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Hippophae salicifolia D. Don.: The "Badri Berry"

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The genus *Hippophae*, also referred to as sea buckthorn, is a rare, valuable, and miraculous plant found in temperate regions. It is well-known for its biological activity and abundance of nutritionally active ingredients, as well as its tremendous therapeutic potential. The natural habitat of sea buckthorn extends widely. *Hippophae* sp. found in the Ladakh region is famous as 'Leh berry,' which is *Hippophae rhamnoides, whereas* in the Himalayas of Uttarakhand the species found is *H. salicifolia*. Ancient Indian literature depicts the forest of this berry in the Uttarakhand Himalayas, especially in the region of Shri Badarinath Dham, but the present scenario is unaware of this.

Hippophae sp. Habit and Habitat Aspect:

Sea buckthorn (*Hippophae* sp. Family: Elaeagnaceae) is a thorny shrub with an actinorhizal habit that is thought to have originated in the Himalayas. It is a very widely distributed genus that has been reported to grow in cold regions of about 30 countries. It typically grows in cold desert areas of Asia and Europe with little rainfall, and it has also been introduced to North and South America. Eighty percent of the world's sea buckthorn resources are reportedly in China. However, rich *Hippophae* sp. deposits have also been discovered in Russia, Mongolia, and the Ladakh region of the Indian Himalayas[1]. The habit of giving sea buckthorn to horses in ancient Greece to give them glossy coats is the source of its generic name, *Hippophae* (Greek: hippos—horse; pharos—shiny)[2,3].

Hippophae are divided into two divisions: coat and coatless groupings. The three species in the coat group—*H. neurocarpa*, *H. tibetana*, and *H. gyantsensis*—have significant frigid brush formation, which is the binding of the fruit rind and seed coat. High altitude areas (3000 to 5300 meters above sea level) are frequently home to coat group species that can withstand the harsh sub-frigid climate of the tree line. These species exhibit complete domination in the region, even in alpine and subalpine zones. The two species in the coatless group are *H. salicifolia* D. Don and *H. rhamnoides* L. Fruit rind and seed coat do not adhere to one another in these species. They are typically found in lower regions, are categorized as temperate brush formations, and are more effective against established trees. They are frequently found in arid and impoverished locations, such as hill ridges or tops, valley terraces, and damp sandy areas like slopes and riversides[4]. They are distributed between 1500 and 3800 meters above sea level, spanning the subtropical to temperate zones[5].

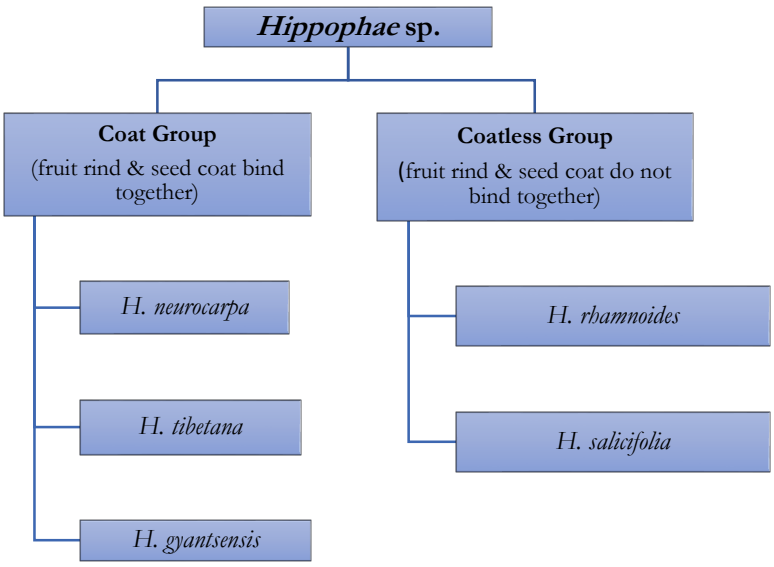

Figure 1: Coat and Coatless groups of the genus *Hippophae*

The three most common species of sea buckthorn in India are *Hippophae rhamnoides*, *H. salicifolia*, and *H. tibetana*. In India, *H. rhamnoides* is found in six valleys: Leh, Nubra, Changthang, Suru, Lahaul, and Spiti. Additionally, it is in a few areas in Nathula, Sikkim, which has been mapped using GIS and remote sensing[6,7]. *H. rhamnoides* popularly known "Leh-berry" is *rhamnoides* species of genera *Hippophae*.

The Himalayan regions of Uttarakhand, Himachal Pradesh, Jammu and Kashmir, and the northeastern regions of India are home to *Hippophae* sp. According to research, *Hippophae salicifolia* D. Don has been found in several regions of Uttarakhand state, including Harsil, Tambara-Kali, Yamunotri valley, Gangotri, Gaurikund, Hanumanchatti, Badrinath valley, Bogdiar, Gori valley, Niti valley, Budhi, Byanse, and Darma vallies[8]. Sea buckthorn is distinguished by its ability to withstand cold, drought, saline-alkaline soils, and strong winds. *Hippophae* sp. has a variety of colloquial names in several languages and regions of the world.[9]

Vernacular Names of *Hippophae* sp.

Hippophae sp. is known by a variety of colloquial names in many parts of the world. *Hippophae* sp. is generally referred to as Sea buckthorn, Sallow-thorn, Sea Berry, Sandthorn, Siberian Pineapple, Seaberry, and Himalaya Berry in various English-speaking regions. It is called Argousier or Argoussier in French; Sanddorn (Stech-dorn) in German; Duindown in Dutch; Olivella spinosa and ventrie marina in Italian; Oblepikha in Russian; and Havtorn in Swedish.

Hippophae sp. is referred to by different local names in different geographical areas. For example, it is called Sastalulu, Sirna, Tasru, Tsarana, and Tsarap in the Ladakh, Lahaul, and Piti area; Chuk, Chuma, Dhurchuk, and Tarwa in the North-Western region of India; Suak in Pangi; and Dhar-bu (Star-bu) in Tibet. In the Ladakh area of Jammu and Kashmir, sea buckthorn is commonly referred to locally as "Tsermang," while the fruits are known as "Tsestalullu." The moniker "Leh berry" has made *Hippophae* sp. in Leh famous.

In Punjabi, sea buckthorn is referred to as Amb, Bautphut, Kalabisa, Kando, Milech, Miles, Rul, Sirma, Suts, Starbu, Tarru, Tsarap, Tsarnang, Tsarmaniechak, Tserkar, and Tswak. It is referred to as Brahmaphal in Hindi. In Ladakhi, one of the colloquial names for sea buckthorn is Sastalulu.

Sea buckthorn is commonly known as Chuk, Ames, and Ameel in Uttarakhand and as Chharma in Himachal. Sea buckthorn is referred to as Tarwa by some local communities, including Bhotia. Sea buckthorn has many colloquial names that reflect its extensive use in Himalayan medicine and cooking[10].

Mythological Aspect

Badrinath Dham is situated in the district Chamoli of Uttrakhand, India. Hindu mythology has a strong connection to Badrinath. The tale of Lord Vishnu's penance is among the most important stories connected to this location. It is thought that Lord Vishnu chose to undertake penance in the hard Himalayan climate after a sage criticized his extravagant lifestyle. He selected this isolated, chilly area, where he reclined in the lotus pose (Padmasana) and spent a considerable amount of time in deep meditation. Vishnu was unaware of the cold when he was in profound meditation. Goddess

Mahalakshmi, his consort, chose to defend him after observing his plight while he was undertaking penance in the difficult circumstances and bad weather. Goddess Lakshmi changed into a Badri tree, a kind of berry tree, to protect her husband while he was meditating.

