisted bioinformatics, long-read and portable sequencing, environmental RNA (eRNA), and the integration of eDNA with remote sensing and geographic information systems, are expected to further enhance biodiversity monitoring and ecosystem assessment. Together, these innovations will strengthen evidence-based conservation and improve the capacity for large-scale, real-time ecological monitoring (Ruppert *et al.*, 2019; Taberlet *et al.*, 2018).

As global biodiversity faces increasing pressures from habitat degradation, climate change, pollution, and biological invasions, reliable monitoring tools are essential for effective conservation. Environmental DNA should be viewed as a complementary approach that enhances, rather than replaces, conventional ecological surveys. Continued interdisciplinary collaboration, technological innovation, and international standardization will enable eDNA to become a cornerstone of global biodiversity monitoring and support evidence-based conservation strategies for achieving long-term ecosystem sustainability.

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MILLETS - FROM ANCIENT GRAINS TO FUNCTIONAL FOODS: NUTRITIONAL AND HEALTH PERSPECTIVES

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1. Introduction

Millets are a class of small-seeded cereal grains that belong to the *Poaceae* (*Gramineae*) family. They are among the earliest food crops humans ever cultivated. The word “millet” comes from the Latin “*millesimum*”, which means “a thousandth part”, referring to the tiny size of the grains. For thousands of years, millets have been grown, mainly in Asia and Africa. There, they have been staple crops and have been essential to maintaining food and nutritional security, particularly for populations residing in semi-arid and desert regions. Millets are extremely important in human diets because of their ability to thrive in unfavourable environmental circumstances. They are perfect for growing in marginal areas where other cereals frequently fail due to their incredible flexibility, short growth season, and outstanding endurance to drought, high temperatures, unpredictable rainfall and poor soil fertility. India contributes over 16% of the world’s sorghum production and roughly 35% of the world’s millet cultivation, making it one of the top producers of millet. Millets are increasingly recognized as climate-smart, climate-resilient crops that can sustain agricultural production amid shifting climatic conditions, owing to their resilience and minimal input requirements (Kumar *et al.* 2024).

In recent decades, consumer preferences have moved from foods that simply fulfill hunger to those that provide additional health advantages and help minimize the risk of chronic diseases. Millets have rekindled scientific and commercial interest due to their exceptional nutritional content and availability of health-promoting phytonutrients. The majority of millet species have lower glycaemic indices and higher quantities of dietary fiber, vital minerals, vitamins, high-quality proteins and antioxidant compounds when compared to main cereals like rice and wheat. As a result, eating millet-based foods on a regular basis has been linked to better gastrointestinal health, improved cardiovascular health, improved glycaemic control, increased antioxidant capacity and a lower risk of obesity, diabetes, some types of cancer and other lifestyle-related disorders.

Millets are a group of cultivated species found in various genera of the *Poaceae* family. The minor millets are foxtail millet, proso millet, little millet, kodo millet, barnyard millet, and

browntop millet. The major millets are sorghum, finger millet, and pearl millet. Even though these species vary in terms of their botanical traits, nutritional makeup and ability to adapt to various agroclimatic situations, taken as a whole, they make a substantial contribution to crop diversification, sustainable agriculture and food and nutritional security.

Millets are frequently referred to as “nutri-cereals” due to their abundance of B-complex vitamins, complex carbohydrates, dietary fiber, high-quality proteins, important amino acids and minerals like calcium, iron, magnesium, phosphorus, potassium and zinc. A variety of bioactive substances, such as phenolic acids, flavonoids, tannins, carotenoids, phytosterols and other phytochemicals with antioxidant, anti-inflammatory, antibacterial, antidiabetic and cardioprotective qualities, are found in millets in addition to their high nutritional content. These bioactive ingredients have sparked significant scientific attention due to their potential use in functional foods, nutraceuticals and therapeutic diets targeted at preventing and controlling transmissible illnesses. The widespread adoption of high-yielding cereals like rice and wheat during the Green Revolution caused millets cultivation and consumption to drop in many nations, despite their significant nutritional, health and agronomic advantages. However, there has been a resurgence of interest in these resilient grains worldwide due to growing worries about climate change, depleting natural resources, food insecurity, malnutrition and the rising incidence of diseases linked to a lifestyle. The United Nations designated 2023 as the International Year of Millets, highlighting their important contribution to resilient food systems, sustainable agriculture, biodiversity conservation, enhanced farmer livelihoods and global food and nutritional security.

Millets are used in many traditional and contemporary food preparations since they are very adaptable grains. They are transformed into flour, porridges, flatbreads, fermented foods, breakfast cereals, bakery items, pasta, noodles, snack foods, extruded products and beverages. While lowering antinutritional elements like phytates and tannins, a variety of processing methods, such as dehulling, milling, soaking, germination, malting, fermentation, roasting, popping and extrusion cooking, enhance their sensory quality, digestibility, shelf life and nutrient bioavailability. The use of millets in gluten-free, functional and value-added food items has been further broadened by recent developments in food processing technologies, increasing their commercial and nutritional significance. Millets are becoming more widely acknowledged as “smart foods” for the future due to their remarkable nutritional content, health-promoting qualities, environmental sustainability and ability to adapt to changing climate circumstances. Their cultivation promotes sustainable agricultural systems, and their inclusion in a variety of cuisines enhances nutritional well-being and public health. The origin, classification, production status, nutritional composition, bioactive compounds, health advantages, grain quality, processing technologies, value-added products and prospects of millets are all covered in detail

in this chapter. Along with highlighting new potential and difficulties related to their production, processing and use, it also emphasizes the critical role millets play in advancing sustainable agriculture, functional nutrition, climate resilience and global food and nutritional security.

2. Origin and Distribution

The *Poaceae* family of small-seeded cereal crops includes the diversified group of millets. They are among the earliest domesticated cereal grains and have been cultivated for thousands of years for their exceptional tolerance to varied agro-climatic conditions. One of the earliest food crops utilized by humans, millets were independently cultivated in several parts of Asia and Africa, according to archaeological data. While pearl millet originated in the Sahel region of West Africa, foxtail millet and proso millet are considered to have originated in northern China. Finger millet is assumed to have originated in the highlands of East Africa, specifically Ethiopia and Uganda, whereas kodo millet, little millet and barnyard millet are said to have originated in the Indian subcontinent and surrounding regions. Through commerce, migration and agricultural growth, millets progressively moved from their centers of origin to various regions of Asia, Africa and Europe. They are now grown all over the world in tropical, subtropical, and semi-arid climates. They can be successfully grown in marginal areas where main cereals like rice and wheat frequently perform inadequately due to their remarkable resistance to drought, high temperatures, poor soil fertility and low rainfall. Additionally, the majority of millet species are suited for rainfed and low-input farming systems due to their short growth length, which typically ranges from 45 to 90 days depending on the species and environmental conditions.

Millions of people still rely on millet as a staple food throughout Asia and Africa, which continue to be the two main millet-producing regions. Almost one-third of the world's millets are grown and produced in India, making it the world's largest producer. The states of Rajasthan, Karnataka, Maharashtra, Uttar Pradesh, Haryana, Gujarat, Madhya Pradesh, Tamil Nadu, Telangana, Andhra Pradesh, Odisha and Chhattisgarh are among those where the crop is widely farmed. While finger millet is primarily grown in southern and eastern India, foxtail, tiny, kodo, barnyard and proso millets are mostly grown in northwest India. Although foxtail, tiny, kodo, barnyard, and proso millets are primarily planted in central and southern India, finger millet is mostly found in southern and eastern regions, while pearl millet is primarily farmed in northwestern India. Outside India, pearl millet is commonly cultivated in sub-Saharan Africa, finger millet in East Africa, foxtail millet in China and other East Asian nations, and proso millet in China, Mongolia, Russia, Ukraine, the United States, and several European countries. Global interest in millet farming has increased due to resource constraints, climate variability, and the growing frequency of droughts. Their resilience, low water requirement, and adaptability to marginal terrain make them ideal crops for climate-resilient agriculture and sustainable food systems. As a result, millets are being encouraged in increasing numbers as future crops that can

enhance farmer livelihoods, food security, and nutritional security, especially in areas that are susceptible to climate change.

3. Taxonomy and Classification of Millets

Taxonomic Position

The general taxonomic classification of millets is as follows: Botanically, millets are classified under the Kingdom *Plantae*, Division *Magnoliophyta* (angiosperms), Class *Liliopsida* (monocotyledons), Order *Poales*, and Family *Poaceae* (*Gramineae*). They comprise a diverse group of small-seeded cereal crops distributed among several genera, including *Sorghum*, *Pennisetum*, *Eleusine*, *Setaria*, *Panicum*, *Paspalum*, *Echinochloa*, and *Urochloa*.

Classification of Millets

Millets are broadly divided into two categories based on their cultivation area, production, and economic significance. Major millets occupy comparatively larger cultivation areas and contribute significantly to global food production. Minor millets are generally cultivated on smaller areas but possess exceptional nutritional quality and resilience under adverse climatic conditions (Table 1).

Table 1: Botanical classification and major and minor growing regions of major millet species

Common Name	Scientific Name	Chromosome Number	Major growing regions
Pearl Millet	<i>Pennisetum glaucum</i>	2n = 14	India, Nigeria, Sudan
Sorghum	<i>Sorghum bicolor</i>	2n = 30	India, Nigeria, USA, Ethiopia
Finger Millet	<i>Eleusine caracana</i>	2n = 36	India, Uganda, Kenya, Nepal
Foxtail millet	<i>Setaria italica</i>	2n = 18	China, India
Proso millet	<i>Panicum miliaceum</i>	2n = 36	China, Russia, USA, India
Little millet	<i>Panicum sumatrense</i>	2n = 36	India
Kodo millet	<i>Paspalum scrobiculatum</i>	2n = 40	India, Africa
Barnyard millet	<i>Echinochloa frumentacea</i>	2n = 36	India, Japan
Browntop millet	<i>Urochloa ramosa</i>	2n = 28	India, South Asia, Africa, Australia

4. Morphological Characteristics

Pearl millet is a tall, erect plant that can reach a height of 1.5-4.0 m. It has broad leaves and a dense cylindrical spike. The oval-shaped grains are typically grey, yellow, or cream in colour and range in length from 3 to 4 mm. Finger millet grows to a height of 0.5-1.5 metres and is

distinguished by digitate inflorescences made up of 4-10 finger-like spikes extending from a central point. The grains are incredibly tiny (1-2 mm in diameter) and range in colour from reddish brown to dark brown. Foxtail millet is a slender annual plant that grows to a height of 60-150 cm and has compact, cylindrical, bristling panicles that resemble a fox tail. The tiny, oval-shaped grains range in colour from cream to yellow. Proso millet is an upright annual crop with a height range of 30 to 100 cm. It bears huge, loose panicles with spherical grains that range in colour from white and yellow to red and black depending on the variety. Little millet is a small, tufted plant with thin stems and compact panicles. The grains are cream, grey, or light brown in colour and are among the smallest of the millet species. Kodo millet is a tufted annual grass that grows to a height of 60-90 cm. It produces thin racemes and small ellipsoidal grains that range from pale brown to dark grey. Barnyard millet is a rapidly growing annual plant with wide leaves and branched panicles. The crop is renowned for its quick maturity and superior adaptation to marginal conditions. The grains are tiny, oval, and white to grey in colour. Browntop millet is a medium-height annual grass with narrow leaves, slender stems, and densely branching panicles. It is regarded for its capacity to adapt to drought-prone environments and poor soils.

5. Production Scenario of Millets

According to FAOSTAT (2025), millets are widely grown around the world due to their flexibility to a variety of agro-climatic conditions and considerable contribution to food and nutritional security. Globally, millets are cultivated on approximately 30.66 million hectares, with a total yield of 30.85 million tons and an average productivity of 1,006 kg per ha. India is the world's largest millet producer, accounting for a significant portion of total millet land and production. In 2025, millets were grown on 8.82 million hectares in India, producing around 12.62 million tons with an average productivity of 1,431 kg per ha, greater than the global average. Figure 2 shows the top ten millet-producing nations worldwide.

According to cumulative production from 2014 to 2024, Rajasthan was the biggest millet-producing state, with 464.6 lakh tonnes, followed by Maharashtra (235.6 lakh tonnes) and Uttar Pradesh (215.3 lakh tonnes). Karnataka, Gujarat, Tamil Nadu, Madhya Pradesh, Andhra Pradesh, and Haryana are some of the other states that produce a lot of millet. Millet production in India was roughly 1,634 lakh tonnes between 2014 and 2024. Pearl millet, sorghum, finger millet, and several small millets are the main millet crops grown in the nation. These crops are essential for boosting food and nutritional security, promoting sustainable agriculture, and raising the standard of living for smallholder farmers.

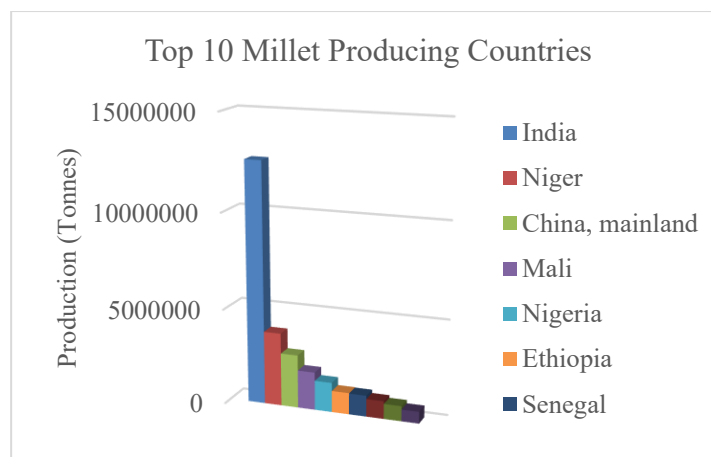


Figure 1: Top 10 Millet-Producing Countries in the World

6. Nutritional Composition

Millet is an extremely nutrient-dense cereal grain that offers a well-balanced mix of macronutrients and micronutrients necessary for human health. They are rich in complex carbohydrates, which constitute the majority of grains and provide long-term energy. In comparison to many other cereals, millets have a slightly balanced amino acid profile and moderate protein content (normally 7-15%), while lysine is typically the limiting amino acid. Depending on the type of millet, the lipid content ranges from about 2 to 7% and is primarily composed of unsaturated fatty acid (Gowda *et al.* 2022). The nutritious value of millets is further enhanced by their abundance of dietary fibre, which includes both soluble and insoluble fractions (IIMR, 2025). Along with vital minerals like calcium, iron, magnesium, phosphorus, potassium, zinc, and manganese, they are excellent providers of key vitamins, especially the B-complex vitamins like thiamine, riboflavin, niacin, pyridoxine, and folate, as given in Table 2. Owing to their rich nutrient profile, millets are considered nutrient-dense grains that contribute significantly to balanced diets and nutritional security.

Millets are increasingly recognized as functional foods because of their rich nutritional composition and diverse health-promoting properties. Being naturally gluten-free, they are suitable for individuals with celiac disease, gluten intolerance, or those seeking gluten-free dietary alternatives.

They also exhibit an alkaline-forming effect after digestion, which may help maintain the body's acid-base balance. Millets are excellent sources of dietary fibre, resistant starch, vitamins, minerals, and bioactive compounds such as phenolic acids and flavonoids, which contribute to improved metabolic health and reduced oxidative stress. Their low glycaemic index and high fibre content help regulate blood glucose levels, improve insulin sensitivity and lower the risk of type 2 diabetes. In addition, dietary fibre and niacin (vitamin B₃) contribute to lowering serum cholesterol levels, thereby promoting cardiovascular health and reducing the risk of hypertension and heart disease. The high dietary fibre and resistant starch content of millets also promote

digestive health by improving bowel regularity, preventing constipation and acting as prebiotics that support beneficial gut microbiota. Furthermore, their antioxidant and anti-inflammatory phytochemicals may help reduce the risk of gastrointestinal disorders, including gastric ulcers and colorectal cancer. Emerging evidence suggests that regular millet consumption may also support liver and kidney function, enhance immune responses, improve respiratory health and lower the risk of certain cancers, although further clinical studies are needed to confirm these benefits. Overall, the regular inclusion of millets in a balanced diet can contribute significantly to better nutritional status and the prevention of several non-communicable diseases (Indian Institute of Millet Research, 2025).

Table 2: Nutritional composition of different millets

Millets	Protein (g)	Fibre (g)	Minerals (g)	Iron (mg)	Calcium (mg)
Sorghum	10	4	1.6	2.6	54
Pearl millet	10.6	1.3	2.3	16.9	38
Finger millet	7.3	3.6	2.7	3.9	344
Foxtail millet	12.3	8	3.3	2.8	31
Proso millet	12.5	2.2	1.9	0.8	14
Kodo Millet	8.3	9	2.6	0.5	27
Little millet	7.7	7.6	1.5	9.3	17
Barnyard millet	11.2	10.1	4.4	15.2	11
Teff	13	8	0.85	7.6	180
Fonio	11	11.3	5.31	84.8	18
Browntop millet	11.5	12.5	4.2	0.65	0.01

7. Bioactive Compounds of Millets

Millets are increasingly recognized not only for their excellent nutritional composition but also for their abundance of bioactive compounds that contribute significantly to human health. These naturally occurring phytochemicals, although present in relatively small quantities, exhibit diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, cardioprotective and anticancer effects. Most bioactive compounds are concentrated in the bran and seed coat, making whole-grain millets nutritionally superior to refined cereals. The quantity and composition of these compounds vary among millet species, genotype, environmental conditions, and processing methods.

Phenolic compounds constitute the most abundant class of bioactive constituents in millets. They occur as free, conjugated, and bound forms, with bound phenolics accounting for the largest proportion. The predominant phenolic acids include ferulic acid, *p*-coumaric acid, caffeic acid, sinapic acid, vanillic acid, protocatechuic acid and gallic acid (Table 3). Ferulic acid is generally the most abundant phenolic acid across most millet species. These compounds effectively

scavenge free radicals, inhibit lipid peroxidation and reduce oxidative stress, thereby protecting cells from oxidative damage associated with ageing and chronic diseases.

Millets are valuable sources of flavonoids, including quercetin, catechin, epicatechin, luteolin, apigenin, kaempferol, vitexin and various anthocyanins. These compounds possess strong antioxidant activity by neutralizing reactive oxygen species and modulating cellular signalling pathways. Flavonoids have also been reported to exhibit anti-inflammatory, antimicrobial, antihypertensive, neuroprotective and anticancer properties. Pigmented millet varieties generally contain higher concentrations of flavonoids than white or cream-coloured grains.

Tannins are polyphenolic compounds primarily concentrated in the outer layers of millet grains. Finger millet, sorghum, kodo millet and barnyard millet are particularly rich in tannins. Although excessive tannins may reduce protein digestibility and mineral bioavailability, moderate levels contribute substantially to antioxidant activity and provide antimicrobial, anti-inflammatory, antidiabetic and anticancer effects. Processing methods such as soaking, germination, fermentation, and decortication effectively reduce excessive tannin content while preserving their beneficial biological functions.

Several millet species contain carotenoid pigments, particularly lutein, zeaxanthin, and β -carotene, which function as natural antioxidants. Pearl millet and proso millet generally contain relatively higher carotenoid concentrations than other millet species. These pigments play an important role in maintaining eye health, enhancing immune function and protecting cells from oxidative stress. Some carotenoids also serve as provitamin A compounds, contributing to improved vitamin A nutrition.

Millets also contain phytosterols, tocopherols, lignans, resistant starch, dietary fibre and bioactive peptides that collectively enhance their functional properties. Phytosterols are structurally similar to cholesterol and help reduce intestinal cholesterol absorption, thereby lowering blood cholesterol levels and reducing the risk of cardiovascular diseases. Tocopherols (vitamin E compounds) protect cellular membranes from oxidative damage, while resistant starch and dietary fibre improve gut microbiota composition and promote gastrointestinal health. The combined presence of phenolic acids, flavonoids, tannins, carotenoids, tocopherols and other phytochemicals provides millets with remarkable antioxidant potential. These compounds neutralize reactive oxygen species, reduce oxidative stress and inhibit inflammatory processes that contribute to the development of chronic diseases such as diabetes, cardiovascular disorders, obesity, neurodegenerative diseases and certain cancers. The antioxidant capacity varies among millet species and is generally higher in pigmented grains than in non-pigmented varieties. Furthermore, appropriate processing techniques such as germination and fermentation can enhance the bioavailability of several antioxidant compounds, thereby improving their physiological effectiveness.

Table 3: Reported concentrations of major phenolic acids in different millet species

Phenolic Acid	Millet Species	Reported Concentration	Reference
Caffeic acid	Finger millet	5.9-10.4 µg/g	Hithamani and Srinivasan (2014)
	Pearl millet	Free: 43.88 ± 0.20 µg/g; Bound: 45.12 ± 0.97 µg/g	Goudar <i>et al.</i> (2023)
	Finger millet	Free: 38.91 ± 0.15 µg/g; Bound: 31.86 ± 0.20 µg/g	Goudar <i>et al.</i> (2023)
	Foxtail millet	Free: 13.59 ± 0.40 µg/g; Bound: 36.27 ± 2.73 µg/g	Goudar <i>et al.</i> (2023)
	Little millet	Free: 17.59 ± 0.26 µg/g; Bound: 23.77 ± 1.28 µg/g	Goudar <i>et al.</i> (2023)
	Barnyard millet	Free: 23.65 ± 0.18 µg/g; Bound: 10.66 ± 0.40 µg/g	Goudar <i>et al.</i> (2023)
	Kodo millet	Free: 12.45 ± 0.36 µg/g; Bound: 20.79 ± 0.30 µg/g	Goudar <i>et al.</i> (2023)
Sinapic acid	Finger millet	11.0-24.8 µg/g	Hithamani and Srinivasan (2014)
	Foxtail millet (whole grain)	Free: 85.36 ± 2.08 µg/g; Bound: 4.13 ± 0.12 µg/g	Pradeep and Sreerama (2017)
	Foxtail millet bran	Free: 121.96 ± 1.56 µg/g; Bound: 5.69 ± 0.13 µg/g	Pradeep and Sreerama (2017)
	Little millet (whole grain)	Free: 63.24 ± 1.04 µg/g; Bound: 2.59 ± 0.07	Pradeep and Sreerama (2017)
	Little millet bran	Free: 113.37 ± 2.01 µg/g; Bound: 6.37 ± 0.18 µg/g	Pradeep and Sreerama (2017)
p-Coumaric acid	Finger millet	1.81-41.1 µg/g	Hithamani and Srinivasan (2014)
	Foxtail millet	Free: 3.66 ± 0.12 µg/g; Bound: 196.62 ± 4.90 µg/g	Pradeep and Sreerama (2017)
	Little millet	Free: 2.71 ± 0.16 µg/g; Bound: 70.36 ± 1.20 µg/g	Pradeep and Sreerama (2017)
	Kodo millet	1.38 ppm	Khare <i>et al.</i> (2020)

Ferulic acid	Finger millet	41-405 µg/g	Shahidi and Chandrasekara (2013)
	Pearl millet	Free: 47.03 ± 0.08 µg/g; Bound: 988.78 ± 8.29 µg/g	Goudar <i>et al.</i> (2023)
	Foxtail millet	Free: 54.65 ± 0.42 µg/g; Bound: 254.20 ± 3.72 µg/g	Goudar <i>et al.</i> (2023)
	Little millet	Free: 29.94 ± 0.15 µg/g; Bound: 133.58 ± 3.85 µg/g	Goudar <i>et al.</i> (2023)
	Barnyard millet	27.88-231.84 µg/g	Goudar <i>et al.</i> (2023)
	Kodo millet	99.35-1445.06 µg/g	Goudar <i>et al.</i> (2023)
Gallic acid	Finger millet	3.91-30.0 µg/g	Hithamani and Srinivasan (2014)
	Pearl millet	Free: 3.87 ± 0.09 µg/g	Goudar <i>et al.</i> (2023)
	Finger millet	Free: 11.93 ± 0.11 µg/g; Bound: 56.12 ± 0.31 µg/g	Goudar <i>et al.</i> (2023)
	Kodo millet	Free: 24.81 ± 0.25 µg/g; Bound: 20.52 ± 0.31 µg/g	Goudar <i>et al.</i> (2023)
Protocatechuic acid	Finger millet	119.8-405 µg/g	Hithamani and Srinivasan (2014)
p-Hydroxybenzoic acid	Finger millet	6.3-370.0 µg/g	Hithamani and Srinivasan (2014)
	Finger millet cultivars	13.5-16.8 mg/kg	Hassan <i>et al.</i> (2020)
Syringic acid	Finger millet	10-60 µg/g	Hithamani and Srinivasan (2014)
	Pearl millet	2.44 ± 0.38 µg/g	Goudar <i>et al.</i> (2023)
	Finger millet	30.05 ± 0.52 µg/g	Goudar <i>et al.</i> (2023)
	Foxtail millet	8.59 ± 0.14 µg/g	Goudar <i>et al.</i> (2023)
	Barnyard millet	21.56 ± 0.29 µg/g	Goudar <i>et al.</i> (2023)
	Kodo millet	28.22 ± 0.46 µg/g	Goudar <i>et al.</i> (2023)
	Proso millet	15.72 ± 0.23 µg/g	Goudar <i>et al.</i> (2023)

8. Health Benefits of Millets

Millets are increasingly recognized as functional cereal grains due to their rich nutritional composition and abundance of bioactive compounds that promote health and reduce the risk of chronic diseases. They contain high levels of dietary fibre, resistant starch, quality proteins, essential minerals, vitamins and phytochemicals, including phenolic acids, flavonoids, tannins, carotenoids and phytosterols. These components collectively contribute to improved metabolic health and disease prevention. One of the most significant health benefits of millets is their role in glycaemic control. Their low glycaemic index, high fibre content, and resistant starch slow carbohydrate digestion and glucose absorption, resulting in a gradual rise in blood glucose levels. In addition, phenolic compounds inhibit digestive enzymes such as α -amylase and α -glucosidase, improving insulin sensitivity and making millets suitable for individuals with diabetes and metabolic syndrome.

Regular consumption of millets also supports cardiovascular health. Dietary fibre, phytosterols and unsaturated fatty acids help reduce total cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides while improving high-density lipoprotein (HDL) cholesterol levels. Furthermore, antioxidant phytochemicals protect blood vessels against oxidative stress and inflammation, thereby lowering the risk of hypertension, atherosclerosis, coronary heart disease and stroke (Saleem *et al.* 2023). The high dietary fibre content of millets promotes gastrointestinal health by improving bowel regularity, preventing constipation and supporting the growth of beneficial gut microbiota. Resistant starch acts as a prebiotic and enhances the production of short-chain fatty acids, which improve intestinal health and nutrient absorption. Owing to their high satiety value and slow digestibility, millets also aid in weight management by reducing hunger, limiting energy intake, and improving lipid metabolism, thereby helping prevent obesity.

Millets possess strong antioxidant and anti-inflammatory properties because of their high concentration of phenolic acids, flavonoids, tannins and carotenoids. These bioactive compounds neutralize reactive oxygen species, reduce oxidative stress, inhibit lipid peroxidation, and suppress inflammatory mediators associated with chronic diseases. Consequently, regular millet consumption may reduce the risk of cardiovascular diseases, neurodegenerative disorders and other oxidative stress-related conditions. Several studies have also highlighted the anticancer potential of millet phytochemicals. Phenolic compounds and flavonoids exhibit antiproliferative, antioxidant and anti-inflammatory activities that may inhibit tumour development and protect cellular DNA from oxidative damage. Although further clinical studies are required, current evidence suggests that millet-based diets may contribute to lowering the risk of certain cancers. Another important advantage of millets is that they are naturally gluten-free, making them an excellent alternative for individuals with celiac disease, gluten intolerance and wheat allergy. In

addition, finger millet is an excellent source of calcium, while other millet species provide significant amounts of iron, magnesium, phosphorus, zinc and B-complex vitamins, supporting bone health, immune function and normal physiological processes. Thus, millets represent nutrient-dense functional grains that offer multiple health benefits and hold great promise for improving human nutrition and preventing lifestyle-related diseases (Jacob *et al.* 2024).

9. Processing Technologies of Millets

Millets are traditionally consumed in whole-grain or minimally processed forms; however, various processing techniques have been developed to improve their nutritional quality, sensory characteristics, digestibility and shelf life. Although millets are rich in nutrients and bioactive compounds, they also contain antinutritional factors such as phytates, tannins and enzyme inhibitors that can reduce the bioavailability of minerals and proteins. Appropriate processing methods not only enhance nutrient utilization but also improve consumer acceptability and expand the application of millets in value-added food products.

Dehulling is the primary processing operation performed to remove the outer husk and bran layers, making millet grains suitable for cooking and milling. While dehulling improves texture, appearance and palatability, excessive removal of the bran may lead to losses of dietary fibre, minerals and phenolic compounds. Milling converts millet grains into flour for the preparation of traditional and modern food products. Depending on the degree of milling, flour characteristics and nutrient retention may vary considerably.

Soaking is a simple and economical method widely used before cooking or further processing. It softens the grains, reduces cooking time, and activates endogenous enzymes that degrade antinutritional compounds, particularly phytates. Germination or sprouting further enhances nutritional quality by increasing the bioavailability of minerals, improving protein digestibility and promoting the synthesis of vitamins, free amino acids and antioxidant compounds. Similarly, malting activates hydrolytic enzymes that improve starch digestibility and modify functional properties, making millet malt suitable for infant foods and beverages.

Fermentation is one of the oldest bioprocessing techniques applied to millet grains. Microbial fermentation improves flavour, texture, digestibility and shelf life while reducing phytates and tannins. It also enhances the availability of essential amino acids, B-complex vitamins, and bioactive compounds. Fermented millet products are widely consumed in many African and Asian countries because of their improved nutritional and sensory qualities.

Thermal processing methods, including roasting, popping, puffing and extrusion cooking, further increase the versatility of millets. Roasting develops desirable flavour and aroma while lowering moisture content and improving storage stability. Popping and puffing produce ready-to-eat snacks with improved texture and digestibility. Extrusion cooking combines high temperature, pressure, and mechanical shear to produce breakfast cereals, expanded snacks, instant mixes and

fortified foods. Extrusion also reduces microbial contamination and antinutritional factors while enhancing product quality and consumer acceptance.

Recent advances in food processing have enabled the incorporation of millet flour into bakery products, gluten-free foods, pasta, noodles, breakfast cereals and functional beverages. Innovative technologies such as composite flour formulation, extrusion and biofortification have further expanded the commercial utilization of millets. These processing approaches not only improve nutritional quality and product diversity but also enhance the market potential of millets as sustainable, health-promoting cereal grains.

10. Value-Added Millet Products

The growing consumer demand for nutritious, convenient, and functional foods has significantly increased the utilization of millets in the development of value-added products. Owing to their excellent nutritional profile, gluten-free nature and health-promoting properties, millets are increasingly incorporated into both traditional and modern food formulations. Advances in food processing technologies have improved the functional properties of millet flour, enabling its use in a wide range of commercial products with enhanced sensory quality, nutritional value and consumer acceptability.

Millet flour is extensively used in bakery products such as bread, biscuits, cookies, cakes, muffins, and crackers, either alone or in combination with wheat and other cereal flours. Composite flour formulations improve the nutritional quality of baked products by increasing dietary fibre, minerals, antioxidants and protein content while reducing the glycaemic response. In addition, millets are widely utilized in gluten-free bakery products for individuals with celiac disease and gluten intolerance. Millets are also processed into breakfast cereals, ready-to-cook mixes, flakes, popped grains, extruded snacks, pasta, noodles, vermicelli and instant porridge formulations. Fermented products such as traditional beverages, malted foods and probiotic drinks prepared from millet grains have gained popularity because of their improved digestibility and enhanced nutritional quality. The high water absorption capacity and functional characteristics of millet flour also make it suitable for soups, infant foods, complementary foods and weaning formulations.

In recent years, millets have emerged as important ingredients in the development of functional foods and nutraceutical products. Their bioactive compounds, including phenolic acids, flavonoids, dietary fibre, and resistant starch, contribute to antioxidant activity, improved glycaemic control, cardiovascular health and gut health. Consequently, millet-based health drinks, nutrition bars, breakfast mixes and fortified food products are receiving increasing commercial attention. The incorporation of millets into dairy products, plant-based beverages, and innovative snack formulations has further expanded their market potential. The development of value-added millet products not only enhances consumer acceptance but also increases the

economic value of millet cultivation by creating new market opportunities for farmers and food industries. Continuous research on product development, processing optimization and quality improvement is expected to further strengthen the role of millets in functional food systems and sustainable nutrition (Kumar *et al.* 2024).

11. Challenges and Future Prospects

Despite their exceptional nutritional, functional, and environmental advantages, the large-scale production and consumption of millets continue to face several challenges. The presence of antinutritional factors such as phytates, tannins, and enzyme inhibitors can reduce the bioavailability of minerals and proteins, although appropriate processing techniques can effectively minimize these limitations. Consumer acceptance remains another challenge due to the coarse texture, characteristic flavour and relatively shorter shelf life of millet-based products resulting from lipid oxidation. In addition, inadequate processing infrastructure, limited value addition, fragmented supply chains, and lower market awareness have restricted the commercialization of millets in many regions. Compared with major cereals such as rice and wheat, investment in millet research, breeding programmes and product development has also been relatively limited.

Nevertheless, the prospects of millets are highly promising. Growing awareness of healthy diets, increasing prevalence of lifestyle-related diseases, and rising demand for gluten-free and functional foods have renewed global interest in millet cultivation and utilization. Advances in breeding, processing technologies, biofortification, and product innovation are improving the nutritional quality, sensory attributes, and commercial value of millet-based foods. Government initiatives, supportive policies, and the declaration of 2023 as the International Year of Millets have further accelerated research, production, and consumer awareness worldwide. Continued efforts in crop improvement, sustainable processing, and value addition are expected to strengthen the role of millets in achieving food and nutritional security while promoting climate-resilient and sustainable agricultural systems.