Seeing Goddess Lakshmi's love and devotion, Vishnu named the place 'Badrikashram', and this is why people worship Lord Narayana as the Lord of Badri – 'Badrinath'. The location was named Badrinath, which translates to "Lord of Badri," as a result of the goddess's deed of devotion. This tale emphasizes the importance of this location in Hindu beliefs and is a lovely depiction of the divine couple's love and devotion for one another.

The Sanskrit word 'Badri' refers to a berry. The Badrinath region was also previously known as 'Badarikavan', meaning the forest of berries. This region was once abundant with wild berries. Even today, small trees/shrubs of the sea buckthorn berry are found in abundance in the Badrinath region. This proves that this sea buckthorn tree is the one "Badri Ber" mentioned in our Puranas, and its story has been recognized in Hindu beliefs for thousands of years.

The *Skanda Purana*, in verse 59 of the *Vaishnava khand*, mentions the Badrinath forest, which is as follows:

अमृतं स्रवते या हि, बदरीतरुयोगतः।।
बदरी कथ्यते प्राज्ञैः, ऋषीणां यत्र संचयः।।59।।

(अर्थ- इस बदरीनारायण आश्रम में बदरीवृक्ष के संग से अमृत बिंदुओं का स्रवण (झरना, वर्षण) होता है। अतः बुद्धिमान इसे बदरीवन कहते हैं यहां पर ऋषियों का समूह रहता है।)

English Translation: In this Badrinarayan ashram, drops of ambrosia flow (drip, shower) from the Badri tree. Therefore, the wise call it Badrivan (Badri forest). A group of sages resides here.

Similarly, Srimad Bhagavatam, First Canto, Chapter 7, Verse 3 mentions the Badri forest, as follows:

तस्मिन् स्व आश्रमे व्यासो बदरीषण्डमण्डिते।
आसीनोऽप उपस्पृश्य प्रणिदध्यौ मनः स्वयम्।।3।।

अर्थ- वहीं व्यास जी का अपना आश्रम है। उसके चारों ओर बेर का सुंदर वन है। उस आश्रम में बैठकर उन्होंने आचमन किया और स्वयं अपने मन को समाहित किया।

English Translation: Vyasa's own hermitage is located there. A beautiful forest of berry trees surrounds it. He sat in that hermitage, performed the ritual of purification, and composed his mind.

The above ancient literature confirms that Badri berry is clearly mentioned in texts that are thousands of years old.

Traditional, Medicinal, Therapeutic and Nutritional Aspects

Sea buckthorn fruits have long been used in Asia and Europe as a source of natural skin care products, healthy meals, and herbal medicines that significantly improve the quality of life for locals. Sea buckthorn's medicinal properties are mentioned in both *Diskorid*'s Classic Tibetan medical publications, such as "*The RGyud Bzi*" (The Four Books of Pharmacopoeia), and Theophrastus's ancient Greek literature. Sea buckthorn has around 190 known and 60 unknown bioactive compounds[11].

It has been referred to as a "wonderful plant," "magic plant," "super food," "functional food," and "bank of vitamins" due to its extensive use. Due to the high nutritional value of its pulp/juice and oil from its berry fruit, sea buckthorn, which is well-known for its medicinal properties, is becoming increasingly popular. Sea buckthorn berries have anti-inflammatory, antihyperlipidemic, antibacterial, anti-obesity, dermatological, pain relief, stimulation of tissue regeneration, immune system activation, neuroprotective, hepatoprotective effects, and protection against cardiovascular disease and cancer. Sea buckthorn is used in several therapeutic compositions. Sea buckthorn is also a rich source of phytochemicals and antioxidants, such as lipids, carotenoids, ascorbic acid, tocopherols, flavonoids, and stress-relieving vitamins (A, B, C, K, and E). Additionally, sea buckthorn's fatty acids, phytosterols, organic acids, amino acids, and minerals are crucial[12].

In addition, sea buckthorn's flavour and nutritional benefits make it a sought-after ingredient for food, medicine, and cosmetics. Sea buckthorn fruits are employed in food industry goods because of its high oil content, high vitamin C content, and abundance of polyunsaturated fatty acids n-3, n-6, and n-9[12]. In urban areas, Leh-berry juice is becoming increasingly popular. Sea buckthorn active principles are being used by more than 150 pharmaceutical and nutraceutical businesses worldwide to manufacture medications and therapeutic nutraceuticals, with China being the top producer.

Every portion (roots, bark, stem, leaves, berries and seeds) of the *Hippophae* (sea buckthorn) plant has a traditional, medicinal and ecological usage. It is

frequently referred to as the "wonder plant" because of its many advantages and adaptability. Different plant parts of *Hippophae* sp. are used for various purposes as shown in table 1.

Table 1: Different plant parts of *Hippophae* sp. and their traditional and professional uses

Fruit (Juice, pulp and seed)	**Leaves**	**Wood**	**Roots**
•Food items and additions • Health foods and juices •Concentrates Liquor and vinegar; jam and jelly; chocolate and ice cream; tea	• Green Tea • Fodder	• Agricultural Implements • Firewood • Charcoal • Timber	• Nitrogen Fixation • Soil Binding
Oil, flavonoids and other bioactive substances from fruit and leaves			
• Medicines • Cosmetics			

The "Badri Berry":

The sea buckthorn the famous Leh berry from the Leh region is *Hippophae rhamnoides*, while the sea buckthorn berry found in the Shri Badrinath Dham region of Uttarakhand is *Hippophae salicifolia*. Based on literature mentioned the berry found in the region of Badarinath Dham, *i.e.*, *Hippophae salicifolia* species should be known as the "Badri Berry" because it has been mentioned in Hindu scriptures and Puranas for thousands of years.

In the Puranas, the Badrinath berry is described as a fruit of immortality. Recent research has shown that it possesses significant medicinal and therapeutic properties. Scientists have now confirmed what was written in the Puranas, further validating the authenticity of the Badrinath berry. i.e. the "Badri Berry".

a **b** **c**

Figure 2. *Hippophae salicifolia* D. Don. plant bearing fruits (a) at initial stage, (b) later stage (c) dried 'Badri Berry'

Data Availability

All data produced or examined in this study are contained within this published article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgements

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OBITUARY

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In Memoriam: Dr. Palpu Pushpangadan (1944–2025)

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On December 19, 2025, Dr. Palpu Pushpangadan, a renowned ethnobotanist, forward-thinking scientific leader, and esteemed member of the PhytoTalks Advisory Board, passed away. With great sadness and reverence for a life dedicated to science, society, and the preservation of traditional knowledge, the Editorial Board documents his passing. For his groundbreaking work in ethnobotany, ethnopharmacology, medicinal and aromatic plants, and biodiversity conservation, Dr. Pushpangadan received recognition on a global scale. He was instrumental in establishing a connection between indigenous knowledge systems and contemporary science, which strengthened the ethical application, scientific validation, and preservation of traditional medical practices[1].

Dr. Pushpangadan held a number of important leadership roles throughout his illustrious career, including Director of the Tropical Botanic Garden & Research Institute (TBGRI), Kerala, and subsequently Director of the CSIR–National Botanical Research Institute (NBRI), Lucknow. These institutions saw significant developments in interdisciplinary research, national capacity building, and international cooperation under his direction. In addition, he held the positions of Director of RGCB in Thiruvananthapuram, Director of CIMAP in Lucknow, and Director General of the Amity Institute for Herbal & Biotech Products Development.