Conclusion

Millets are nutrient-dense, climate-resilient cereal grains that offer significant nutritional, health and environmental benefits. Rich in dietary fibre, essential nutrients, and bioactive compounds, they contribute to the prevention of lifestyle-related diseases while supporting sustainable agriculture and food security. Advances in processing technologies and value-added product development have further expanded their utilization. Promoting millet cultivation and consumption can play a crucial role in achieving sustainable food systems, improved nutrition, and enhanced public health in the face of changing climatic and dietary challenges.

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SIGNIFICANCE AND IMPORTANCE OF MEDICINAL PLANTS IN IMMUNE BOOSTING

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Abstract

For ages, traditional healthcare systems have relied heavily on medicinal herbs, which are still essential for boosting human immunity. Natural and plant-based treatments that can boost immunity with few adverse effects are becoming more and more popular as infectious diseases and lifestyle-related problems become more common. The bioactive substances found in medicinal plants, such as flavonoids, alkaloids, phenolics, and terpenoids, which have immunomodulatory, antioxidant, and anti-inflammatory qualities, are the focus of this chapter's investigation of the role of these plants in strengthening immunity. The traditional uses of important medicinal plants, including as Tulsi (*Ocimum sanctum*), Ashwagandha (*Withania somnifera*), Giloy (*Tinospora cordifolia*), Turmeric (*Curcuma longa*), and Ginger (*Zingiber officinale*), are addressed together with their scientific validity.

The chapter also discusses recent research results, safety issues, and potential future developments for plant-based immunotherapeutic. All things considered, medicinal plants present viable, affordable, and long-lasting methods of enhancing immune function and averting illness.

Keywords: Medicinal Plants, Traditional Medicine, Immune System, Healthcare, Immunity.

1. Introduction

The immune system is a complex network of organs, tissues, and cells that work together to protect the body from illnesses and harmful substances. Its main function is to defend the body from diseases, infections, and other threats. Although the immune system protects the body in most cases, it can occasionally go wrong. Autoimmune diseases can be caused by immune system disorders. This is the state in which the body's tissues are attacked by the immune system or immunodeficiency disorders, which impair immunity and increase susceptibility to infections. Maintaining a strong immune system is essential for overall wellbeing. The goal of this research is to investigate how plants affect the immune system. Immunity is the capacity of humans to fend against harmful microbes, aberrant cells, and foreign substances. The immune system is a group of organs, tissues, cells, and enzymes that work together to defend the body. The host's protection against harmful germs is known as immunity. It is made up of an intricate web of cells

and molecules. The immune system is the culmination of all the cells, tissues, and chemicals that protect the host. The immune system's primary physiological role is to either avoid or eradicate illnesses (Centimole, 2022). Globally, there is currently a growing trend and desire for organic and green living. Herbal items made from plants or trees are rich in aromatic compounds and nutritional additives. Natural plant extracts are used in aromatherapy and folk medicine, which are holistic forms of healing that enhance health and wellbeing. (Golechha, 2022; Boukhatem & Setzer, 2020). Herbs are used as bases of medicine in many ways in human beings in their life. Research interest has focused on various herbs that possess immune stimulating properties as a useful feature in helping diminish the risk of cancer. In different herbs, a wide ranging of phytochemicals, have been identified such as the flavonoids, lignans, terpenoids, polyphenolics, sulfides, saponins, carotenoids, curcumins, plant sterols and phthalides. (Khodadadi, 2015). Echinacea, garlic, ginger, and turmeric are examples of medicinal plants that have been used for generations to strengthen immunity and fend off illness. These plants have been demonstrated to have immunomodulatory qualities, which can help strengthen the immune system and fend off illnesses. (Khunteta, 2025)

1.1 Importance of a Strong Immune System

To protect the body from dangerous pathogens including bacteria, viruses, fungi, and parasites, the immune system is a highly developed network of cells, tissues, and organs. In order to prevent illness and preserve general health, its effectiveness and strength are essential. In addition to providing defense against infections, a healthy immune system is essential for long-term wellbeing, healing, and illness prevention. The primary function of immune system is to prevent illness. The body can fend against common infections like the flu, colds, and other infectious disorders when it has a strong immune system. The World Health Organization states that maintaining immunity through healthy eating, good cleanliness, immunizations, rest, and exercise is crucial for general health and illness prevention (WHO report 2023).

1.2 Factors affecting immunity

The immune system is composed of many biological elements and functions that cooperate to maintain your health. The innate immune system and the adaptive immune system, or humoral immunity and cell-mediated immunity, are two different forms of immunity. As the first line of protection against foreign organisms and materials, the innate immune system is composed of biological (pH, temperature, and oxygen levels), chemical (enzymes), and mechanical (mucous membranes and skin) barriers. According to Bergmans *et al.*, (2020) the adaptive immune system is composed of antigen-specific leukocytes known as lymphocytes. Pollution, stress, and other variables that lead to immunological dysfunction have increased despite the fact that modern lives have decreased contact with microorganisms, and it is evident that the current diet damages the immune system. (Deng *et al.*, 2017)



Figure 1: Factors responsible for effecting immune system

2. Overview of Medicinal Plants

Everything was based on experience because there was not enough knowledge available at the time about the causes of the ailments or about which plant could be used as a remedy. As the rationale behind the use of particular medicinal plants to cure particular illnesses emerged throughout time, the use of these plants increasingly moved away from the empirical framework and toward explanatory facts. Prior to the development of iatrochemistry in the sixteenth century, plants were used for both prophylaxis and treatment. (Kelly 2009). Nowadays, there is a growing demand for and acceptance of medicinal herbs. Without a question, plants contribute significantly to ecosystems by offering vital services. Humans and other living things cannot exist as they ought to without plants.

In any case, medicinal herbs in particular have consistently served as a general indicator of the health of ecosystems. (Singh, 2002). Humans have definitely thought about medicinal plants since prehistoric times. One could argue that prior to history, early humans were more or less aware of the qualities of the plants they used for clothing, food, shelter, and fuel. One of the oldest sciences in nations like China, Greece, Egypt, and India is the study of medicinal plants. Plants were widely used in ancient Persia as fragrant agents, medicines, and disinfectants (Hamilton, 2004). In actuality, the use of medicinal plants to treat illnesses has existed throughout human history; that is, since people have always looked to their surroundings for tools to help them recover from illnesses, using plants has been their only option (Halberstein, 2005). The use of different plants as Medicinal Plants has become increasingly important in the global health system for both humans and plants (Rehman *et al.*, 2020; Ali *et al.*, 2021).

Table 1: Some common medicinal plants their ingredient and purpose (Gulave *et al.*, 2025)

Botanical Name	Common Name	Ingredient	Purpose
<i>Zingiber officinale</i>	Ginger	Gingerols, Shogaols	Has anti-inflammatory, antioxidant, and digestive-enhancing qualities that support immunological response.
<i>Ocimum tenuiflorum</i>	Tulsi	Eugenol, Ursolic acid, Rosmarinic Acid	Its antibacterial, anti-inflammatory, and stress-relieving properties strengthen immunity.
<i>Curcuma longa</i>	Turmeric	Curcumin	Provides anti-inflammatory and immunological modulation through the potent pharmacological properties of curcumin.
<i>Beta vulgaris</i>	Beetroot	Betalins, Dietary nitrates	Prevents the overreaction of the immune system and lowers inflammation to regulate immunological function.
<i>Glycyrrhiza glabra</i>	Liquorice	Glycyrrhizin, Licirigenin	Enhances immunity through hormone-like action, antiviral, and anti-inflammatory qualities.
<i>Moringa oleifera</i>	Moringa	Quercetin, Chlorogenic acid	Increases immunity due to its rich vitamin, mineral, protein, and antioxidant content.
<i>Withania somnifera</i>	Ashwagandha	Withanolides	Its rich vitamin, mineral, protein, and antioxidant content boosts immunity.
<i>Linum usitatissimum</i>	Flaxseed	Alpha-linolenic acid	Provides fiber, antioxidants, lignans, and omega-3 fatty acids to support immunological and gastrointestinal health.

2.1 Traditional systems of medicine

The traditional system of medicine is a vast system that is present all over the world. The use of traditional medicine has become very popular in different areas of the world because of the numerous significant effects toward the sustainability of human health and the prevention of several infections and diseases. Traditional medicine is a combination of experience, belief, skill, and knowledge that improves the health, the diagnosis, and the mental and physical condition of individuals.

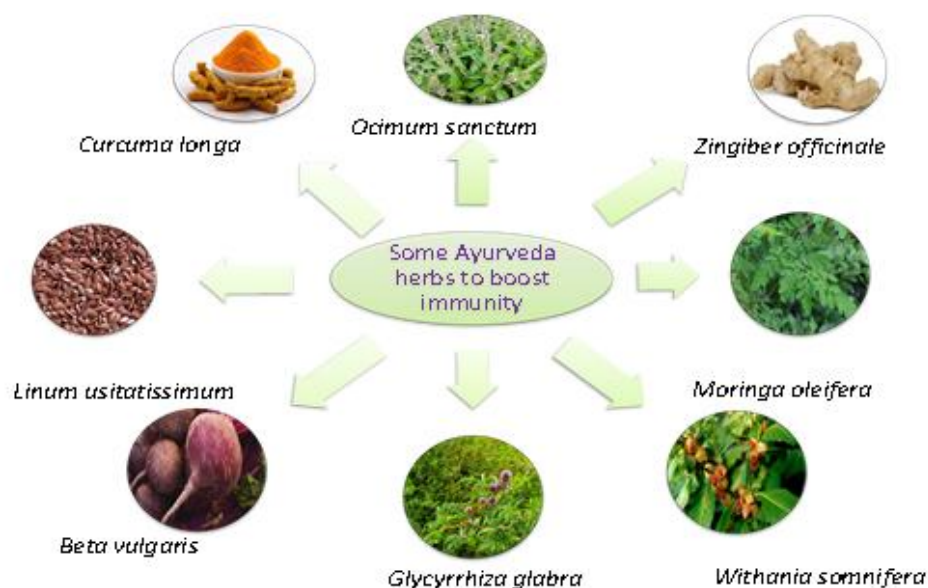


Figure 2: Some local Ayurveda herbs to boost immunity

People use natural medicines in the form of alternative and complementary medicine. The traditional system of medicine is a vast system that is present all over the world. The use of traditional medicine has become very popular in different areas of the world because of the numerous significant effects toward the sustainability of human health and the prevention of several infections and diseases. Traditional medicine is a combination of experience, belief, skill, and knowledge that improves the health, the diagnosis, and the mental and physical condition of individuals. People use natural medicines in the form of alternative and complementary medicine. The traditional system of medicine is a vast system that is present all over the world. The use of traditional medicine has become very popular in different areas of the world because of the numerous significant effects toward the sustainability of human health and the prevention of several infections and diseases. Traditional medicine is a combination of experience, belief, skill, and knowledge that improves the health, the diagnosis, and the mental and physical condition of individuals. People use natural medicines in the form of alternative and complementary medicine. The traditional system of medicine is a vast system that is present all over the world. The use of traditional medicine has become very popular in different areas of the world because of the numerous significant effects toward the sustainability of human health and the prevention of several infections and diseases. Traditional medicine is a combination of experience, belief, skill, and knowledge that improves the health, the diagnosis, and the mental and physical condition of individuals. People use natural medicines in the form of alternative and complementary medicine. The traditional system of medicine is a vast system that is present all over the world. The use of traditional medicine has become very popular in different areas of the world because of the numerous significant effects toward the sustainability of human health and

the prevention of several infections and diseases. Traditional medicine is a combination of experience, belief, skill, and knowledge that improves the health, the diagnosis, and the mental and physical condition of individuals. People use natural medicines in the form of alternative and complementary medicine. The use of natural plants has been documented from various parts of the globe, where almost 70% of the population uses natural medicine to improve health-related conditions. The role of natural plant medicine has been documented across the globe for effective and protective health care. They are found in different countries on different continents. All over the world, the traditional medical system is a large system. Due to its many important benefits for maintaining human health and preventing many illnesses and infections, traditional medicine has grown in popularity throughout the world. Traditional medicine improves people's health, diagnosis, and mental and physical conditions by combining experience, belief, skill, and knowledge. Natural remedies are used by people as additional and alternative medicine. Traditional medicine is defined by World Health Organization (WHO) as the entirety of knowledge, skills, and practices based on the theories, beliefs, experiences indigenous to different cultures, whether explicable or not, used in maintenance of health, as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness. Furthermore, the lengthy history of using herbal remedies is referred to as traditional use. National authorities may approve their use since it is well-established and generally considered as safe and effective (WHO Report, 2017). There are about 20,000 known medicinal plants in India, but approximately 7,000–7,500 of them are used by traditional healers to treat various illnesses. Ayurveda uses 2000 plants, Siddha 1300, Unani 1000, Homeopathy 800, Tibetan 500, Modern 200, and folk 4500, according to the various Indian medical systems. There are about 25,000 potent plant-based remedies utilized in traditional and folk medicine in India. In India, around 1.5 million practitioners provide medical care within the traditional medical system. Over 7800 manufacturing facilities are thought to produce traditional plant-based formulations and natural health products in India, requiring over 2000 tons of raw materials from medicinal plants each year (Pandey *et al.*, 2008). According to (Patwardhan *et al.*, 2005), around 1500 herbals are marketed as nutritional supplements or traditional ethnic remedies. The traditional medicine in India is the most popular system called the system of Indian medicine. These natural plant-derived systems in India have become so vast that physiotherapists who work in India have divided them into six categories: naturopathy, yoga, Unani, homeopathy, Ayurveda, and Siddha. Each system has gained specific importance over time and proved helpful for human health. In the 18th century, homeopathy came to India and became a greater part of this system; so in the traditional Indian system, homeopathy is also important. Ayurveda has been increasingly significant in India's traditional system. Ayurveda is founded on a conceptual framework, which sets it apart from other systems. Ayurveda is a comprehensive medical system that enhances

people's health on a philosophical, physical, ethical, and psychological level. It is more than merely ethnomedicine. Over 10,000 plants are grown for medicinal purposes in India; some of these plants are tasty, while others are utilized for medical purposes. Similar to Ayurveda, Siddha is another medical system in India. Siddhars, who were extremely knowledgeable about the medical system, made the initial discovery of this system. Materia Medica prepares medications using metal and mineral-based materials. Greece is where the Unani medical system first appeared. Hippocrates was the first doctor to propose the idea of medicines derived from plants. Traditional medicine is primarily used in India for primary healthcare. Plant herbs are typically used in infusions and decoctions. Some important medicinal plant mostly cultivated in India shown in table 1

3. Phytochemicals, Pahrmacological and Their Immunomodulatory Effects

Table 2: Bioactive compounds obtained from medicinal plants and their therapeutic effects

Bioactive compound	Potential	Sources
Alkaloids	Antimicrobial agents, antiviral, antimutagenic, anticarcinogenic, antibacterial, anticonvulsant, analgesic, antifungal, anthelmintic, cardiotonic, anti-bacterial, and inflammatory; lower fever, treat intestinal colic, and prevent peptic ulcers; decongestant, uterine contraction agents, angina therapy, treatment for altitude sickness and antirheumatic; Parkinson's illness; treatment for traumatic nervous system damage, myasthenia gravis, treatment for hypertension and depression.	Adamski <i>et al.</i> , (2020); Thawabteh <i>et al.</i> , (2019); Heinrich <i>et al.</i> , (2021)
Flavonoids	Preventing coronary heart disease, hepatoprotective, anti-inflammatory, antiviral, anticancer, Parkinson's disease, and managing hypertension.	Kumar & Pandey, (2013)
Terpenoids	Anti-tumor, anti-malarial, anxiolytic, and anesthetic; asthma, gastric ulcers, wound healing, anti-inflammatory, antimicrobial, antiallergic, and anticancer activities.	Masyita <i>et al.</i> , (2022)
Phenolics	Anti-inflammatory medications; high blood pressure, metabolic issues; diseases caused by fire, Alzheimer's disease, neurodegenerative disorders, skin conditions, rheumatoid arthritis, bowel inflammation; antiviral, antibacterial, anticancer, and anti-aging properties; diseases associated with allergies.	Rahman <i>et al.</i> , (2021)

Medicinal plants include bioactive compounds with a variety of structural and functional characteristics. The primary classes of phytochemicals and their pharmacological potentials are summarized in Table 2. The efficacy of plant bioactive chemicals against neurodegenerative illnesses, metabolic syndromes, and infectious diseases is part of their pharmacological spectrum. Because of their high efficacy and minimal toxicity, compounds including curcumin, quercetin, resveratrol, and andrographolide have moved from laboratory research to clinical studies. Furthermore, the synergistic effects of several phytochemicals present in plant extracts might improve therapeutic results a advantage that is sometimes disregarded in synthetic medications that only include one ingredient.