The creation of the Jeevani herbal remedy, which is derived from Trichopus zeylanicus, and the internationally recognized benefit-sharing model, which guaranteed indigenous communities proper recognition and rewards, are two of his most enduring legacies[2]. Many people consider this work to be a turning point in the governance of biodiversity and ethical bioprospecting. Numerous national and international awards, such as the Padma Shri (2010), UN-Equator Initiative Prize, UNEP Borlaug Award, and John W. Harshberger Medal, were given to Dr. Pushpangadan. In addition to serving as President of national and international ethnopharmacological societies, he was a Fellow of several esteemed academies. His lifetime of exceptional scientific productivity was reflected in his more than 500 research publications, more than 20 books, and more than 200 patents.

Dr. Pushpangadan provided invaluable advice, intellectual support, and encouragement as a member of PhytoTalks' advisory board, especially in the early years of the publication. His involvement significantly enhanced the journal's academic culture and dedication to moral, superior research.

The Editorial Board sends its deepest sympathies to his loved ones, coworkers, students, and admirers worldwide. Future generations of scientists and decision-makers committed to using science to advance society will be motivated by Dr. Palpu Pushpangadan's legacy.

Biographical Snapshot

Dr. Palpu Pushpangadan
(23 January 1944 – 19 December 2025)
Fields: Ethnobotany, Ethnopharmacology, Medicinal & Aromatic Plants
Former Director: TBGRI, Kerala; CSIR–NBRI, Lucknow
Honours: Padma Shri; UN-Equator Initiative Prize; UNEP Borlaug Award
Publications: 500+ papers; 20+ books
Patents: 200+
Association: Advisory Board Member, PhytoTalks

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The Cultural and Healing Worth of Pteridophytes: An Ethnobotanical Impression

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Abstract

Pteridophytes, one of the ancient plant groups, have been utilized in the ethnic and therapeutic practices of many cultures. They comprise lycophytes and ferns. These primitive "vascular cryptogams" are much more than decorative plants from the evolutionary past; pteridophytes have been used multifariously in customary medications as a valuable constituent of ethnobotany. Apart from therapeutic uses, these plants are used as sacrament plants, foods, homely items, and culturally distinct symbols all through Asia, Africa, Europe, and America. For centuries, species like *Adiantum capillus-veneris*, *Drynaria quercifolia*, *Pteris multifida*, and *Selaginella bryopteris* have been employed in conventional medical systems like Unani, Ayurveda, and Traditional Chinese Medicine. Currently, many indigenous peoples still use locally grown species of pteridophytes for daily nutrition and useful remedies. Regardless of their widespread cultural presence, these plants are still less studied in contemporary materia medica. However, an array of bioactive chemicals with antimicrobial, antioxidant, anti-inflammatory, and anticancer potentials have recently been found. This review reveals the perpetual implication of pteridophytes and gives importance to the need to sustain traditional knowledge, lift the sustainable use, and explore their potential in upcoming drug invention by amalgamating global ethnobotanical acquaintance with modern scientific understandings.

Keywords: Pteridophytes, Ethnobotany, Traditional Medicine, Indigenous Knowledge, Cultural Uses, Biodiversity Conservation

1. Introduction

J.W. Harshberger established the term "ethnobotany" in 1895 to classify the study of the intricate relationships that occur amongst plants and humans, particularly in ecological, cultural, and social circumstances. Ethnobotany, which was primarily demarcated as the study of plants used by native and ethnic societies, has developed into a multidisciplinary discipline that cartels traditional acquaintance with modern methods of resource management, biodiversity safety, and viable development. Exploring the ecological, figurative, ceremonial, and socio-economic characteristics of human-plant interactions goes beyond merely registering plant uses[1-3].

With fossil records going back to the Silurian period, i.e., more than 400 million years ago, these primitive vascular cryptogams represent some of the most primitive land-dwelling flora on earth. They display a staggering diversity of morphological and physiological variations that allow them to establish in terrestrial, epiphytic, xeric, and aquatic environments with about 10,500 species reported worldwide. Their placement amid bryophytes and spermatophytes, marks a pivotal alteration toward the progress of vascular tissues, dominant sporophytic generations in their life cycle, and, in certain groups (Lycophytes), the rise of heterospory—an initiation to the progression of seeds that makes them evolutionary very significant[4-6].

Since pteridophytes tend to grow almost all over the world, several ethnic groups in Asia, Africa, Europe, and America have been able to incorporate these plants deeply into their customs. Their ethnobotanical impact holds ritualistic practices, food uses, ecological functions, and medicinal uses. For more than two millennia, pteridophytes have been used as therapeutic plants in various ethnic medicinal systems. The medicinal capacities of species like *Adiantum capillus-veneris*, *Marsilea minuta*, and *Selaginella bryopteris* are described in ancient Indian texts like 'Charaka Samhita' and 'Sushruta Samhita'. Akin to this, ferns like *Lygodium japonicum*, *Selaginella tamariscina* and *Pteris multifida* are valued for their hemostatic, decontaminating, and anti-inflammatory qualities in Unani Ayurveda, and Traditional Chinese Medicine. For instance, in Unani medicine system, *Adiantum capillus-veneris* is used to treat respiratory and urinary ill conditions, which reflects its worth in various cultural healing philosophies[7].

Pteridophytes have been assimilated into daily life, food systems, and rituals by aboriginal and ancestral populations around the ecosphere, who have established rich ethnomedicinal traditions including them. In South Asia and Southeast Asia, species like *Diplazium esculentum* are used as nutraceuticals and medicines, and epiphytic ferns species like *Drynaria quercifolia* and *Nephrolepis cordifolia* are used in treatments for skeletal related problems, respiratory diseases, and reproductive conditions[8]. Ferns also have several cultural uses besides their medicinal uses: in some ethnic societies, they are used as body beautifications, ornamentals, protecting talismans, and bioresources for insulation. Since a large percentage of this traditional knowledge is passed down vocally, it is inclined to decline in the face of modernization and altering cultural customs[9].

Compared to angiosperms, pteridophytes have been used for a long time, but their incorporation into contemporary pharmacology is still quite limited. However, current studies are highlighting their bioactive potential more and more, finding substances with anti-inflammatory, diuretic, anticancer, antioxidant, and antimicrobial qualities. This increasing interest highlights the necessity of methodically recording and assessing the traditional knowledge about pteridophytes in order to promote conservation and support their potential role in future drug discovery[10].

This review summarizes ethnobotanical acquaintance from around the world regarding the cultural, medicinal, and ecological value of vascular cryptogams. It explores their phytochemical and pharmacological potential, highlights their traditional uses in various societies, and emphasizes the value of conserving ethnobotanical knowledge systems for long-term use and further scientific research.

2. Literature Search Strategy

A comprehensive literature survey was conducted to compile ethnobotanical and medicinal information on pteridophytes. Relevant peer-reviewed research articles, review papers, ethnofloristic surveys, books, theses, and reports were retrieved from major scientific databases, including Scopus, Web of Science, PubMed, Google Scholar, JSTOR, and ScienceDirect. Additional information was sourced from classical ethnobotanical texts and regional floras.

3. The Review's Objectives

The accumulated knowledge of indigenous and local communities regarding the use of plants for food, medicine, and cultural customs is known as ethnobotanical knowledge. Because of migration, habitat loss, modernization, and the deterioration of oral traditions, this knowledge is rapidly disappearing. In order to prevent irreversible cultural and biological loss and to promote the sustainable use of plant resources, many studies conducted over the past ten years have stressed the importance of recording traditional practices.

Despite being frequently disregarded in ethnobotanical literature, pteridophytes have significant ecological, cultural, and medicinal value. Their various bioactive compounds have wound-healing, antimicrobial, anti-inflammatory,

and antioxidant properties. Despite this potential, their ethnobotanical applications are still less common than those of angiosperms, highlighting the necessity of a thorough synthesis of the available data to comprehend their function in indigenous healthcare systems[11]. Therefore, this review addresses the need to: (i) maintain dwindling traditional knowledge of pteridophytes; (ii) emphasize its ethnopharmacological potential; and (iii) support conservation and sustainable utilization measures.

3.1 The Value of Ethnobotanical Research in Maintaining Conventional Knowledge

The importance of ethnobotanical research in preserving traditional knowledge before it is lost is highlighted by recent studies. Pteridophytes continue to be an essential part of primary healthcare in many tribal and rural areas for the treatment of wounds, fevers, respiratory conditions, digestive issues, and other common illnesses. In addition to providing crucial baseline data on species use, preparation techniques, and regional healthcare systems, documentation of such practices protects cultural traditions.

The pharmacological potential of pteridophytes is also revealed by ethnobotanical records. Numerous fern species that have long been used in traditional medicine have been found to have scientifically proven antioxidant, antimicrobial, anti-inflammatory, and wound-healing qualities. These documents direct bioprospecting activities and provide information for later phytochemical and pharmacological studies[12].

Additionally, by emphasizing culturally significant or heavily harvested species, ethnobotanical documentation aids in conservation planning. Sustainable-use strategies are strengthened and participatory approaches are encouraged when formal conservation frameworks are integrated with local knowledge. In general, ethnobotanical research provides a basis for protecting cultural heritage, directing the development of new drugs, and encouraging the preservation of biodiversity[13].

3.2 Worldwide Distribution

There are about 11,500 species of ferns worldwide, with tropical and subtropical mountainous areas having the highest diversity. Despite making up only around 7% of the planet's land area, eight equatorial and semitropical mountain biodiversity hotspots are home to nearly 58% of all fern species. Central America, the Caribbean, the Andean tropics, eastern Brazil, northeastern South America, Madagascar, Malesia, and East Asia are notable areas of richness. [14–17]. Climate and elevation gradients are closely correlated with fern diversity. The highest species richness is found in humid, topographically complex areas with high temperature and precipitation variability. The ecological significance of montane habitats, which make up less than 2% of the world's land area, is highlighted by the fact that nearly half of all fern species are found there[14].

3.3 Distribution by Region (India)

More than 1,000 species of pteridophytes from 67 families and 191 genera can be found in India. The main hubs of fern diversity are found in the Eastern, Western, and Himalayan regions[18]. A rich pteridophytic flora, including several rare, threatened, and endemic taxa, is supported by the nation's diverse climates, altitudes, and soil types. The Western Ghats, northeastern India, and the Himalayan belt are especially important for endemism and species richness.

3.4 Literature Review

Pteridophytes are special vascular cryptogams with distinctive foliage morphology and separate sporophytic and gametophytic generations. Approximately 1,300 species have been identified in India across major phytogeographical zones like the Himalayas, Western and Eastern Ghats, Central India, and the Andaman & Nicobar Islands, out of the approximately 13,600 extant species found worldwide[19].

As early as Theophrastus and Dioscorides' writings, the medicinal applications of pteridophytes were acknowledged. Ayurvedic, Unani, Siddha, homeopathic, and tribal medical

systems all make extensive use of them in India. Indian pteridophyte taxonomy is still based on Beddome's groundbreaking "Handbook of the Ferns of British India, Ceylon, and the Malay Peninsula" (1883).

Ferns are prized for their aesthetic qualities and are frequently used as ornamentals and horticultural plants in addition to their therapeutic uses. They are increasingly being used in landscaping and nurseries.

Pteridophytes are used as vegetables, fodder, fibers, oils, natural dyes, and fragrances, among other cultural, medicinal, and commercial uses, according to international ethnobotanical research. Their pharmacological potential is highlighted by recent research: for instance, *Drynaria fortunei* exhibits bone-strengthening properties[20], while *Diplazium esculentum* demonstrates anti-inflammatory and chemopreventive activities[21]. As interest in plant-based and alternative medicine grows, pteridophytes are emerging as promising subjects for contemporary ethnopharmacological research[22].

4. Representative Ethnobotanically Important Pteridophytes

1. ***Adiantum capillus-veneris*** **L. (Maidenhair fern)**
   - **Habit/Habitat:** Small lithophytic fern; grows on limestone rocks, cliffs, and moist ravines.
   - **Key Uses:** Paste of leaves/rhizome used for wounds, hair tonic, bronchial ailments, fever, and menstrual issues. Decoction used for childbirth support and gynecological disorders. Fresh juice used for cough and diabetes.
   - **Ethnopharmacology:** Shows bronchodilatory, anti-inflammatory, anxiolytic, and antidepressant activity.
2. ***Diplazium esculentum*** **(Retz.) Sw. (Vegetable fern)**
   - **Habit/Habitat:** Large terrestrial fern common along streams and moist slopes.
   - **Key Uses:** Popular edible fern used as seasonal vegetable; also applied for constipation, wounds, rheumatism, skin issues, fever, and pregnancy-related care.
   - **Ethnopharmacology:** Rich in polyphenols with anti-inflammatory and anticancer potential.
3. ***Selaginella bryopteris*** **(L.) Baker (Sanjeevani Booti)**
   - **Habit/Habitat:** Lithophyte of arid, rocky habitats; renowned for desiccation tolerance.
   - **Key Uses:** Cooling agent for heat stroke, remedy for diarrhea and urinary issues; decoctions used for menstrual problems and postpartum care. Culturally revered as a "life-restoring herb."
   - **Ethnopharmacology:** Exhibits antioxidant, antibacterial, and wound-healing activities.
4. ***Pteris vittata*** **L. (Chinese brake fern)**
   - **Habit/Habitat:** Lithophytic fern on limestone walls, stream margins, and disturbed habitats.
   - **Key Uses:** Decoction for dysentery, glandular swellings, fever, urinary troubles; fronds used ceremonially and as cattle-shed cushions.
   - **Ethnopharmacology:** Contains rutin and other flavonoids with antibacterial, antifungal, and anti-inflammatory effects.
5. ***Osmunda regalis*** **L. (Royal fern)**
   - **Habit/Habitat:** Moist slopes near waterfalls, swamps, and humid soils.
   - **Key Uses:** Rhizome decoction used as abortifacient and for menstrual disorders; leaf paste for birth control; also used for rheumatism, wounds, and renal issues.
   - **Ethnopharmacology:** Traditionally used for musculoskeletal disorders and internal inflammations.
6. ***Lygodium flexuosum*** **(L.) Sw. (Climbing fern)**

- **Habit/Habitat:** Moist forest edges, hedges, and stream banks.
- **Key Uses:** Treats menorrhagia, dysmenorrhea, infertility, arthritis, jaundice, skin infections, and leprosy. Widely used in rituals (e.g., Nira festival, Kerala).
- **Ethnopharmacology:** Known for anti-inflammatory, antimicrobial, and wound-healing effects.