3.1 Mechanisms of immune modulation

Immune system modulation is essential for controlling human health and disease processes. The immune system's necessity to eradicate and regulate pathogenic and nonpathogenic microorganisms that can impair the body's capacity to maintain homeostasis is the reason for its significance. To sustain a successful symbiotic effect, a microbiome of nonpathogenic bacteria must exist in addition to a functioning host immune system. Half of the 35 trillion cells in the human body are descended from humans, and the other half are descended from microorganisms. (Sender *et al.*, 2016).

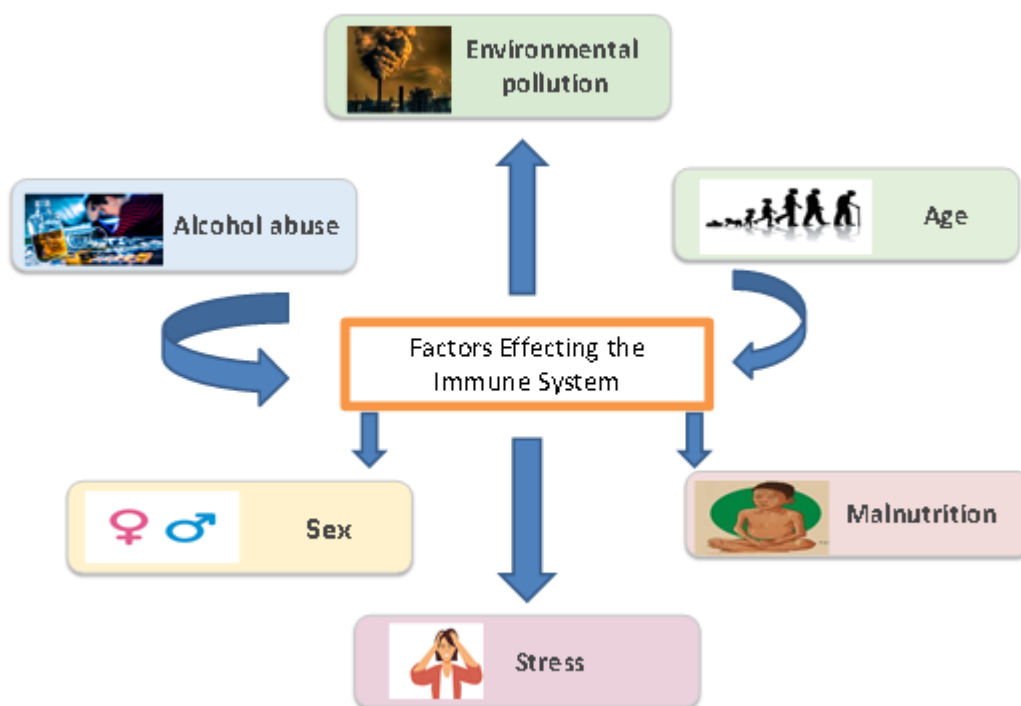


Figure 3: Factor effecting the immune system

Other antimicrobial medicines have immunomodulatory qualities in addition to being antiviral medications. This issue included a thorough analysis of specific immunomodulatory effects for antifungal, antimalarial, and regularly used antibiotics (macrolides and azole) (Ruh *et al.*, 2017). Antimicrobial medicines' most prevalent immunomodulatory effect is their ability to reduce the

inflammatory response to a persistent infection. While most of these medications directly reduce the generation and release of pro-inflammatory cytokines, the reported reductions in the degree of inflammation may also be due to a reduction in the load of pathogens. The adaptive immune response is a protective system that becomes more specialized and customized over time. It involves specialized immune cells called lymphocytes, such as T and B cells. According to Bonilla & Oettgen (2010), lymphocytes' capacity to identify and form memory against certain antigens is essential for the adaptive immune response. In conclusion, the lymphatic system serves as a crucial conduit between the innate and adaptive immune responses, which are the two branches of the immune system. By carrying immune cells and antigens, lymphatic capillaries promote immune cell activation, antigen presentation, and immune response coordination (Dieterich *et al.*, 2014).

3.2 Medicinal Plants for Immunity Boosting

The herbal immunity booster, composed of natural ingredients such as Ginger, Tulsi, Turmeric, Beetroot, Liquorice, Moringa, Ashwagandha, and Flaxseeds demonstrated promising characteristics in terms of sensory appeal, physical stability, and flow properties. Organoleptic assessments confirmed a pleasant taste, aroma, and texture suitable for all age groups, while physicochemical analyses demonstrated low moisture content, ideal pH, and good compressibility, supporting product stability and shelf life. It is even better suited for powder-based supplements because of its uniform particle size and superior flow behavior.

Table 3: Some important traditional medicinal plants mostly cultivated in India

Plant Name	Local Name	Part Used	Medicinal Uses
<i>Aegle marmelos</i>	Bael	Fruit	Chemopreventive and hypoglycemic activity
<i>Acorus calamus</i>	Vach	Rhizome	Antispasmodic effect and a nervine tonic
<i>Aloe barbadensis</i>	Aloe vera	Gel	Treatment of skin disease and wound healing
<i>Andrographis paniculate</i>	Kalmegh	Whole plant	Treatment of flu and cold and shows hepatoprotective effect
<i>Berberis aristata</i>	Daruhaldi or Daruharidra	Stem, root, fruit, bark, and wood	Hypoglycemic, antiprotozoal, and antitrichoma effects
<i>Bacopa monnieri</i>	Brhami	Whole plant	Show antioxidant activity and enhances memory
<i>Callicarpa macrophylla</i>	Priyangu or Daya	Mostly root and leaves	Uterine problems

Source: Akram *et al.*, 2021

Conclusion

For generations, medicinal herbs have been essential to preserving and improving human health, especially when it comes to boosting the immune system. These plants, which are rich in bioactive substances such as phenolic acids, terpenoids, flavonoids, and alkaloids, have important anti-inflammatory, antioxidant, and immunomodulatory qualities. They are useful in controlling and preventing a variety of illnesses due to their capacity to control both innate and adaptive immune responses. In recent years, there has been a renewed interest in plant-based therapeutics, driven by increasing awareness of the limitations and side effects associated with synthetic drugs. Medicinal plants such as Tulsi (*Ocimum sanctum*), Ashwagandha (*Withania somnifera*), Moringa (*Moringa oleifera*), Beetroot (*Beta vulgaris*), Liquorice (*Glycyrrhiza glabra*), *Linum usitatissimum* (Flaxseed) and Turmeric (*Curcuma longa*) have demonstrated promising potential in enhancing immune resilience and overall well-being. Scientific validation through pharmacological and clinical studies has further strengthened their credibility in modern healthcare systems. A important and sustainable resource for boosting immunity is medicinal herbs. Global health could greatly benefit from their incorporation into preventive healthcare measures, particularly in light of the emergence of infectious diseases. To fully realize their potential and further their sensible application in modern medicine, more research and legislative backing will be necessary.

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SMART IPM STRATEGIES FOR MODERN PROTECTED CULTIVATION

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Introduction

Protected cultivation has emerged as a transformative approach in modern horticulture, allowing growers to manipulate environmental conditions and optimize crop productivity. Alongside the benefits of improved yield and quality, the enclosed environment of protected structures also poses unique challenges for insect pest management. Integrated Pest Management (IPM) under protected cultivation therefore plays a critical role in sustaining plant health and ensuring economic viability.

Protected cultivation refers to production systems where the crop-growing environment is partially or fully modified to meet the optimal requirements of plants. Temperature, humidity, light intensity, ventilation, and soil moisture can be regulated using structures such as greenhouses, shade houses, net houses, polyhouses, tunnels, and other protective setups. By moderating the microclimate, these structures extend cropping seasons, safeguard crops from harsh weather events, and significantly reduce biotic stresses including insect pests and diseases.

Historical Evolution of Protected Cultivation

In ancient times, crop protection using artificial structures was already practiced — for example, early records from the 1st century CE describe wheeled beds and glazed frames used to grow cucurbit species year-round for the Roman emperor Tiberius Caesar, a proto-greenhouse practise now regarded as the earliest documentation of controlled-environment horticulture (Janick and Paris, 2022). In the 16th–17th centuries, European gardeners in England and the Netherlands developed early glass-based greenhouses or orangeries to cultivate tender plants under protection, marking the beginning of what is now modern greenhouse horticulture (Sonneveld and Voogt, 2009). Over the past few decades, advances in structure design, glazing materials, climate control, and energy optimization have driven the rapid global expansion of greenhouse and protected cultivation systems — enabling year-round production, improved resource-use efficiency, and adaptability to diverse climatic zones (Tiwari *et al.*, 2025).

Protected cultivation has witnessed remarkable growth over the past few decades, expanding into agro-ecological regions that previously were ill-suited for reliable, year-round horticultural production. This expansion is fueled by rising demand for high-value vegetables, flowers and

fruit; the increasing need for climate-resilient farming systems; and the broader adoption of precision agriculture and resource-efficient farming methods. As technologies advance, diverse types of protected structures have evolved — greenhouses, polyhouses, net houses, shade/cloth houses, low- and high-plastic tunnels, and combinations thereof — each tailored to specific agro-climatic zones, crop types and farm scales.

Different kinds of structures — from fully enveloped greenhouses to simpler plastic tunnels or net houses — allow growers to manipulate the microclimate around the crop more efficiently: controlling temperature, moderating light, shielding from wind/rain/hail, and reducing pest and disease pressure. According to Singh & Singh (2020), protected cultivation provides stability in production, ensuring yield and quality even under erratic or extreme external climatic conditions. In regions where temperature, rainfall, or season length are major constraints, protective structures offer a viable alternative to open-field cultivation (Singh and Singh, 2020).

For instance, high tunnels / plastic tunnels (semi-controlled structures) have become especially popular because they offer many of the benefits of greenhouses — improved microclimate, extended season — but at lower investment and operating cost. A study by Iqbal *et al.* (2023) found that high-tunnel tomato and lettuce production yielded significantly higher and more market-ready produce compared to open-field crops, owing to favorable soil and air temperature regimes, better water-use efficiency, and improved protection from abiotic stresses and pests.

Moreover, in hot and semi-arid zones (such as many parts of Central and North India), protected cultivation can mitigate the damaging effects of high temperatures and erratic rainfall. According to Patil *et al.* (2022), in tomato grown under polyhouse in arid conditions, protected structures helped maintain optimal day/night temperatures ($\approx 25\text{--}30\text{ }^{\circ}\text{C}$ / $20\text{ }^{\circ}\text{C}$) — critical for successful flowering and fruit set — thereby significantly improving yield and reducing crop failure risk compared to open-field farming.

Low-plastic tunnels, net-houses, and shade-net houses also provide accessible options for resource-limited farmers or smallholders. As described by Verma & Kumar (2018), these low-cost structures have enabled off-season (winter or early-season) cultivation of vegetables and cucurbits, helping growers fetch premium prices in markets when open-field supply is low; such off-season cultivation under tunnels often provides higher returns per unit area, with reduced water and external input requirements.

In summary, the rapid spread and diversification of protected cultivation structures — from high-tech greenhouses to low-cost plastic tunnels — reflect a global shift in horticultural production strategies. By enabling microclimate manipulation, extending production seasons, safeguarding crops against abiotic and biotic stresses, and facilitating off-season or early-season production, these structures offer farmers — especially in regions with variable or harsh climates — a way to

stabilize output, improve quality, and potentially increase income, while making better use of land, water, and inputs.

Types of Tunnels in Protected Cultivation

Protected cultivation uses different forms of tunnels to create favorable microclimates that support early, off-season, and high-quality crop production. Among these, low tunnels, walk-in tunnels, and high tunnels are the most widely used. Though they share the common purpose of modifying the crop environment, they differ significantly in structure, crop suitability, management convenience, and yield potential.

a. Low Tunnels

Low tunnels are the simplest and least expensive form of protected cultivation. These structures are constructed close to the ground using flexible hoops made of bamboo, PVC pipes, or galvanized wires, over which transparent plastic or polythene sheets are stretched. Their small size makes them relatively cheap to install and ideal for small and marginal farmers.

These tunnels are primarily used for early season production of cucurbitaceous vegetables such as cucumbers grown on flat beds, melons, watermelons, bitter gourd, snake gourd, and various squashes. By protecting crops during the early growth stages from frost, cold winds, and heavy rains, low tunnels help initiate crop growth earlier than open-field planting.

b. Walk-in Tunnels

Walk-in tunnels are mid-sized structures that provide enough height for farmers to comfortably enter and perform routine cultural operations. They are less expensive than high tunnels but offer better accessibility and microclimate control than low tunnels, making them a preferred choice for many vegetable growers.

These tunnels are widely used for cultivating solanaceous and cucurbitaceous vegetables such as tomatoes, cucumbers, sweet peppers, and hot peppers. The improved space inside the structure allows uniform training, staking, pruning, and harvesting, which ultimately contributes to higher yields. Walk-in tunnels also facilitate more efficient pest and disease management, as farmers can monitor crop health closely and apply required treatments with greater ease.

c. High Tunnels

High tunnels are the most advanced and productive type of tunnel structures. They are designed with greater width and height, allowing convenient movement of workers, equipment, and sometimes even small machinery. High tunnels use strong frames covered with UV-stabilized polyethylene film, providing durability and better environmental regulation.

These tunnels are especially suitable for high-value crops such as tomatoes, sweet peppers, and cucumbers. The spacious interior allows for excellent air circulation, efficient temperature management, and proper training of tall or vining crops. High tunnels support maximum crop yield among all tunnel types because they enable precise control over humidity, temperature, and

irrigation. Their well-ventilated environment reduces disease incidence, and the larger growing area enhances overall productivity.

Overall, tunnel farming—whether low, walk-in, or high—helps farmers achieve early harvests, reduce water usage, and produce significantly higher yields per acre compared to traditional open-field farming.

Greenhouses and Other Protective Structures

Greenhouses represent a more sophisticated form of protected cultivation. Historically, greenhouses emerged when transparent glass became widely available, allowing growers to manipulate light and temperature effectively. Modern greenhouses protect crops from adverse conditions such as cold, heavy rain, hailstorms, and strong winds. They provide an optimized environment that surpasses open-field cultivation by ensuring controlled temperature, humidity, and light.

Greenhouses enable off-season and year-round production of vegetables, flowers, and nursery crops. They provide the stability needed to introduce new and exotic crops into local production systems. Two basic types of greenhouses are commonly used:

- Fully controlled greenhouses designed for year-round production where heating, cooling, and automated climate systems maintain ideal growing conditions.
- Partially controlled greenhouses with minimal climate regulation, used primarily for seasonal production.

In addition to greenhouses, several other protective structures support specialized crop needs:

Plastic Houses

Constructed mainly from polythene sheets, plastic houses are cost-effective and widely used. They offer partial protection from temperature extremes and rainfall and are suitable for short-duration vegetable production.

Shade Houses

Shade houses consist of frames covered with shade cloth or light-transmitting plastic. They slightly reduce the intensity of sunlight, lower daily maximum temperature, and increase relative humidity. These structures are ideal for shade-loving plants, nurseries, and transplant production.

Lath Houses

Lath houses resemble shade houses but use movable lath slats as covers. They protect high light-sensitive plants and allow adjustable light filtration.

Hot Beds

Hot beds are framed structures, often built with concrete or polyester plastic, designed to supply bottom heat through hot air, hot water, or electrical systems. They are used for raising seedlings during winter and for rooting cuttings.

Cold Frames

Cold frames consist of simple frames with covers used primarily for hardening seedlings, protecting plants from frost, wind, and heavy rains. They can be temporary (usually made of wood) or permanent (constructed from bricks or concrete blocks).

Integrated Pest Management (IPM) in Protected Cultivation

Protected cultivation systems—such as greenhouses, net houses, polyhouses, and other enclosed structures—create highly favorable environments for rapid plant growth. However, these same conditions also support the fast and aggressive development of insect pests. Warm temperatures, high humidity, abundant foliage, and reduced natural enemy activity all contribute to pest outbreaks that are often more severe than in open-field agriculture. Therefore, a well-structured Integrated Pest Management (IPM) programme becomes an essential component of modern protected agriculture.

Importance of IPM in Protected Environments

Unlike open fields, where climatic variations and natural enemies help regulate pest populations, protected structures provide stable microclimates that support uninterrupted pest multiplication. Leafminers, whiteflies, aphids, thrips, mites, and caterpillars can flourish within a very short span of time. Additionally, the aesthetic and market standards differ based on crop type. For instance, vegetable growers may accept minor cosmetic feeding damage, while ornamental growers require near-perfect foliage and flowers, making even small infestations economically significant. Efficient IPM in protected structures thus demands continuous attention, early detection, and highly coordinated control measures.

1. Pest Identification

Accurate diagnosis forms the foundation of any successful IPM programme. Each crop grown under protected conditions has a predictable set of insect pests and associated risk periods. A clear understanding of pest biology, life cycle, host preferences, and environmental triggers enables growers to select the right preventive, cultural, biological, and chemical measures. Since closely related species often differ in their susceptibility to pesticides or natural enemies, misidentification can lead to control failures and increased crop losses.

2. IPM Management Framework

IPM in protected cultivation can be visualized as a pyramid consisting of three integrated pillars:

- Avoidance of pest entry and establishment
- Regular sampling and early detection
- Curative measures through biological or chemical means

These three layers function in harmony. Moreover, the strategy for managing one pest must not negatively interfere with the management of another.

Although cultural and physical measures may not eradicate severe infestations, they are indispensable for delaying pest establishment and keeping pest levels manageable.

3. Avoidance Measures

3.1 Physical Barriers

3.1.1 Insect-Proof Screens

Screens are the first line of defense against pests entering the greenhouse. Properly selected screens can restrict thrips, aphids, whiteflies, and leafminers effectively. However, mesh size must be optimized to balance pest exclusion and ventilation needs.

Recommended mesh sizes:

Insect Pest	Hole Size (µm)	Mesh (Threads per inch)
Leafminer	610	34
Cotton whitefly	462	42
Aphid	340	52
Greenhouse whitefly	290	58
Thrips	192	76

Thrips exclusion requires a fine 76-mesh screen, which also stops larger pests. Because finer screens restrict airflow, growers often compensate by increasing screened surface area, especially at vents.

3.1.2 Double-Door Entry System

A double-door or vestibule prevents pests from being blown into the structure when the main door opens. Workers may unknowingly carry insects on clothing; therefore, reducing air turbulence and limiting direct entry both help minimize contamination.

3.1.3 UV-Absorbing Films

Spectrally modified greenhouse covers block ultraviolet wavelengths (below ~370–380 nm) that insects use for navigation, host-finding, and mating. Studies from Japan and other countries have shown:

- Reduced entry of whiteflies, aphids, thrips, and leafminers
- 10 to 100-fold reduction in insects caught on sticky traps
- 50–80% fewer insecticide sprays in crops like tomato

These films selectively filter UV light without impairing photosynthetically active radiation (PAR), making them highly suitable for IPM programmes.

3.2 Early Detection

3.2.1 Sanitation and Cultural Practices

Sanitation and cultural practices form the foundation of integrated pest management (IPM) inside protected cultivation structures. Because greenhouses and polyhouses provide stable environmental conditions—warmth, humidity, and continuous food availability—pest

populations can multiply rapidly once introduced. Therefore, preventing initial infestation is far more economical and effective than attempting to control pests after establishment.

3.2.2 Pre-Season Cleanup

Pre-season sanitation is one of the most important steps for reducing pest carryover between cropping cycles. Pests generally enter protected structures through three key pathways: (1) infested planting material, (2) residual populations surviving from previous crops, and (3) migration from external weeds or alternative hosts around the greenhouse.

Before planting a new crop, the structure should be thoroughly cleaned by removing all plant debris, volunteer plants, weeds, and organic residues from the floor, gutters, benches, and corners. These often harbor whiteflies, mites, aphids, thrips, and soil-dwelling pests such as fungus gnats. A fallow period of 2–4 weeks, during which no host plants are present, significantly helps in breaking pest life cycles. During this fallow time, installing a few yellow sticky cards can help detect any remaining flying pests and confirm that the structure is pest-free before transplanting.

3.2.3 Inspection of Incoming Plants

Planting material is one of the most common sources of pest introduction. Seedlings and cuttings must therefore be inspected carefully for eggs, larvae, nymphs, pupae, or feeding symptoms. Early infestations are much easier to remove at the seedling or nursery stage than after canopy establishment. Leaves showing stippling, mines, curling, frass deposits, or honeydew must be removed immediately. If any suspicious symptoms are detected, trays should be isolated, and the affected plants either treated or discarded depending on severity. Preventive inspection reduces the risk of introducing whiteflies, thrips, leafminers, and mealybugs.

3.2.4 Balanced Fertilizer Use

Nutrient management plays a significant role in pest susceptibility. Excessive nitrogen fertilization encourages lush, succulent growth that attracts sap feeders such as aphids and whiteflies and predisposes plants to outbreaks. A balanced application of NPK and micronutrients promotes stronger plants with better resistance to insect attack. Monitoring electrical conductivity (EC) and pH of the growing medium also helps maintain optimal nutrient balance.

3.2.5 Pinching, Pruning, and Debris Removal

Regular canopy management improves airflow, reduces humidity pockets, and removes pest hotspots. Pinching and pruning of overcrowded shoots, removal of infested leaves, and disposal of fallen plant material all contribute to reducing pest breeding sites. Trimmings must be immediately collected and removed from the greenhouse, never left on walkways or soil where pests can continue to develop. In crops like tomato, pruning lower leaves after fruit clusters are

harvested is particularly effective for controlling whiteflies and leafminers, which commonly colonize shaded lower foliage.

3.2.6 Worker Hygiene and Quarantine

Human movement is a major route of pest spread inside greenhouses. Workers should avoid handling infested plants before touching uninfested ones. Tools such as clippers, knives, and tying materials must be regularly disinfected. Where possible, the greenhouse can be divided into zones, and movement from “clean” to “infested” sections should be minimized. Visitors should be limited during high-risk periods.

3.2.7 Trap Crops and Indicator Plants

Trap crops or indicator plants help in early detection and diversion of pests. Certain species attract specific pests more strongly than commercial crops. For example, *Portulaca oleracea* is known to attract the tobacco caterpillar (*Spodoptera litura*) and can be strategically planted at greenhouse entry points or corners to monitor early moth activity. Similarly, marigold or castor may be used as indicator plants for *Helicoverpa* species. Monitoring these plants provides an early warning system for timely intervention.

4.1 Scouting

Regular and systematic scouting is one of the most important components of integrated pest management (IPM) in protected cultivation. Early detection of pests allows growers to intervene before the infestation reaches economic damage levels. As emphasized by Lopes (2009), structured visual inspection remains the foundation of pest monitoring in greenhouse crops.

A thorough scouting procedure includes examining the entire plant from bottom to top, with special attention to the undersides of leaves, where many pests such as whiteflies, mites, thrips, and leafminers prefer to feed and oviposit. Vangapandu (2017) demonstrated that focusing on lower leaf surfaces greatly improves early diagnosis of sucking pests in tomato and chilli under protected conditions.

Scouts should also inspect the soil surface and substrate for larvae, pupae, and fungus gnat activity; Cloyd (2007) highlighted that soil-dwelling life stages are often missed during routine canopy checks, leading to delayed management decisions. Recording observations using standardized field data sheets or mobile apps ensures accuracy and helps track population trends over time. According to Binns (2000), consistent documentation is crucial for setting action thresholds and predicting future outbreaks.

A practical guideline is to monitor at least 1–2% of total plants weekly, or more frequently during high-risk periods, a recommendation supported by commercial greenhouse research in Europe and North America (Gerson 2003). Frequent scouting helps identify localized hotspots, improves the timing of interventions, and supports integration of biological control agents before pest populations escalate.

4.2 Monitoring with Traps

Monitoring traps greatly supplement visual scouting in protected cultivation or field crops. For example, yellow sticky traps (e.g. 4" × 12" or 8" × 12") are widely used to attract and capture adults of many flying pests such as whiteflies, thrips, aphids, leafminers, fungus gnats and adult psyllids, giving an early indication of pest presence and relative abundance. blue sticky traps can be used to monitor thrips more specifically; in a comparative study on pea crops, blue traps caught significantly more thrips (e.g. *Thrips tabaci*, *Frankliniella intonsa*) than white or yellow traps — making blue the optimal color for thrips monitoring in that context (Michał-Lech *et al.* 2020).

Suggested trap densities in greenhouse or protected-cultivation contexts are consistent with recommendations in literature: one to two yellow sticky traps per 50–100 m² (equivalent to ~1–2/100 m²) for monitoring purposes, and higher densities (five or more) when striving for mass-trapping. Reports also highlight that traps should be placed just above the crop canopy (e.g. 4–6 inches), and raised as plants grow, to maximize capture of flying. Sticky traps must be replaced when their surfaces become saturated (e.g., 60–70% covered), because clogged surfaces reduce trapping efficiency (Lu *et al.*, 2012).

In addition, pheromone traps (or light/funnel-type traps) are effective for detecting moth pests such as *Helicoverpa armigera* (tomato fruit-borer / pod borer) and *Spodoptera litura* (armyworm / cutworm), helping to time interventions (chemical or biocontrol) based on actual moth flight and presence. In India, light and pheromone trap networks have been successfully used to monitor *H. armigera* across different cropping systems (Srivastava *et al.* 2011). A recent survey also emphasized that funnel-type pheromone traps reliably detect *H. armigera* adult catches — making them a valuable early warning tool rather than a direct control method.

Hence, a trap-based monitoring strategy should be part of integrated pest management (IPM) in protected cultivation or open field — to enable early detection, guide timing of interventions (chemical or biological), and potentially reduce reliance on pesticides by targeting infestations before they escalate.

5. Curative Measures

5.1 Biological Control

Most insect pests in protected cultivation have natural enemies—predators, parasitoids, and entomopathogens—that can be effectively used for biological control. When introduced early, these agents can prevent pests from reaching damaging levels. Hoddle (2002) demonstrated that the parasitoid *Encarsia formosa* performs best when released at the initial stages of whitefly infestation in greenhouse crops. Likewise, the predatory mite *Amblyseius swirskii* has shown strong suppression of thrips and whiteflies in vegetable and ornamental greenhouses when released preventively, as reported by Calvo (2011).

Biological control success in protected structures depends heavily on pesticide compatibility. Several studies show that broad-spectrum insecticides can severely reduce survival or reproduction of key natural enemies. For example, Van de Veire (2002) reported that many commonly used insecticides greatly reduce the activity of predatory mites and parasitoids, underscoring the need to select selective or biocompatible products. Maintaining favorable microclimatic conditions is another requirement; Shipp (1996) showed that temperature and humidity strongly influence performance of predatory mites and parasitoids in greenhouse crops. To maximize success, growers must:

- Use pesticides compatible with natural enemies
- Establish natural enemies early (preventive releases)
- Maintain optimal microclimate (temperature, humidity, canopy structure)
- Avoid broad-spectrum, highly persistent insecticides
- Integrate microbial biocontrol agents where appropriate

Western Europe and North America have already adopted biological control widely, where natural enemies form the backbone of commercial greenhouse IPM programs and significantly reduce reliance on synthetic chemicals.

5.2 Chemical Control

Chemical insecticides remain important for managing severe outbreaks. However, their use in protected cultivation must be judicious because:

- High temperature inside greenhouses increases pesticide volatility
- Restricted air circulation increases exposure risk for handlers
- Risk of chemical residues on produce is higher
- Pest populations develop resistance rapidly

To ensure safe and effective use:

- Select pesticides with low persistence
- Follow recommended waiting periods; Denholm (1992)
- Avoid repeated use of the same insecticide class; Doan Ngoc *et al.*, 2015)
- Incorporate botanicals and microbial pesticides (e.g., neem products, *Bacillus thuringiensis*)
- Observe a minimum 12-hour re-entry period
- Avoid fumigant-type insecticides inside enclosed structures

Resistance management requires rotation among modes of action and integration with non-chemical tools.

6. Future Thrust Areas

Several gaps remain in the IPM strategies for protected cultivation:

- Need for more research on pest avoidance techniques
- Development of location-specific biological control modules
- Establishment of safe waiting periods for greenhouse crops
- Training growers in diagnosis, scouting, and safe pesticide usage
- Increased availability of affordable biocontrol agents in India
- Greater emphasis on climate-smart pest management solutions

Strengthening awareness and building capacity among growers are essential to ensure sustainable pest management in protected environments

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SUSTAINABLE LIVESTOCK PRODUCTION UNDER CHANGING CLIMATE: IMPACTS, ADAPTATION, AND MITIGATION

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Abstract

Climate change has emerged as one of the greatest challenges to global livestock production, affecting animal health, productivity, welfare, and the sustainability of livestock production systems. Rising temperatures, altered precipitation patterns, increased atmospheric carbon dioxide concentrations, and the growing frequency of extreme weather events directly and indirectly influence livestock through heat stress, water scarcity, declining feed quality and quantity, reduced pasture productivity, and the emergence of infectious diseases and parasites. These changes adversely affect growth performance, reproductive efficiency, milk and meat production, and overall farm profitability, while threatening food security and rural livelihoods, particularly in developing countries. Simultaneously, the livestock sector contributes substantially to greenhouse gas emissions through enteric fermentation, manure management, feed production, and land-use change, highlighting its dual role as both a contributor to and a victim of climate change. This chapter comprehensively reviews the impacts of climate change on livestock production, including its effects on animal welfare, feed and water resources, disease dynamics, livestock biodiversity, and economic sustainability. It also discusses climate-smart adaptation and mitigation strategies such as heat stress management, sustainable feed and pasture management, efficient water use, improved animal health, genetic improvement, precision livestock farming, methane mitigation, manure management, renewable energy utilization, and integrated crop–livestock systems. These approaches are essential for developing resilient, low-emission livestock systems capable of ensuring sustainable productivity, environmental conservation, and global food security under changing climatic conditions.

Keywords: Climate Change, Livestock Production, Heat Stress, Greenhouse Gas Emissions, Feed and Fodder, Water Scarcity, Animal Health, Disease Dynamics, Climate-Smart Livestock, Adaptation, Mitigation, Sustainable Livestock Systems.

Introduction

Livestock production is a fundamental component of global agricultural systems, contributing significantly to food security, nutrition, rural livelihoods, and economic development. The sector provides a diverse range of products, including milk, meat, eggs, wool, hides, and manure, while also supplying draft power and serving as a financial asset for millions of farming households. Livestock production systems in developing countries are undergoing rapid transformation due to population growth, urbanization, rising incomes, and changing dietary preferences. The global

population is projected to increase from approximately 6.5 billion to 9.2 billion by 2050, with more than one billion of this growth occurring in Africa (Thornton *et al.*, 2009). As urban populations expand and consumer demand for animal-derived foods rises, the demand for livestock products such as milk, meat, and eggs is expected to increase substantially, driving the intensification and modernization of livestock production systems (Delgado *et al.*, 1999; Cheng *et al.*, 2022).

Simultaneously, the global climate is undergoing significant changes (Abbass *et al.*, 2022). Climate change has emerged as one of the most critical threats to global livestock production, influencing animal health, productivity, feed resources, water availability, and overall farming sustainability (Cheng *et al.*, 2022). Rising atmospheric concentrations of greenhouse gases, primarily carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O), have increased global mean temperatures, altered precipitation patterns, and intensified the frequency of extreme weather events such as heat waves, droughts, floods, cyclones, and wildfires. These climatic changes directly and indirectly affect livestock systems across diverse agroecological regions. The relationship between climate change and livestock production is bidirectional. While livestock systems are vulnerable to climate change, they are also important contributors to greenhouse gas emissions. The livestock sector generates emissions through enteric fermentation, manure management, feed production, land-use change, and energy consumption. Methane emissions from ruminants and nitrous oxide emissions from manure represent major sources of agricultural greenhouse gases. Consequently, improving livestock sustainability is essential not only for climate adaptation but also for climate change mitigation. This chapter aims to provide a comprehensive overview of the interactions between climate change and livestock production, with particular emphasis on the impacts of changing climatic conditions on animal health, productivity, resource availability, and environmental sustainability. In addition, the chapter reviews climate adaptation measures designed to improve the resilience of livestock production under changing environmental conditions.

2. Livestock and Climate Change: A Two-Way Relationship

Livestock production and climate change share a complex and interdependent relationship. While livestock systems are highly vulnerable to changing climatic conditions, they also contribute significantly to greenhouse gas (GHG) emissions that drive global warming. Rising temperatures, altered precipitation patterns, prolonged droughts, and the increasing frequency of extreme weather events adversely affect livestock productivity, animal health, feed and water availability, reproductive performance, and disease dynamics. Heat stress reduces feed intake, milk yield, growth rate, and fertility while increasing susceptibility to diseases and mortality, particularly in high-producing dairy cattle and poultry. Climate-induced changes in pasture productivity and forage quality further constrain livestock production, especially in rainfed and grazing-based production systems (Thornton *et al.*, 2009).

Conversely, the livestock sector contributes substantially to anthropogenic greenhouse gas emissions through enteric fermentation, manure management, feed production, land-use change, and energy consumption throughout the livestock supply chain. Ruminant animals such as cattle, buffaloes, sheep, and goats emit considerable quantities of methane (CH₄) during enteric fermentation, whereas manure management and nitrogen fertilizer application release methane and nitrous oxide (N₂O), both of which possess high global warming potentials. Feed cultivation, transportation, processing, and conversion of forests into grazing lands or feed-producing areas further increase carbon dioxide (CO₂) emissions, making livestock production an important contributor to climate change (Gerber *et al.*, 2013).