7. ***Equisetum ramosissimum*** **Desf. (Horsetail)**
   - **Habit/Habitat:** Moist, sandy-alluvial soils; often in open to semi-shaded areas.
   - **Key Uses:** Used for fertility, kidney stones, skin issues, fractures, urinary troubles, hypertension, and digestive ailments.
   - **Ethnopharmacology:** Contains apigenin, kaempferol, quercetin, terpenes, and sterols with strong antioxidant and diuretic properties.
8. ***Marsilea quadrifolia*** **L. (Water Clover)**
   - **Habit/Habitat:** Ponds, paddy fields, and shallow wetlands.
   - **Key Uses:** Paste for cold, cough, skin issues; used for fever, dysentery, urinary disorders, neurological problems, and as famine food.
   - **Ethnopharmacology:** Rich in flavonoids and novel polyphenols; shows antioxidant and neuroprotective potential.
9. ***Cyathea gigantea*** **(Wall. ex Hook) (Tree fern)**
   - **Habit/Habitat:** Moist valleys, evergreen forests, stream sides.
   - **Key Uses:** Caudex paste used for wounds, pain relief, diabetes management, gynecological issues, and indigestion. Pith (tashe) consumed as supplementary famine food.
   - **Ethnopharmacology:** Contains saponins, triterpenes, sterols, and oleanolic acid with antimicrobial, anti-inflammatory, and antidiarrheal effects.
10. ***Nephrolepis cordifolia*** **(L.) Presl (Ladder fern)**
    - **Habit/Habitat:** Moist areas near water; roadside fern with creeping rhizomes and tubers.
    - **Key Uses:** Tubers used for indigestion, ulcers, fever, jaundice, dehydration, and wounds. Rhizome extract used in traditional female sterilization practices.
    - **Ethnopharmacology:** Exhibits antihypertensive, antidiabetic, antimicrobial, and hepatoprotective properties.
11. ***Cheilanthes farinosa*** **(Forssk.) Kaulf. (Silver fern)**
    - **Habit/Habitat:** Dry, rocky crevices; lime-rich soils.
    - **Key Uses:** Extract used for menstrual issues, fever, ulcers, eczema, stomach pain, wounds, and veterinary infections. Stipes used for crafting ear/nose ornaments.
    - **Ethnopharmacology:** Fresh fronds show antibacterial activity; root paste effective for skin inflammations.
12. ***Drynaria quercifolia*** **(L.) J.Sm. (Oak-leaf fern)**
    - **Habit/Habitat:** Epiphytic fern of humid tropical forests.
    - **Key Uses:** Rhizome paste for heart problems; fronds used for typhoid, inflammation, migraine, and gastric issues; acts as expectorant and antiseptic.
    - **Ethnopharmacology:** Noted for antibacterial and anti-inflammatory effects.

5. Pteridophytes' Secondary Metabolites:

Secondary metabolites are organic compounds that perform essential ecological tasks but do not

directly aid plant growth or reproduction. In pteridophytes, they aid in defense, allelopathy, UV protection, and environmental adaptation. These compounds enhance stress tolerance, mediate interactions between microbes and plants, and significantly boost the medicinal value of ferns. They help plants adapt to biotic and abiotic stresses, such as herbivory, pathogens, intense light, temperature fluctuations, salt, and drought, according to Bennett and Wallsgrove[23].

5.1 Principal Ecological Roles

Ferns and related plants protect themselves through a combination of chemical and physiological strategies. Phytoecdysteroids help defend against herbivorous insects by disrupting their growth and development, while tannins reduce palatability and discourage feeding. Some ferns also exhibit allelopathy, releasing bioactive compounds into the surrounding soil that suppress the growth of neighboring plants and reduce competition. In addition, harmful UV-B radiation is absorbed by epidermal flavonoids, which act as natural sunscreens and protect plant tissues from ultraviolet damage.

5.2 Saccharides

Cyanogenic glycosides release hydrogen cyanide upon tissue damage, deterring herbivores by inhibiting cytochrome c oxidase[24]. They are mainly reported in *Pteridium* species and *Microgramma vacciniifolia*. Prunasin is the most common cyanogenic glycoside in ferns[25].

5.3 Terpenoids

Terpenoids, which are biosynthesized from isoprene units, represent a diverse class of secondary metabolites in ferns and include mono-, sesqui-, di- and triterpenes, as well as carotenoids. Among them, phytoecdysteroids act as analogues of insect moulting hormones and disrupt normal insect development, contributing to plant defense[26]. Saponins, reported in *Adiantum* species, impart a bitter taste that deters herbivory[27]. Carotenoids such as β-carotene, common in members of Dryopteridaceae, play an essential role in light harvesting and protect photosynthetic tissues from photo-oxidative damage[28]. Sesquiterpenes, particularly pterosins found in *Pteris* species, are of notable pharmacological interest due to their reported antitumor, cytotoxic, antidiabetic, and anti-obesity activities[29], and they also participate in microbial interactions while providing antioxidant, anti-inflammatory, and neuroprotective benefits[30].

5.4 Alkaloids

Although alkaloids are relatively rare in true ferns, they are abundant in fern allies, particularly members of the Lycopodiaceae. Notable examples include huperzine A from *Huperzia serrata*, a potent acetylcholinesterase inhibitor that has been widely investigated for the treatment of Alzheimer's disease[31]. Related compounds such as huperzines A, B, and R exhibit strong neuroprotective properties[32], while lycodine derivatives are known for their antimicrobial activity[33]. In addition, hordenine, an alkaloid found in some lycophytes, mimics the action of dopamine and epinephrine and influences cardiovascular as well as metabolic responses[34].

5.5 Phenolic Compounds

Phenolic compounds are the most widespread secondary metabolites in ferns, where they function as powerful antioxidants, UV protectants, and antimicrobial agents[35]. Among these, caffeic and chlorogenic acids are known for their anti-inflammatory and cardioprotective effects, while tannic and gallic acids—classified as hydrolysable tannins—exhibit strong antioxidant, astringent, and wound-healing properties[34]. The high abundance of phenolics also helps explain why many phenolic-rich ferns successfully thrive in high-altitude environments, as these compounds enhance tolerance to intense ultraviolet radiation[36].

5.6 Flavonoids

Flavonoids, a diverse group of ~4000 polyphenols, show strong antioxidant, anti-inflammatory, antibacterial, and anticancer activities[37]. *Dryopteris* species possess flavonoids with cytotoxic effects on ovarian, liver, breast, and lung cancer cell lines[11, 38].

6. Pteridophytes' Biological Activities

To adapt to terrestrial stressors like UV radiation, pathogens, and herbivores, pteridophytes developed a variety of biochemical pathways. They produce a variety of bioactive compounds, such as flavonoids, steroids, phenolics, alkaloids, and terpenoids[39], thanks to their lengthy evolutionary history. Antioxidant, antibacterial, anti-diabetic, antitumor, antifungal, antiseptic, and anti-inflammatory qualities are provided by these substances[40]. Calaguline[41] and Huperzine A/B are two notable examples.

6.1 Antioxidant Characteristics

Many ferns have strong radical-scavenging activity due to their high polyphenol content[42]. These antioxidants reduce oxidative stress, which is associated with aging, cancer, atherosclerosis, and cardiovascular diseases. Significant antioxidant activity is exhibited by species such as *Drynaria*, *Davallia*, *Dicranopteris*, *Marsilea*, *Equisetum*, *Pteris*, and *Sellaginella*[43]. Antioxidants aid in preventing reactive oxygen species-induced damage to proteins, lipids, and DNA[44].

6.2 Antimicrobial Characteristics

Alkaloids, flavonoids, tannins, and glycosides found in the epidermal glands of pteridophytes prevent the growth of microorganisms[45–48]. *Adiantum*, *Lygodium*, *Pteris*, *Christella*, *Salvinia*, and *Ceratopteris* species have been shown to exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria[49]. *Cheilanthes* and *Marsilea* species exhibit antifungal effects[50]. *Blechnum orientale*, *Woodwardia, Lygodium*, *Asplenium*, *Microsorum*, *Pteris*, and *Selaginella*[51] are among the ferns that exhibit antiviral activity.

6.3 Anti-inflammatory Characteristics

Inflammation and cellular damage are caused by an excess of reactive oxygen species. Phenolics, flavonoids, diterpenes, and tannins derived from ferns aid in reducing inflammatory reactions and neutralizing free radicals[52].

6.4 Anticancer Properties

Fern phytochemicals reduce oxidative stress, inhibit angiogenesis, modulate the cell cycle, and prevent metastasis, all of which have anticancer and apoptosis-inducing effects. Serratenediol, lycopodine, and lycojaponicumin analogs from Lycopodium species exhibit significant anticancer potential[53, 54].

7. Pharmaceutical Products

Huperzine A, a reversible acetylcholinesterase inhibitor isolated from *Huperzia serrata*, has gained considerable attention for its potential in the treatment of Alzheimer's disease and has also been used traditionally to manage conditions such as swelling, fractures, and organophosphate poisoning[55, 56]. Another notable compound, calaguline, extracted from *Polypodium* leaves, exhibits anti-inflammatory and anti-proliferative activities by inhibiting NF-κB activation[57, 58].

8. Preservation and Ecological Use

Because pteridophytes require steady, humid microclimates, habitat disruption quickly lowers their populations[59]. The following are major threats: pollution and invasive species; habitat loss, deforestation, and land-use change; overharvesting for medicinal and ornamental purposes; and climate change that modifies moisture regimes. Epiphytic ferns usually destroy their host trees hence numerous species are currently classified as endangered or vulnerable[60].

9. Conservation Strategies

These strategies may be *In situ* i.e., preservation of microclimate-dependent ecosystems, and protection of habitats and establishment of protected areas, or *Ex situ* by *in vitro* culture and cryopreservation of gametophytes and sporophytes; living collections; spore banking[61]. DNA banks, germplasm, and botanical gardens are other supportive strategies[62].

9.1 Ecological Harvesting

This procedure meant to create controlled harvesting procedures; to encourage agroforestry for the production of medicinal ferns; to involve local populations and provide incentives for sustainable practices; and to develop mechanisms for certifying materials sourced responsibly[63].

9.2 Indigenous Knowledge

Ethnobotanical study, bioprospecting, cultural recognition, and biodiversity conservation are all aided by the documentation of traditional usage[64, 65].

10. Future Prospects and Conclusion

Pteridophytes have long served traditional medicine for treating inflammation, infections, and tumors. Despite this, their phytochemistry and pharmacology remain underexplored compared to angiosperms. Most species have not yet been chemically profiled or bioassayed. Future studies should concentrate on the following areas: isolating and characterizing novel bioactive compounds; comprehending biosynthetic pathways; creating phytopharmaceuticals through computational drug design; combining biochemical research with ethnobotanical knowledge; and sustainable conservation and cultivation to stop biodiversity loss. Pteridophytes protection guarantees the survival of ecologically significant species and maintains access to essential therapeutic resources.

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Author contributions

SS –Research concept and design, **S** –Collection and assembly of data, Data analysis and interpretation, writing the article, **SS, S** – Critical revision and final approval of the article.

Data Availability

Not Applicable.

Conflicts of interest

The authors declare that there are no conflicts of interest related to this article. No ethical issues

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Ophiocordyceps sinensis (Keera Jari/ Himalayan Viagra): Pharmacological properties and their therapeutic potential

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Abstract

Ophiocordyceps sinensis (Keera jari), also known as caterpillar fungus, is a member of the Ophiocordycipitaceae family of fungi that parasitizes ghost moth larvae and produces a fruiting body valued as herbal medicine. Due to its numerous uses in the pharmaceutical and medical fields, *Cordyceps* species are commonly referred to as traditional Chinese medicine (TCM). Keera jari has been acknowledged in Nepal for many years, and in India it is known as 'keera jari' or 'keera ghas.' It is among the most promising therapeutic mushrooms in the world. People all throughout the world use the caterpillar fungus for its tremendous therapeutic properties. It grows between 3500 and 4500 meters above sea level on the upper Himalayan plateau. This review's goal is to gather information on Keera jari (Yarsagumba), including its history, cultivation, taxonomic traits, several medicinal applications, and phytochemical research that has been done thus far.

Keywords: *Ophiocordyceps sinensis,* Keera Jari, Himalayan Viagra, Medicinal applications, Himalayan plateau.

1. Introduction

Ophiocordyceps sinensis (Berk.) G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora [Syn. *Cordyceps sinensis* (Berk.) Sacc.] is also referred to as Yarsagumba (fungus cum larvae) in Nepali[1,2] and caterpillar fungus[3, 4]. This fungus is employed extensively in research and clinical therapies. *Thitarodes armoricanus* (*Hepialus armoricanus*) is a species of ghost moth (family Hepialidae) that acts as the host for *Ophiocordyceps sinensis*.

Lepidopteran larvae are infected by the parasitic fungus *Ophiocordyceps sinensis* (syn. *Cordyceps sinensis*). The diseased larva is prized for its therapeutic qualities and is usually referred to as a fungal caterpillar. Locally referred to as "keera jari" in Uttarakhand, it is typically found in meadows between 3,500 and 5,000 meters above sea level. The fungus infects the larva at the end of autumn and gradually spreads throughout its entire body, killing it[5, 6]. The larva stays buried in the ground throughout the winter. However, the fungus-infected larva sporulates and forms a structure resembling a plant's shoot after the snow begins to melt[7]. *O. sinensis* is quite expensive and has a large market because of its possible medical usefulness. As a result, the natives now have a reliable stream of money from its collection. The Pithoragarh district's Chiplakot, Ultapara, Brahmkot, Najari, and Nangnidhura–Munshyari regions, as well as the Chamoli district's Ghat, Dewal, Niti, and Mana valleys, are the main locations for *O. sinensis* in Uttarakhand.

However, the supply frequently falls short of demand since *Ophiocordyceps* is an uncommon and challenging material to collect. The harvesting season in Central Himalaya lasts from April to May. After gathering it in a camp with friends and family, the local collectors sell it to approved organizations like the Forest Department Corporation.

Local collectors exhibit a more balanced male-to-female ratio, but non-residents—outsiders who travel to the collection areas are predominately male. 'Keera jari' can be collected using a variety of techniques, such as digging a tiny hole or using a knife to delicately remove it from the ground[8]. Prior to or early in the spring, the caterpillar fungus is more useful. The upper portion of the mushroom may split during the last stages of sporulation, and the host larva becomes mushy and unattractive[9]. China has long been familiar with the uncommon and exotic medicinal mushroom *O. sinensis*. One that is said to have several extensive therapeutic benefits.