Overall, livestock production is both a contributor to and a victim of climate change. Sustainable intensification, technological innovation, and evidence-based management practices are essential to balance the increasing global demand for animal-source foods with environmental conservation. Developing climate-resilient and low-emission livestock production systems will play a critical role in ensuring food security, protecting natural resources, and achieving global climate mitigation and sustainable development goals (Herrero *et al.*, 2016).

3. Climate Change: Causes and Trends

Climate change refers to long-term alterations in the Earth's climate system resulting from both natural processes and anthropogenic activities. The extensive combustion of fossil fuels, rapid industrialization, urbanization, deforestation, and intensified agricultural activities have substantially increased atmospheric concentrations of greenhouse gases, leading to global warming and widespread climatic changes. Among agricultural sectors, livestock production is especially sensitive to climatic variability due to its dependence on feed resources, water availability, pasture productivity, and animal physiological responses to environmental stress (Papakonstantinou *et al.*, 2024). Consequently, understanding the causes and trends of climate change is essential for developing resilient and sustainable livestock production systems.

3.1 Greenhouse Gases and Global Warming

The greenhouse effect is a natural atmospheric process that maintains Earth's average surface temperature at approximately 15°C, making the planet suitable for life (Thweatt *et al.*, 2024). Solar radiation reaches the Earth's surface as shortwave energy, where a portion is absorbed and later emitted as longwave infrared radiation. Greenhouse gases present in the atmosphere absorb part of this outgoing infrared radiation and re-radiate it back toward the Earth's surface, thereby maintaining a stable thermal balance. Human activities have significantly intensified the natural greenhouse effect by increasing atmospheric concentrations of greenhouse gases (Bajoria *et al.*, 2024). Carbon dioxide (CO₂) is the most abundant anthropogenic greenhouse gas and is primarily released through the combustion of coal, petroleum, and natural gas, cement production, industrial activities, and deforestation (Nunes, 2023). Methane (CH₄), although present in lower atmospheric concentrations than CO₂, possesses a much higher global warming

potential over a 100-year period. Major sources of methane include enteric fermentation in ruminant animals, manure management, rice cultivation, wetlands, and fossil fuel extraction. Nitrous oxide (N₂O) is another potent greenhouse gas generated mainly through excessive nitrogen fertilizer application, manure decomposition, biomass burning, and industrial activities. Other greenhouse gases, including hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), sulfur hexafluoride (SF₆), and nitrogen trifluoride (NF₃), are synthetic compounds with extremely high global warming potentials despite their relatively low atmospheric concentrations. The continuous increase in greenhouse gas concentrations has resulted in rising global mean temperatures, accelerated glacier retreat, melting polar ice sheets, increasing sea levels, changing precipitation patterns, and greater frequency of extreme weather events.

3.2 Major Drivers of Climate Change

Climate change is driven by both natural and anthropogenic factors, although recent climatic changes are overwhelmingly associated with human activities.

Fossil Fuel Combustion

The largest contributor to anthropogenic climate change is the burning of fossil fuels for electricity generation, transportation, industrial production, and domestic energy use. These activities release massive quantities of carbon dioxide into the atmosphere, accounting for the majority of global greenhouse gas emissions (IPCC, 2023; Friedlingstein *et al.*, 2023)

Deforestation and Land Use Change

Forests act as major carbon sinks by absorbing atmospheric carbon dioxide through photosynthesis. However, widespread deforestation for agriculture, urban expansion, mining, and infrastructure development reduces carbon sequestration capacity while simultaneously releasing stored carbon into the atmosphere.

Agricultural Activities

Agriculture contributes significantly to greenhouse gas emissions through livestock production, rice cultivation, fertilizer application, crop residue burning, and manure management. Livestock production is an important source of methane emissions due to enteric fermentation in ruminants, while nitrogen fertilizers applied to croplands generate nitrous oxide emissions through microbial processes in soils (Gerber *et al.*, 2013).

Industrialization and Urbanization

Rapid industrial growth has substantially increased energy demand and greenhouse gas emissions from manufacturing processes, chemical production, construction, and transportation. Urbanization further intensifies emissions through increased electricity consumption, waste generation, transportation networks, and expanding infrastructure.

Population Growth and Consumption Patterns

Global population growth, increasing incomes, and changing lifestyles have accelerated demand for food, energy, transportation, and manufactured goods. Rising consumption of animal-source

foods has stimulated expansion of intensive livestock production systems, increasing demand for feed crops, water resources, and energy inputs.

Natural Drivers

Natural factors such as volcanic eruptions, solar variability, and ocean-atmosphere interactions influence climate over long timescales. However, these factors cannot explain the rapid increase in global temperatures observed during the past century.

Impact of climate change on livestock

Climate change significantly affects livestock production by increasing heat stress, altering feed and water availability, and increasing the prevalence of diseases, ultimately reducing animal health, productivity, and reproductive performance.

Impact of Climate Change on Feed

Feed Quality and Quantity

Climate change significantly affects both the quantity and quality of livestock feed resources by altering temperature, precipitation patterns, and atmospheric carbon dioxide (CO₂) concentrations. Rising temperatures, prolonged droughts, and erratic rainfall reduce pasture productivity, forage biomass, and fodder crop yields, leading to seasonal feed shortages and increased reliance on expensive commercial feeds. Elevated CO₂ concentrations may increase herbage growth; however, they often alter pasture composition by changing the ratio of grasses to legumes and reduce forage nutritional quality by lowering nitrogen (protein) and mineral concentrations while increasing structural carbohydrates, thereby decreasing digestibility and nutrient utilization (Hopkins and Del Prado, 2007). Climate change also modifies the concentration of water-soluble carbohydrates in herbage, influences dry matter production, and increases the frequency of droughts, which may offset any gains in forage biomass. Furthermore, more intense rainfall events can accelerate nutrient losses through nitrogen leaching, reducing soil fertility and pasture productivity. Warmer and more humid conditions also increase the risk of fungal growth and mycotoxin contamination in feed. Collectively, these changes reduce animal growth, milk production, reproductive efficiency, and overall livestock productivity, emphasizing the need for climate-resilient forage species, improved pasture management, fodder conservation, and balanced nutritional strategies to sustain livestock production under changing climatic conditions (Thornton *et al.*, 2009). Numerous studies conducted under controlled growth chamber and Free-Air CO₂ Enrichment (FACE) conditions have demonstrated that elevated atmospheric carbon dioxide (CO₂) concentrations enhance photosynthesis, biomass accumulation, and crop yield, particularly in C₃ plant species. A comprehensive review by Long *et al.* (2006) reported that increasing atmospheric CO₂ concentrations to approximately 550 ppm can increase the yields of several C₃ crops by 20–30%, primarily through enhanced photosynthetic efficiency and improved water-use efficiency. However, these potential gains

may be reduced by concurrent increases in temperature, drought stress, nutrient limitations, and other climate-related constraints.

Heat Stress

Heat stress is one of the most severe direct impacts of climate change on livestock production, resulting from rising temperatures, increased humidity, and more frequent heat waves. When environmental heat exceeds an animal's capacity to dissipate body heat, physiological and behavioral changes such as increased respiration rate, panting, sweating, water intake, and reduced feed consumption occur to maintain thermal balance. These responses divert energy away from productive functions, leading to reduced growth, milk yield, reproductive efficiency, and feed conversion while compromising immune function and increasing susceptibility to diseases. Heat stress also adversely affects animal welfare by causing dehydration, oxidative stress, discomfort, and, in severe cases, mortality, particularly in high-producing dairy cattle, poultry, and pigs. As climate change intensifies thermal stress, the adoption of climate-smart adaptation measures—including improved housing, shade provision, cooling systems, adequate water supply, nutritional interventions, and selection of heat-tolerant breeds—is essential to enhance animal welfare, maintain productivity, and improve the resilience of livestock systems (Bernabucci *et al.*, 2010; Collier & Gebremedhin, 2015).

Water Scarcity

Climate change is intensifying water scarcity, posing a significant threat to livestock production worldwide. Rising temperatures, altered precipitation patterns, prolonged droughts, and increased evapotranspiration have reduced the availability and reliability of freshwater resources for livestock farming. Water is essential not only for animal drinking but also for feed and fodder production, sanitation, cooling systems, and processing of livestock products. Consequently, reduced water availability adversely affects animal health, productivity, and overall farm sustainability.

Water scarcity directly compromises livestock performance by limiting drinking water intake, leading to dehydration, reduced feed consumption, impaired thermoregulation, and decreased growth, milk yield, reproductive efficiency, and disease resistance. During periods of heat stress, animals require substantially greater quantities of water to maintain normal physiological functions, making water shortages even more detrimental. In addition, inadequate water availability reduces the irrigation of fodder crops and pasturelands, resulting in lower forage production and poorer feed quality, thereby indirectly affecting livestock productivity.

These challenges increase production costs and threaten the livelihoods of livestock-dependent communities, particularly in arid and semi-arid regions.

Disease Dynamics and Emerging Pathogens

Climate change is altering the epidemiology, distribution, and transmission dynamics of livestock diseases by creating favorable environmental conditions for the emergence and spread

of pathogens and their vectors. Rising temperatures, changing rainfall patterns, increased humidity, and the growing frequency of extreme weather events influence the survival, reproduction, and geographical distribution of disease vectors such as ticks, mosquitoes, biting flies, and midges, leading to the expansion of vector-borne diseases into previously unaffected regions. Warmer climates also enhance the persistence and transmission of bacterial, viral, fungal, and parasitic pathogens, increasing the incidence of diseases such as bluetongue, Rift Valley fever, anaplasmosis, babesiosis, and tick-borne theileriosis. Furthermore, heat stress weakens the immune system of livestock, making animals more susceptible to infectious diseases, parasitic infestations, and metabolic disorders. Climate-induced flooding and droughts can contaminate water and feed resources, facilitate the spread of waterborne and foodborne pathogens while disrupt veterinary services and disease surveillance. These changes not only reduce animal productivity, reproductive performance, and welfare but also increase the risk of zoonotic disease transmission, posing significant threats to public health, food security, and livestock-based livelihoods.

Milk Production and Quality

Climate change adversely affects milk production and quality by exposing dairy animals to heat stress, reducing feed and water availability, and increasing the prevalence of diseases. Elevated ambient temperatures decrease feed intake and alter metabolic and endocrine functions, resulting in negative energy balance, reduced nutrient utilization, and lower milk synthesis. High-producing dairy cows are particularly susceptible to heat stress, with milk yield declining by 10–25% or more during prolonged periods of elevated temperature and humidity. Climate-induced deterioration in forage quality and quantity further limits nutrient intake, while water scarcity compromises thermoregulation and lactation performance. Heat stress also affects milk composition by reducing milk fat, protein, lactose, and total solids, thereby lowering nutritional value and processing quality. In addition, increased incidences of mastitis and other infectious diseases under warmer climatic conditions negatively influence milk quality by increasing somatic cell counts and reducing shelf life.

Meat Production and Quality

Climate change negatively affects meat production by impairing animal growth, feed conversion efficiency, carcass characteristics, and meat quality through increased heat stress, reduced feed availability, water scarcity, and greater disease incidence. Elevated temperatures decrease feed intake and increase maintenance energy requirements, leaving less energy available for muscle growth and weight gain. Consequently, livestock exhibit reduced average daily gain, lower carcass weight, and poorer feed efficiency, leading to decreased meat production and higher production costs. Heat stress also alters muscle metabolism before slaughter, reducing glycogen reserves and increasing the incidence of meat quality defects such as pale, soft, exudative (PSE) meat in pigs and poultry and dark, firm, dry (DFD) meat in cattle and sheep. In addition, climate-

induced deterioration in forage quality and nutritional deficiencies adversely affect carcass composition, intramuscular fat deposition, tenderness, and overall meat quality. Increased prevalence of infectious and parasitic diseases further compromises animal health, increases mortality, and reduces meat safety through a higher risk of pathogen contamination.

Adaptation and Mitigation Measures to Climate Change in Livestock Systems

Developing resilient livestock systems requires a combination of adaptation and mitigation strategies. Adaptation focuses on enhancing the capacity of livestock and farming systems to cope with changing climatic conditions, whereas mitigation aims to reduce greenhouse gas (GHG) emissions from livestock production while improving resource-use efficiency. Integrating these approaches is fundamental to achieving climate-smart livestock production that ensures food security, environmental sustainability, and improved livelihoods.

Adaptation Measures

1. Heat Stress Management

- Climate-resilient livestock housing with improved ventilation and insulation.
- Provision of shade, cooling systems (fans, sprinklers, foggers), and adequate drinking water.
- Modification of feeding schedules to cooler periods of the day.
- Nutritional supplementation with antioxidants, electrolytes, minerals, and vitamins to alleviate heat stress.

2. Climate-Resilient Breeding

- Conservation and utilization of indigenous breeds possessing superior heat tolerance and disease resistance.
- Genetic selection and genomic breeding for heat tolerance, feed efficiency, and climate resilience.
- Crossbreeding programs combining productivity with environmental adaptability.

3. Sustainable Feed and Fodder Management

- Cultivation of drought- and heat-tolerant forage crops.
- Improved pasture management through rotational grazing and reseeded.
- Silage and hay production for fodder conservation during feed-scarce periods.
- Use of alternative feed resources, agro-industrial by-products, and feed supplements.

4. Water Resource Management

- Rainwater harvesting and on-farm water storage.
- Efficient irrigation systems for fodder production.
- Recycling and reuse of water where feasible.
- Precision water management to improve water-use efficiency.

5. Animal Health Management

- Strengthened disease surveillance and early warning systems.
- Strategic vaccination and parasite control programs.
- Improved farm biosecurity and hygiene.
- Regular veterinary health monitoring.

6. Precision Livestock Farming

- Internet of Things (IoT)-based sensors for monitoring animal health and environmental conditions.
- Wearable devices for tracking body temperature, activity, and physiological responses.
- Artificial intelligence (AI) and machine learning for disease prediction, heat-stress detection, and precision feeding.
- Remote sensing and Geographic Information Systems (GIS) for pasture and drought monitoring.

7. Integrated Farming Systems

- Integrated crop–livestock production for efficient nutrient recycling.
- Agroforestry and silvopastoral systems providing shade, carbon sequestration, and improved biodiversity.
- Circular bioeconomy approaches utilizing livestock waste for energy and organic fertilizers.

Mitigation Measures

1. Reducing Enteric Methane Emissions

- Improving forage quality and digestibility.
- Precision feeding and balanced ration formulation.
- Supplementation with methane-reducing feed additives such as 3-nitrooxypropanol (3-NOP), seaweed (*Asparagopsis* spp.), essential oils, tannins, and nitrates.
- Increasing feed conversion efficiency through improved nutrition.

2. Improved Manure Management

- Biogas production through anaerobic digestion.
- Composting and vermicomposting.
- Covered manure storage systems to reduce methane emissions.
- Nutrient recycling through precision manure application.

3. Carbon Sequestration

- Rotational and regenerative grazing.
- Restoration of degraded pasturelands.
- Silvopastoral and agroforestry systems.
- Increasing soil organic carbon through improved grassland management.

4. Enhancing Feed Efficiency

- Precision nutrition to match nutrient supply with animal requirements.
- High-quality forage production.
- Feed processing technologies to improve digestibility.
- Selection of highly efficient animals with superior feed conversion ratios.

5. Renewable Energy Adoption

- Solar-powered water pumps and farm equipment.
- Wind energy where suitable.
- Biogas utilization for electricity generation and cooking fuel.
- Energy-efficient lighting, ventilation, and milking systems.

6. Waste Valorization and Circular Economy

- Conversion of livestock waste into biofertilizers.
- Biochar production from manure.
- Nutrient recovery technologies.
- Sustainable recycling of agricultural residues.

7. Policy and Institutional Support

- Climate-smart livestock policies.
- Incentives for low-carbon livestock production.
- Carbon credit and payment-for-ecosystem-services schemes.
- Capacity building, farmer training, and extension services.
- Investment in climate-resilient infrastructure and research.

Conclusion

Climate change poses significant challenges to livestock production by affecting animal health, productivity, feed and water resources, and farm profitability. Developing climate-resilient livestock systems requires the integration of adaptation and mitigation strategies that simultaneously enhance productivity, reduce greenhouse gas emissions, and improve resource-use efficiency. Climate-smart livestock production combines improved breeding, nutrition, animal health, precision farming technologies, sustainable pasture management, renewable energy, and efficient manure management to address the challenges posed by climate change. Strong policy support, technological innovation, and farmer capacity building will be essential to ensure sustainable livestock production, environmental conservation, and global food security under future climatic conditions.