In the last twenty years or so, most Westerners have become aware of this uncommon herbal remedy. Chinese practitioners have known for generations that it is effective in treating a wide range of medical issues, and contemporary scientific research into its seemingly remarkable spectrum of therapeutic powers has confirmed this. The description and use of this once-rare medicinal gem are summarized in this chapter[7, 10].

1.1 Name and general description

Ophiocordyceps sinensis, an Ascomycetes fungus that is closely related to mushrooms and has a long and distinguished history as a medicinal herb. Despite not being a true mushroom in the taxonomic sense, it has long been considered and referred to as a therapeutic mushroom. The Latin words cord and ceps, which imply "club" and "head," respectively, are the source of the name *Cordyceps*. The club fungus *Ophiocordyceps sinensis,* whose stroma or fruitbody extends from the mummified bodies of insect larvae—typically caterpillar larvae of the Himalayan Bat Moth, *Hepialis armoricanus*—is appropriately described by the Latin conjugation.

Although many other closely similar species fall under the general category of *Cordyceps*, the term "Cordyceps" typically refers primarily to the species *Ophiocordyceps sinensis* in historical and common usage. The most well-known species of *Ophiocordyceps* in the world may be *O. sinensis*, however there are numerous additional species in the genus *Ophiocordyceps* that have been shown to have useful therapeutic qualities by contemporary research. Since many of the several species of *Ophiocordyceps* suit the description and applications disclosed here, we shall generally use the name *Ophiocordyceps* without the species designator in this study. In cases when a particular species identification is significant, the species name will also be provided[11, 12].

For many decades, Traditional Chinese Medicine (TCM) has recognized and utilized *Ophiocordyceps sinensis*. It is hard to locate and harvest because it is only found at high elevations on the Himalayan Plateau. *Cordyceps* has long been one of the costliest medications known due to the challenges associated with harvesting this exotic medicinal. Due of its expensive cost, it was historically out of the reach of the typical Chinese subject and was only available to members of the emperor's court and other Chinese nobles. The extraordinary list of therapeutic applications for *Ophiocordyceps* has made it a highly prized mainstay of Chinese medicine, despite its high price and scarcity.

Only in 1726, when it was presented at a scientific conference in Paris, did Western scientific audiences learn about these amazing phenomena, which had been acknowledged as a natural wonder for more than 2000 years in China and the surrounding Orient. The first specimens were brought back to France by a Jesuit priest who wrote about his encounters with the *Ophiocordyceps* mushroom while he was at the Chinese Emperor's court. Although *Ophiocordyceps* has always been rare in nature, modern technological advancements in cultivation have made the possibility of affordable *Cordyceps* a reality[11].

1. Taxonomy and Description

Kingdom: Fungi; Phylum: Ascomycota; Class: Ascomycetes; Order: Hypocreales; Family: Clavicipataceae; Genus: *Ophiocordyceps*; Species: *Ophiocordyceps sinensis*

Basionym: *Sphaeria sinensis*; Synonym: *Cordyceps sinensis*

Vernacular names: Cordyceps mushroom, caterpillar fungus, totsu kasu, tochukasu (Japanese), hia tsao tong tchong, and dongchongxiacao (Chinese) are examples of colloquial names.

1.2 *Description*

The *Ophiocordyceps sinensis* mushroom's ascocarp, or fruitbody, begins at its base on an insect larval host, most commonly the larva of the Himalayan bat moth, *Hepialis armoricanus*, though other insect hosts are occasionally found. It concludes with the stipe and stroma at the club-like cap. The fruitbody is dark brown to black, and the organism's "root," the larval body that has been infected by the mycelium of the mushroom, is yellowish to brown in color. It has a caterpillar-like shape and color, measuring 5–15 cm in length and 0.14–0.4 cm in thickness. Its stem, which is between two and five centimeters long, is either brown or black. There are two varieties based on color; the white yellow variety is larger and of higher quality. The other kind is smaller, copper-colored, and of lower quality. Under ideal circumstances, the spores are finally released and either fall within a few centimeters of their source or are carried away by wind[13].

1.3 *Habitat*

The fungus *Ophiocordyceps* appears annually. The months of April through June are typically when harvesting occurs. *Ophiocordyceps*, which feeds on the moths' larvae, only grows at elevations higher than 3,800 meters above sea level in the chilly, grassy alpine meadows of the Himalayan Plateau, which includes modern-day India, Tibet, Nepal, and the Chinese provinces of Sichuan, Gansu, Hubei, Zhejiang, Shanxi, Guizhon, Qinghai, and Yunnan. In the spring, the caterpillar exhibits subsurface symptoms of the fungal infection, at which point the mycelium starts to break down the host until fruiting is triggered. This occurs when winter gives way to spring and summer, when the thawing of snow at lower elevations makes it easier for foragers to locate the mushroom, and the food source (the caterpillar) has been exhausted. Although it seems improbable, it is currently unknown if it also bears fruit under the snow during the harsher months[14].

1.4 *Ophiocordyceps*: *Parasite or Symbiont?*

It is important to note that the entomopathogenic aspects of the *Ophiocordyceps* mushroom is debatable, even though the spore may be an "infectious" agent that targets the moth larvae. Many eminent researchers believe that keera jari (*O. sinensis*) truly has a symbiotic relationship with the host, meaning that the interaction is mutually beneficial rather than pathogenic, based on an increasing amount of logical and empirical data. Given the isolated and hostile setting in which the moth/*Ophiocordyceps* partnership takes place, this makes sense. Because parasites typically cause the host's mortality, nature tends to choose against them. As is known when other animals eat *Ophiocordyceps*, a more plausible explanation for the unusual combination of an insect and this fungus would be a mutually beneficial symbiosis, in which the moth might get an energy boost from the *Ophiocordyceps* living in its body[15].

Ophiocordyceps frequently displays a single-celled, yeast-like anamorph development stage when cultivated. Other insects have been shown to have similar yeast-like symbionts of the genus *Cordyceps*, which are most likely beneficial to the host insect[3]. If this is the case with the *Ophiocordyceps* /moth relationship, the stressor that causes the *Cordyceps* to generate its fruitbody could be the insect host's death. The *Ophiocordyceps* would have to enter a "reproduce or die" mode once the host insect perished. The mycelium, as opposed to the more commonly observed fruitbody, is the stable-state life form in most fungi. In the kingdom of fungi, it is most usual for fruitbody production to occur only when a significant stressor forces this defensive reproductive-phase response. In the

natural world, these stressors are typically heat and cold, fire and flood, or the full use of the food supply and the consequent lack of nutrients. It is quite challenging to get *Ophiocordyceps* to produce fruiting body in the lab, but when it does, it usually reacts to one or more of these stresses[15].

1.5 *Edibility*

Because of its small size, scarcity, and harsh texture, it is not typically regarded as an edible fungus. *Ophiocordyceps* has historically been eaten as a medicinal soup with a variety of meats, depending on the intended medical condition[16]. In modern medicine, *Ophiocordyceps* is frequently taken alongside vitamin C, which has been shown to help the body absorb and digest the mushroom's therapeutic ingredients. A parasitic fungus called *O. sinensis* infects lepidopteran larvae. The diseased larva is prized for its therapeutic qualities and is usually referred to as a fungal caterpillar.