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EMERGING TRENDS IN PLANT BIOTECHNOLOGY FOR SUSTAINABLE AGRICULTURE

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Abstract

Global agriculture faces unprecedented challenges arising from climate change, population growth, declining soil fertility, emerging pests and diseases and increasing pressure on natural resources. Sustainable agricultural practices have become essential to ensure long-term food security while minimizing environmental impacts. Plant biotechnology has emerged as one of the most promising scientific approaches for developing climate-resilient, high-yielding and nutritionally enhanced crops. Recent advances in genome editing, genetic engineering, molecular breeding, synthetic biology, plant tissue culture, omics technologies, artificial intelligence, nanobiotechnology and microbiome engineering have transformed modern crop improvement strategies. These technologies enable precise manipulation of plant genomes, accelerate breeding cycles, improve resistance against biotic and abiotic stresses, enhance nutrient-use efficiency and reduce dependence on chemical fertilizers and pesticides. Furthermore, precision agriculture integrated with biotechnology supports efficient resource utilization through real-time monitoring and data-driven crop management. This chapter discusses the latest developments in plant biotechnology and their contributions toward sustainable agriculture. It also highlights current challenges, biosafety considerations, ethical issues, regulatory frameworks, and future research directions. The integration of biotechnology with digital agriculture and climate-smart farming is expected to revolutionize global agricultural systems while promoting environmental sustainability and food security.

Keywords: Plant Biotechnology, Sustainable Agriculture, Genome Editing, CRISPR-Cas9, Molecular Breeding, Plant Tissue Culture, Omics Technologies, Synthetic Biology, Precision Agriculture, Climate-Smart Agriculture.

1. Introduction

Agriculture remains the backbone of global food production and rural livelihoods. However, increasing population growth, projected to exceed 9.7 billion by 2050, places enormous pressure on agricultural productivity. Simultaneously, climate change, water scarcity, soil degradation, biodiversity loss, and emerging pathogens significantly threaten crop production. Conventional

breeding techniques have substantially contributed to agricultural development but require long breeding cycles and often lack precision when dealing with complex traits.

Plant biotechnology has revolutionized crop improvement by enabling precise genetic modifications, rapid multiplication of elite cultivars, and development of stress-tolerant varieties. Modern biotechnology combines molecular biology, genomics, bioinformatics, tissue culture, synthetic biology, nanotechnology, and artificial intelligence to improve agricultural sustainability. These innovations facilitate the development of crops with enhanced nutritional quality, disease resistance, improved photosynthetic efficiency, and better adaptation to changing climatic conditions.

This chapter presents emerging trends in plant biotechnology that are reshaping sustainable agriculture worldwide.

2. Fundamentals of Plant Biotechnology

Plant biotechnology involves the application of biological techniques to modify, improve, and propagate plants for agricultural purposes.

Major areas include

- Plant tissue culture
- Genetic engineering
- Marker-assisted breeding
- Genome editing
- Molecular diagnostics
- Synthetic biology
- Plant genomics
- Functional genomics
- Metabolomics
- Precision agriculture

These technologies improve crop productivity while reducing environmental degradation.

3. Emerging Technologies in Plant Biotechnology

3.1 CRISPR-Cas Genome Editing

Genome editing represents one of the greatest breakthroughs in modern agriculture.

CRISPR-Cas9 enables researchers to

- Precisely modify genes
- Knock out undesirable traits
- Improve drought tolerance
- Enhance disease resistance
- Increase nutritional quality

Recent developments include

- Base editing
- Prime editing
- Multiplex genome editing
- CRISPR-Cas12
- CRISPR-Cas13

3.2 Molecular Breeding and Genomic Selection

Molecular breeding integrates DNA markers with conventional breeding.

Important approaches include

- Marker-Assisted Selection (MAS)
- Marker-Assisted Backcrossing
- Genomic Selection
- Quantitative Trait Locus (QTL) Mapping
- Genome-Wide Association Studies (GWAS)

Advantages

- Shorter breeding cycles
- Higher breeding accuracy
- Better disease resistance
- Improved grain quality
- Climate resilience

3.3 Plant Tissue Culture

Plant tissue culture remains a cornerstone of biotechnology.

Major techniques include

- Micropropagation
- Organogenesis
- Somatic embryogenesis
- Embryo rescue
- Haploid production
- Synthetic seed technology

Applications

- Rapid multiplication
- Virus-free planting material
- Germplasm conservation
- Rare species conservation
- Commercial horticulture

Countries increasingly use tissue culture for banana, potato, sugarcane, orchids and medicinal plants.

4. Plant Microbiome Engineering

The plant microbiome significantly influences crop productivity.

Beneficial microorganisms include

- Rhizobacteria
- Endophytes
- Mycorrhizae
- Nitrogen-fixing bacteria

Applications

- Biological nitrogen fixation
- Phosphate solubilization
- Disease suppression
- Stress tolerance
- Reduced fertilizer use

Microbiome engineering is becoming an environmentally friendly alternative to chemical agriculture.

5. Nanobiotechnology in Agriculture

Nanotechnology enhances the delivery of agricultural inputs.

Applications include

- Nano-fertilizers
- Nano-pesticides
- Nano-sensors
- Controlled nutrient release
- Seed nano-priming

Benefits

- Reduced chemical usage
- Higher nutrient efficiency
- Lower environmental pollution
- Precision nutrient management

6. Artificial Intelligence in Plant Biotechnology

Artificial intelligence complements biotechnology through

- Gene discovery
- Genomic prediction
- Plant disease diagnosis
- Yield forecasting
- Phenotyping
- Breeding optimization

Machine learning models analyse massive genomic datasets to identify candidate genes associated with desirable traits.

Deep learning improves

- Plant disease recognition
- Weed detection
- Crop monitoring
- Stress prediction

7. Biotechnology for Climate-Resilient Crops

Climate change necessitates resilient crop varieties.

Biotechnology contributes through development of

- Heat-tolerant wheat
- Flood-resistant rice
- Drought-resistant maize
- Salt-tolerant barley
- Disease-resistant cassava

Stress-responsive genes include:

- DREB
- NAC
- HSP
- WRKY
- MYB transcription factors

8. Biofortification and Nutritional Enhancement

Hidden hunger affects billions worldwide.

Biotechnology addresses micronutrient deficiencies through biofortification.

Examples include

- Vitamin A-rich rice
- Iron-rich beans
- Zinc-enriched wheat
- High-protein maize
- Folate-rich rice

Genome editing further accelerates nutritional improvement.

9. Precision Agriculture and Digital Biotechnology

Modern farming increasingly integrates

- Internet of Things (IoT)
- Remote sensing
- UAVs

- Satellite imaging
- AI decision support
- Smart irrigation

Biotechnology-generated crop varieties combined with precision farming maximize productivity while minimizing resource consumption.

10. Biosafety, Ethical Issues, and Regulatory Challenges

Despite tremendous benefits, biotechnology faces several concerns.

Biosafety

- Gene flow
- Non-target effects
- Ecological impacts

Ethical Considerations

- Public acceptance
- Food labelling
- Intellectual property
- Access to technology

11. Future Perspectives

Future plant biotechnology will increasingly integrate

- Artificial Intelligence
- Quantum computing
- Digital twins
- Multi-omics
- Synthetic biology
- Gene editing
- Climate-smart agriculture
- Carbon farming
- Autonomous agriculture

Conclusion

Plant biotechnology has become indispensable for achieving sustainable agriculture under changing climatic and socio-economic conditions. Emerging technologies such as CRISPR-based genome editing, synthetic biology, omics sciences, microbiome engineering, nanobiotechnology, and AI-assisted breeding are transforming crop improvement with unprecedented precision and efficiency. These innovations enable the development of resilient, nutrient-rich, and resource-efficient crops while reducing dependence on chemical inputs and conserving natural resources. However, widespread adoption requires robust biosafety regulations, ethical governance, public engagement, and equitable access to advanced

technologies. Continued interdisciplinary collaboration among plant scientists, breeders, data scientists, policymakers, and farmers will be essential for translating scientific discoveries into sustainable agricultural practices. Future integration of biotechnology with precision agriculture and climate-smart farming offers immense potential to ensure global food security, environmental conservation, and agricultural resilience.

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NANOSCALE MATERIALS AND THEIR EMERGING ROLE IN SUSTAINABLE INSECT PEST CONTROL

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Introduction

Nanotechnology has emerged as one of the most transformative scientific domains in recent decades due to the exceptional properties exhibited by materials at the nanoscale. Targeted nanoparticles frequently display unique characteristics, including remarkable mechanical strength, enhanced chemical reactivity, and high electrical conductivity—features that distinguish them from their bulk counterparts and make them highly valuable across scientific disciplines (Ulrich *et al.*, 2006). Their extraordinary behavior is largely attributed to the atomic-level structure and interactions that become significant when matter is reduced to sizes between 1 and 100 nm. At this scale, nanoparticles express distinct physical, biological, and chemical properties that arise from quantum effects and high surface-area-to-volume ratios (Leiderer and Dekorsy, 2008).

Nanoparticles are typically constructed atom by atom or molecule by molecule, and their size, and occasionally their shape, can be precisely controlled through specific experimental methods. Once synthesized, nanoparticles have the ability to organize themselves into ordered patterns, layers, or multilayered structures. This intrinsic capacity for self-assembly is driven by various intermolecular and interatomic forces, including hydrogen bonding, hydrophobic and hydrophilic interactions, dipole–dipole attractions, gravitational influences, and surface tension effects. Interestingly, self-assembly is not limited to engineered nanosystems; many natural biological architectures—such as cellular membranes, vesicles, and deoxyribonucleic acid (DNA)—are also formed through spontaneous assembly processes governed by similar fundamental forces (Atanu *et al.*, 2010).

The term "nano" originates from the Greek word for "dwarf," symbolizing extreme smallness. Scientifically, “nano” refers to 10^{-9} , or one-billionth of a unit, and it is commonly used to describe structures or particles measured in nanometers. To illustrate this scale, many viruses measure around 100 nm in diameter. The concept of nanotechnology evolved with the manipulation and application of materials within the range of 1–100 nm, where their properties

differ dramatically from larger-scale forms. Two foundational approaches dominate nanotechnology: the top-down approach, in which larger structures are miniaturized to the nanoscale (as seen in nanoelectronics, photonics, and nanoengineering), and the bottom-up approach, where individual atoms and molecules assemble into nanoscale architectures. These methods underpin numerous natural and engineered processes across chemistry, biology, physics, and materials science.

The strength of nanotechnology lies in its ability to harness the novel behaviors nanoparticles exhibit—whether related to their mechanical solidity, magnetic properties, electrical conductance, or chemical efficiency. As a rapidly advancing field, nanotechnology offers boundless opportunities for innovation and is anticipated to contribute significantly to future industrial and technological development. Its applications already span a wide range of areas including medicine, electronics, environmental engineering, agriculture, and biotechnology.

Within the context of agriculture and entomology, the application of nanoparticles is gaining considerable attention. The current focus is increasingly shifting toward understanding how nanoparticles interact with insects and evaluating their potential uses in insect pest management. Interest in this emerging area stems from the possibility that nanoparticles can provide targeted, effective, and environmentally safer alternatives to conventional pest control strategies. Research suggests that engineered nanoparticles may be capable of influencing insect physiology, behavior, or survival in unique ways due to their nanoscale properties and ability to interact with biological systems at the molecular level (Nykypanchuk *et al.*, 2008). As investigations continue, nanotechnology is expected to provide modern tools and novel approaches that could transform existing pest management practices and contribute to more sustainable agricultural production systems.

Nanoscale Materials and Their Application

Nanoscale materials represent one of the most transformative scientific developments of the present century. Their unique structural, chemical, magnetic and mechanical characteristics have enabled a broad spectrum of applications across biological, environmental and industrial systems. Living organisms—from simple bacteria to complex insects—naturally depend on nanometer-scale protein assemblies that perform essential tasks such as the whipping movement of bacterial flagella or the contraction and relaxation of muscle fibres. These naturally occurring nanoscale machines illustrate how biological efficiency and precision often originate at dimensions well below the micrometer scale, a concept that has greatly inspired modern nanoscience (Goodsell, 2004).

The commercial significance of nanoscale materials is not new. Nanometer-sized carbon particles, commonly known as carbon black, have long been incorporated into tyres to enhance their mechanical strength and durability. Similarly, nanosilver particles have been crucial in the

initiation of photographic film development, while a variety of nanostructured catalysts are indispensable to the petrochemical industry. These examples highlight how the manipulation of materials at the nanoscale provides physicochemical advantages not attainable with bulk materials (Ajayan, 2003).

Most engineered nanoparticles today are synthesized through controlled chemical processes that allow precise manipulation of particle size, morphology and surface characteristics. Accurate identification and characterization of these nanoparticles require advanced tools such as the scanning tunneling microscope (STM) and the atomic force microscope (AFM). The invention of the STM by Gerd Binnig and Heinrich Rohrer, for which they received the 1986 Nobel Prize in Physics, marked a pivotal moment in nanoscale science by enabling the visualization and manipulation of individual atoms on material surfaces (Binnig & Rohrer, 1986). This breakthrough laid the foundation for the rapid expansion of nanotechnology in diverse scientific domains.

Environmental applications of nanotechnology have gained exceptional momentum in recent years. Nanostructured materials are now widely used in devices designed for pollution detection, treatment and remediation. Their high surface area, strong adsorption capacity and tunable surface chemistry allow nanoparticles to capture pollutants, catalyze their breakdown, or facilitate environmentally benign manufacturing processes. Such approaches fall under the broader umbrella of green nanotechnology, which emphasizes minimal environmental impact and long-term sustainability (Klaine, 2012).

Beyond environmental utilities, nanomaterials are increasingly integrated into industrial manufacturing. They are used as fillers and strengthening agents in composite materials, significantly improving mechanical performance without increasing weight. In the health sector, nanotechnology has contributed to the development of antimicrobial wound dressings, targeted drug-delivery systems and diagnostic tools with high sensitivity. Nanotechnology-enabled textiles offering stain resistance, enhanced durability, or self-cleaning properties are now being commercialized as well. The electronics and communication sectors benefit from nanoscale conductors, semiconductors and photonic components that allow miniaturization, greater processing efficiency and lower energy consumption (Roco, 2011).

Agriculture is another major beneficiary of nanoscale innovation. Recent advancements include nanoparticle-based pesticide formulations designed for controlled release, higher efficacy and reduced environmental toxicity. Nanoparticles are also being explored for plant nutrient delivery, stress management and crop protection. Products such as transparent sunscreen lotions, nano-enabled coatings, and biocompatible insecticidal formulations further demonstrate the broad applicability of nanotechnology across consumer and agricultural markets (Scrinis & Lyons, 2007). As research progresses, the integration of nanoparticles into environmentally safe pest-

management strategies is emerging as a frontier area, with the potential to redefine conventional agricultural practices.

Natural Nanoparticles in Several Insects

Although naturally occurring nano-structures are being neglected, they are a potentially rich source of products that meet certain specifications. The emerging industries based on nanotechnology have so far made little use of 'free' technology available in nature. A good example is the ordered hexagonal packed array of structures in the wings of cicada for instance, *Psaltoda claripennis* Ashton and termite for example, family *Rhinotermitidae*. Studies have shown that the size of the nanoparticles may vary from 200 to 1000 nm. The structures tend to have a rounded shape at the apex and protrude some 150-350 nm out from the surface plane. These wing nanoparticles help in the aerodynamic efficiency of the insect.

Nowadays, we know that insects possess ferromagnetic resonance which is temperature dependent. The magnetic material is present in the head, thorax and abdomen of insects like *Solenopsis substitute* (Fabricius), an ant. These magnetic nanoparticles in social insects act as geomagnetic sensors. The magnetic material content is slightly higher in heads with antennae than in abdomen of the ants. It is known that the behaviour of a great variety of higher animals is influenced by changes in the local magnetic field within their environment.

More specifically, it has been shown that honey bees use geomagnetic field information for orientation, homing and foraging. However, the process that animals use to detect the geomagnetic field is still not known. Several observations indicate that intracellular biomineralized magnetite could interact with the geomagnetic field monitoring information on its intensity and direction. Identifying the presence of magnetite particles in different species of insects and thus showing that insects' behaviour is influenced by the geomagnetic field is a first step towards demonstrating that biogenic magnetite is involved in geomagnetic field detection. It has been shown that the ant *Formica rufa* Linnaeus uses information from the geomagnetic field for orientation during the foraging process. The same observation has been made in respect of the ant *Solenopsis invicta* Buren. The presence of ferric iron in the abdomen of *S. invicta* workers was also observed. The observation which was made through electron microscope technique clearly demonstrated that several species of ants recognize magnetic signals with the help of magnetite nanoparticles. Isolated nanoparticles of insects have diameters of about 12 and 11 nm in abdomen with petiole and head with antennae, respectively. It is noteworthy that magnetite particles were also observed in the stingless bee *Schwarziana quadripunctata* Lepeletier especially in the head, antennae, thorax and abdomen.

The magneto sensor is present in *Pachycondyla marginata* Roger, a migratory and termitephagous ant, hunting only the termite species *Neocapritermes opacus* Hagen. Also, ferromagnetic material has been detected in *Apis mellifera* Linnaeus abdomens and identified as

suitable for magnetic reception. Magnetic nanoparticles in the *A. mellifera* abdomens are well accepted as involved in their magnetoreception mechanism. The biogenic magnetic properties of the honeybee *A. mellifera* were investigated with a view to understanding the bee's physiological response to a magnetic field. The magnetizations of bee abdomens on one hand, and heads and thoraxes on the other hand, were measured separately as functions of temperature and field.

Both the antiferromagnetic responses of the ferrihydrite cores of the iron storage protein ferritin, and the ferrimagnetic responses of nanoscale magnetite (Fe₃O₄) particles, were observed, large magnetite particles (ca. 30 nm or more), capable of retaining remanence magnetization at room temperature were found in the abdomens, but were absent in the heads and thoraxes. Fire ant (*S. invicta*) workers, queens and alates were analyzed by magnetic resonance imaging (MRI) for the detection of natural magnetism. All ferromagnetic materials are magnetic nanoparticles, which are solely responsible for localization of specific direction for food and host of insects. Recently, cicada wings have been investigated by atomic force microscopy (AFM) used for observing nanoparticles. The structures consist of hexagonal close-packed protrusions with a lateral spacing of 200 nm and may have multiple functionalities.

Nanostructure components are also present in compound eyes of insects. Wings of butterflies possess bright colour components and these colour components are nothing but nanoparticles (Tai., 2008). Recently, a novel photodegradable insecticide involving nano particles has been prepared. Naturally, volatile phytochemicals and nanoparticles of insects are solely responsible for plant-insect interaction. Moreover, in the case of silkworm, electrospun silk fibroin-based fibers with average diameter of 700 nm were prepared from aqueous regenerated silkworm silk solutions (Zhang *et al.*, 2007). The electrospun nanocomposite of silkworm silk helps in producing single wall carbon nanotubes (SWNT) for drug delivery system (Scrinis and Lyons., 2007).

Nanoparticles: A Green Revolution in Future

The potential uses and benefits of nanotechnology are enormous. These include agricultural productivity enhancement involving nanoporous zeolites for slow release and efficient dosage of water and fertilizer, nanocapsules for herbicide delivery and vector and pest management and nanosensors for pest detection. Nanoparticles help to produce new pesticides, insecticides and insect repellants.

Nanoencapsulation is a process through which a chemical such as an insecticide is slowly but efficiently released to a particular host plant for insect pest control. Nanoencapsulation with nanoparticles in form of pesticides allows for proper absorption of the chemical into the plants unlike the case of larger particles. This process can also deliver DNA and other desired chemicals into plant tissues for protection of host plants against insect pests. Release mechanisms of nanoencapsulation include diffusion, dissolution, biodegradation and osmotic

pressure with specific. It has been observed that nanoparticles loaded with garlic essential oil is efficacious against *Tribolium castaneum* Herbst (Yang *et al.*, 2008). Also, it is known that aluminosilicate filled nanotube can stick to plant surfaces while nano ingredients of nanotube have the ability to stick to the surface hair of insect pests and ultimately enters the body and influences certain physiological functions have reported that potentized drugs significantly increased plant growth, chlorophyll, protein and water content in the leaves as compared to the control. CCC 30 (nano) was found to be more effective than CCC 30.

Potentized drugs are thought to initiate their action on the integral membrane proteins of leaves and modulate cell physiology towards growth. Ambitious uses of nanoparticles are bio-remediation of contaminated environments, biocides and antifungals on textiles. Surface-modified hydrophobic as well as lipophilic nanosilica could be effectively used as novel drugs for treatment of nuclear polyhedrosis virus (BmNPV), a scourge in the silkworm industry. Also, research on silkworm, *Bombyx mori* L. race Nistari clearly demonstrates that nano particle could stimulate more production of fibroin protein which can help in producing carbon nano tube in future. The above highlights the putative effects of nanoparticles on insects, as these small particles are present in their entire body parts. Research on nanoparticles and insect control should be geared toward introduction of faster and ecofriendly pesticides in future. It high time therefore that leading chemical companies to focus on formulation of nano scale pesticides for delivery into the target host tissue through nanoencapsulation.

At present, the toxicological and ecotoxicological risks linked to this expanding technology ("emerging technology") cannot be assessed yet. While nanotechnology is increasingly moving into the centre of public attention, it is currently not yet linked to any great degree to concerns about health and the environment. Nanoencapsulation is currently the most promising technology for protection of host plants against insect pests. Thus, nanotechnology will revolutionize agriculture including pest management in the near future. Over the next two decades, the Green Revolution would be accelerated by means of nanotechnology.

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A REVIEW OF PHYTOCHEMICAL EXTRACTION TECHNIQUES, PRINCIPLES, AND SKINCARE FORMULATIONS IN HERBAL COSMETICS

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Abstract

Herbal cosmetics have experienced a significant surge in demand due to consumer preference for natural, biodegradable, and safer alternatives to synthetic cosmetic products. The formulation of effective herbal Cosmeceuticals depends on selecting appropriate processing techniques to extract and preserve bioactive phytochemicals while maintaining cosmetic stability, safety, and sensory appeal. This review summarizes traditional and modern preparation methods—including powders, infusions, decoctions, ointments, balms, creams, oils, pastes, liniments, and ubtans—and analyzes their underlying principles, extraction efficiency, quality control metrics, and dermatological applications.

Keywords: Herbal Cosmetics, Cosmeceuticals, Bioactive Phytochemicals, Extraction Techniques, Quality Control, Dermatological Applications.

1. Introduction

Herbal cosmetics are topical preparations designed to cleanse, protect, nourish, and beautify human skin, hair, and oral cavities using plant-derived ingredients as active constituents. Modern cosmetic science integrates traditional phytotherapy with contemporary pharmaceutical technology to produce formulations that offer both cosmetic appeal and therapeutic efficacy.

The choice of preparation method depends on:

- The structural characteristics of the plant matrix.
- The solubility, volatility, and thermolability of active phytochemicals (e.g., flavonoids, tannins, essential oils, alkaloids).
- The intended delivery system and target tissue.

2. Fundamental Extraction and Preparation Techniques

2.1 Solid and Aqueous Preparation Methods

2.1.1 Herbal Powders

Herbal powders are finely divided solid dosage forms produced by drying and pulverizing plant materials such as leaves, roots, bark, seeds, or flowers. They serve as active ingredients,

adsorbents, exfoliating agents, or bases in face packs, body scrubs, tooth powders, and hair cleansers.

- **Preparation Steps:** Collection and authentication, cleaning, drying (shade drying for heat-sensitive constituents, sun/oven drying for hard structures), size reduction (pulverization), and sieving to ensure uniform particle size.
- **Applications:** Skin cleansing, oil absorption (e.g., Multani mitti), hair cleansing (e.g., Reetha, Amla), and oral care (e.g., Babool, Neem).

[Raw Plant Material] → [Cleaning & Drying] → [Pulverization] → [Sieving (Uniform Mesh)]
→ [Herbal Powder]

2.1.2 Herbal Infusions vs. Herbal Decoctions

Aqueous extractions are divided based on the hardness of the plant tissue and the thermolability of the target phytochemicals.

- **Infusions:** Prepared by steeping soft plant parts (leaves, flowers, tender stems) in hot or cold water without prolonged boiling. This process preserves volatile, aromatic, and heat-sensitive constituents.
- **Decoctions:** Involves gentle, prolonged boiling of hard, dense materials (roots, barks, seeds, rhizomes) in water. Heating breaks cellular walls to release water-soluble, heat-stable phytochemicals such as tannins, saponins, and glycosides.

Table 1: Comparison of Herbal Infusions and Herbal Decoctions Used in Cosmetic Formulations

Feature	Herbal Infusions	Herbal Decoctions
Target Plant Material	Soft parts (Leaves, Flowers, Soft Fruits)	Hard parts (Roots, Barks, Rhizomes, Seeds)
Extraction Process	Steeping in hot/cold water (10–20 min)	Extended boiling (volume reduction to 1/3 or 1/4)
Phytochemical Profile	Volatile, heat-sensitive compounds	Water-soluble, heat-stable compounds (tannins, saponins)
Stability	Low; requires fresh preparation or preservation	Relatively higher; requires refrigeration
Cosmetic Base Use	Toners, face mists, delicate rinses	Cleansers, anti-acne washes, mouthwashes

2.2 Semi-Solid and Anhydrous Systems

2.2.1 Herbal Ointments and Balms

Semi-solid topical forms provide prolonged skin contact, improving the absorption of lipophilic active compounds.

- **Herbal Ointments:** Semi-solid preparations containing active extracts, powders, or oils incorporated into oleaginous, absorption, or water-washable bases via fusion or incorporation methods. They provide protective barrier formation, deep moisturization, and wound-healing support.
- **Herbal Balms:** Concentrated anhydrous (water-free) formulations consisting of waxes (beeswax, candelilla wax), butter (shea, cocoa), and fixed/essential oils. Because they lack water, balms have long stability profiles and generally do not require synthetic preservatives. They are primarily used for lip care, heel repair, and local counter-irritant action.

2.2.2 Herbal Pastes and Ubtans

- **Herbal Pastes:** Prepared by triturating fresh or dried plant material with water, milk, rose water, or fixed oils to create a thick mass. Fresh pastes provide high initial phytochemical activity but have limited shelf stability.
- **Herbal Ubtan:** A traditional Ayurvedic formulation composed of flour bases (chickpea, rice), adsorbent clays, and herbal powders (turmeric, sandalwood, neem). Used as a natural cleanser and exfoliant, ubtan removes dead skin cells, controls excess oil, and brightens complexion.

2.3 Liquid Topical Formulations

2.3.1 Herbal Oils

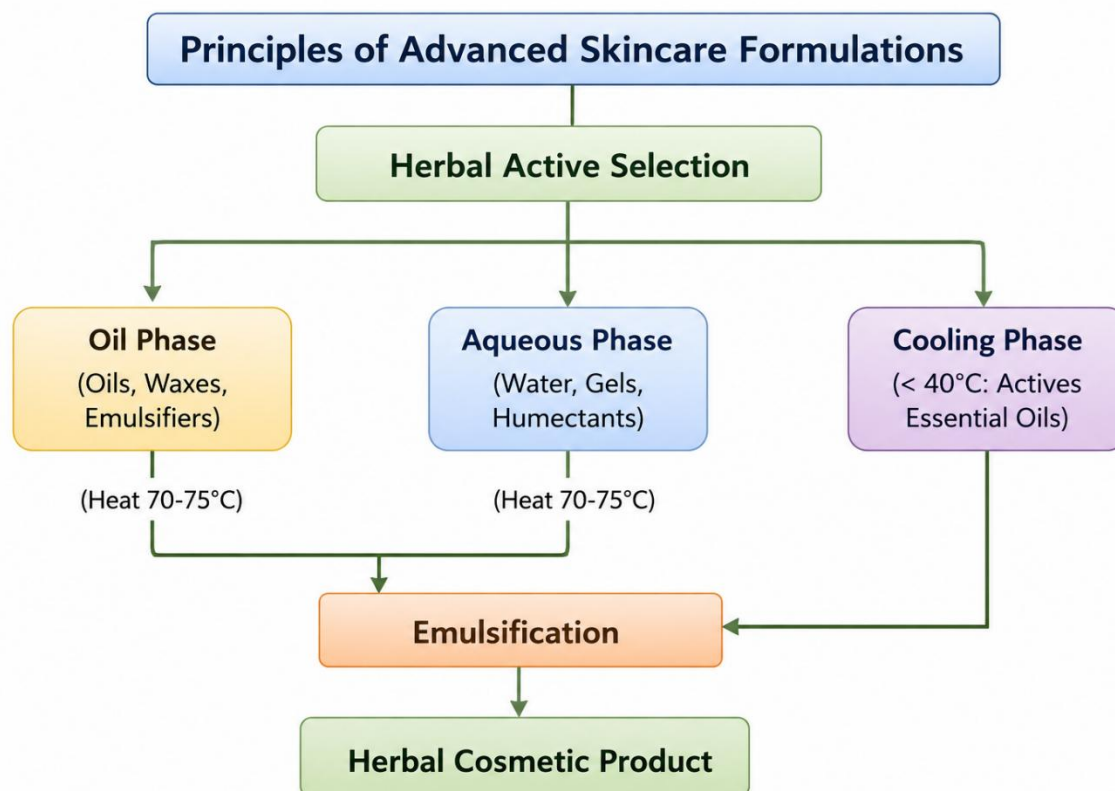
Herbal oils function both as active phytochemical delivery systems and as emollients. They are produced by extracting lipophilic active constituents into fixed carrier oils (e.g., sesame, coconut, almond, jojoba oil).

- **Extraction Methods:** Cold infusion (maceration for 2–4 weeks for heat-sensitive herbs), hot infusion (gentle heating at 50–60 °C), traditional decoction-evaporation (boiling aqueous decoctions with oil until all water evaporates), or essential oil blending.
- **Applications:** Hair growth stimulation, anti-dandruff therapy, barrier restoration, and facial massage.

2.3.2 Herbal Liniments

Liniments are liquid or semi-liquid external herbal preparations intended for application by rubbing onto the skin. They are commonly prepared using alcoholic, hydro-alcoholic, or oily bases containing volatile oils and resins such as camphor, menthol, and eucalyptus oil. These formulations promote rapid dermal absorption and provide localized therapeutic effects, including counter-irritant, warming, and cooling actions. Liniments are widely used to relieve muscular pain, joint stiffness, sprains, and minor aches, while also offering soothing aromatherapeutic benefits through their volatile herbal constituents.

3. Principles of Advanced Skincare Formulations



3.1 Emulsion Technology: Herbal Creams and Lotions

Emulsion-based formulations allow the simultaneous incorporation of both hydrophilic and lipophilic active ingredients.

3.1.1 Emulsion Types

- **Oil-in-Water (O/W):** Preferred for general cosmetic applications due to its non-greasy feel, rapid skin absorption, and wash ability.
- **Water-in-Oil (W/O):** Offers occlusive, deeply moisturizing, and protective properties suited for dry skin and barrier repair.

3.1.2 Formulations

- **Herbal Creams:** Semi-solid emulsions requiring balanced oil (15–20%) and aqueous phases (60–75%), stabilized by natural emulsifiers (beeswax, lecithin, acetyl alcohol).
- **Herbal Lotions:** Low-viscosity emulsions featuring higher water content (65–80%) and lower oil content (5–15%), allowing easy spreadability over larger body areas.

3.2 Model Formulation Profiles

Model 1: Oil-in-Water (O/W) Anti-Acne Herbal Cream

Total Formula Weight = 100gm

Phase A: Oil Phase (Heat to 70–75 °C)

- **Almond Oil-** Light emollient, lipophilic carrier.
- **Jobaba Oil** -Sebum-balancing lipid.

- **Beeswax** -Emulsifier and consistency regulator.
- **Cetyl Alcohol** - Viscosity builder.

Phase B: Aqueous Phase (Heat to 70–75 °C)

- **Purified Water** - Aqueous vehicle.
- **Aloe Vera Gel** - Soothing and hydrating agent.
- **Glycerin** - Humectant.

Phase C: Active/Cooling Phase (Add below 40 °C)

- **Neem Extract** - Antibacterial and anti-acne agent.
- **Turmeric Extract** - Anti-inflammatory agent.
- **Tea Tree Essential Oil** -Antimicrobial agent.
- **Vitamin E** - Lipophilic antioxidant.

Model 2: Herbal Cleansing and Brightening Face Pack (Powder Base)

Total Weight = 100 gm

Ingredients

- **Multani Mitti (Fuller's Earth)** - Oil adsorbent and deep cleanser.
- **Kaolin Clay** - Gentle skin adsorbent.
- **Sandalwood Powder** - Skin cooling and complexion enhancement.
- **Neem Leaf Powder** - Antimicrobial agent.
- **Orange Peel Powder** - Natural antioxidant and brightening agent.
- **Rose Petal Powder** - Skin toner.
- **Turmeric Powder** - Anti-inflammatory agent.
- **Aloe Vera Powder** - Hydrating agent.

4. Quality Control and Standardization

Ensuring safety, stability, and reproducibility in herbal cosmetics requires evaluation across several parameters:

Organoleptic Evaluation: Testing color, odor, texture, and visual appearance.

- **Physical Constants:** Checking pH compatibility with human skin pH 5.5 - 7.0, measuring spreadability, viscosity, and particle size distribution (passing through Sieve No. 80/100).
- **Chemical & Oxidation Stability:** Measuring acid value and peroxide value for oil-based preparations to monitor rancidity.
- **Microbial Load Testing:** Assessing total viable count, yeast, and mold levels to ensure formulations meet regulatory standards.
- **Dermal Safety:** Performing human patch testing to confirm non-irritating profiles.

5. Summary and Future Directions

Herbal cosmetics present a viable alternative to synthetic formulations by combining traditional botanical knowledge with modern delivery technologies. Key challenges include batch-to-batch variability in plant raw materials, susceptibility to microbial contamination in high-water formulations, and lipid oxidation in oil bases.

Addressing these challenges requires enhanced standardization of plant extracts, advanced green extraction methods, and natural preservative systems to extend shelf life while maintaining consumer safety.

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