Locally referred to as "keera jari" in Uttarakhand, it is typically found in meadows between 3,500 and 5,000 meters above sea level. At the end of autumn, the larva contracts the fungus, which gradually spreads throughout its entire body until it dies[14]. Throughout the winter, the larva remains hidden in the dirt. However, the fungus-infected larva sporulates when the snow starts to thaw, forming a structure that looks like a shoot. Because of its potential medical benefits, O. sinensis has a sizable market and is pricey. The villagers now have a steady source of income from their collection as a result. However, excessive exploitation is problematic[17].

2. Review of Literature

Ophiocordyceps sinensis (syn. *C. sinensis*) is generally found in China from the Central Yunnan Plateau to the Qilian Mountains in Qinghai Province, and from Mount Daloushan in Guizhou Province to the vast regions of the Himalaya. However, this fungus's strong host-specificity on moth insects and restricted geographic distribution seem to limit its environmental association. According to reports, the caterpillar fungus can be found between 2200 and 5000 meters in China, between 4200 and 5200 meters in Bhutan, between 3500 and 5050 meters in Nepal, and between 3200 and 4800 meters in India. The caterpillar fungus was initially discovered in India in the late 1990s[18]. The species has been found in alpine meadows in several protected areas, including Askot Wildlife Sanctuary in Uttarakhand, Dehang-Debang Biosphere Reserve in Arunachal Pradesh, Nanda Devi Biosphere Reserve, and Kanchendzonga Biosphere Reserve in Sikkim. Most of the product for commerce is reported from Uttarakhand along the borders with China and Nepal, despite reports of the species coming from three states in the Indian Himalaya[19]. Caterpillar fungi often inhabit alpine and subalpine meadows and shrublands with an average annual precipitation of at least 350 mm[4]. Although the upper altitude limit may reach the snowline areas above 5000 m asl, it grows best in the altitudinal range of 3000–5000 m asl[12].

2.1 *Ecology*

During the monsoon, or wet season, the fungus grows on the caterpillar. The fungus's spores terminate and develop on a living caterpillar. Eventually, the caterpillar perishes. The fungus needs five to seven years to finish its life cycle and generate the natural product. Keera jari only thrives in extremely particular, difficult-to-replicate environments[19]. Only the Himalayas are home to the species. All year round, the temperature is low there. The amount of oxygen available is limited by the high altitude. Growing *Cordyceps* is ideal in these extreme conditions, which make life challenging even for most fungus. Kutki (*Picrorhiza scrophulariiflora*), Jatamansi (*Nardostachys grandiflora*), Bukiphool (*Anaphalis* sp.), and other high-altitude grasses are linked with this species[20].

2.2 *Medicinal Properties*

For decades, the insect and fungal remains have been manually gathered, dried, and utilized in traditional Chinese medicine to treat weariness, sickness, kidney disease, and low libido. When it came to the use of *O. sinensis* parts, those who lived in the uplands used to just gather the elevated fungal portion. Individuals are wise enough to use *O. sinensis* for a variety of objectives. Practically

speaking, this fungus is used by the majority of highland indigenous people to treat a variety of conditions, including headaches, rheumatism, liver disease, diarrhea, and—most importantly—as an aphrodisiac. Nyamnyi Dorje, a Tibetan physician and lama who lived from 1439 to 1475, provided the first known account of Yarsagumba. Yarsagumba is described as a sexual tonic in his textbook, An Ocean of Aphrodisiacal Qualities. Yarsagumba contains bioactive compounds with immunomodulating, anti-inflammatory, and anticancer activities, according to in vitro research[20]. *Ophiocordyceps* extract-containing products and supplements have rapidly gained popularity because of their many health advantages. The medicinal uses of the fungus by locals and traditional healers, claims that Sikkim's traditional healers have been using the fungus for more than eighteen ailments, with the folk healers recommending its use as an aphrodisiac. The number of stoners for a disease is used to gauge the strength of the allegations. In the launching and Lachen region of Sikkim, this fungus is traditionally utilized by both macho and womanly people to treat sexual dysfunction, improve general health and appetite, and boost life. In order to increase their sexual energy and desire, people of both sexes typically consume one fragment of *O. sinensis* with one cup of milk[21]. As an aphrodisiac, the Bhutia communities mix one piece of *O. sinensis* with one cup of locally produced alcohol (chang), leave it for an hour, and then drink it in the morning and evening. Instead of using alcohol, some people utilize hot water. The original people claim that fungus is more effective than ginseng and that it is also useful to treat tuberculosis, cancer, exhaustion, chronic pain, liver, and other ailments. Though mindfulness has only grown since 1995, the indigenous people of Sikkim have been acknowledging that their ancestors utilized this medication prior to the Chogel period, which occurred between 1200 and 1600 BC. The primary active components of *O. sinensis* are cordycepin and cordycepic acid. This tiny plant is a remarkable hybrid of a mushroom (fungus) and a yellow caterpillar. It has all the essential amino acids, water-soluble vitamins B1, B2, and B12, and vitamins E and K. Numerous sugars, including mono-, di-, and oligosaccharides; proteins; sterols; nucleosides; and macro- and microelements (Fe, Cu, K, Na, Ca, Mg, Mn, Zn, Al, Si, Pi, Se, Ni, Sr, Ti, Ga, Cr, V, and Zr) are also present. Men's testosterone levels are raised by *Ophiocordyceps*. In Oriental society, it has long been used as a tonic supplement for reproductive and sexual dysfunctions. Additionally, it raises energy levels. Men's testosterone levels are raised by *Ophiocordyceps*. In Oriental society, it has long been used as a tonic supplement for reproductive and sexual dysfunctions. Additionally, it boosts both men's and women's energy levels and reproductive capacities. For many decades, Traditional Chinese Medicine (TCM) has valued *C. sinensis* for its potential to promote health and stimulate sexual desire[22].

2.3 *Sustainability Issues*

The availability and abundance of the species in its natural habitat are shown to be unaffected by the current Keera jari collection practices. Once the 'Keera Jari' is selected or harvested, more of them are reportedly discovered growing around the pit of the previous year's harvest the next year[26].

2.4 *Socio-Economic and Policy Issues*

For almost ten years, the government has prohibited the trade and collection of this substance without any scientific justification. Nonetheless, this commodity is still being collected and traded illegally. It is one of the main sources of income for people in the Himalayas[24, 26].

2.5 *Current Policy*

In some countries it is prohibited to gather, use, sell, distribute, transport, or export this commodity. Before being gathered for trade, the harvestable fungus body disperses its spores for renewal (part of the spores meet the larvae). However, illegal collecting is made possible by differences in the prices set by the government and the market. This necessitates legislation to prevent

overexploitation and conservation. It is recommended that the keera jari be collected should be made alternate year. Strict measures should be taken to stop illicit supply and collection [13, 25, 26].

3. Conclusion

The elucidation of *O. sinensis* host insects may provide a fundamental understanding for efficient insect resource management techniques as well as for the preservation and sustainable usage of the fungus. Therefore, as soon as the moth population starts to drop, they should be reintroduced to the ecosystem and spread in any way possible. Construction, farming, livestock grazing, large-scale human migration, and other practices that upset the ecology should be discouraged. Furthermore, more research is obviously needed to develop effective management measures to ensure the long-term survival of this fungus due to growing harvest pressure and the lack of trustworthy basal data. It is necessary to develop alternative revenue streams that are specifically designed to sustain indigenous people who depend on *Ophiocordyceps*. Only when local communities are involved in decision-making and execution do government-issued regulations and directives have a decent chance of succeeding. Due to its limited range, this species is likely to experience genetic erosion (rapid habitat fragmentation leading to decreasing gene pools, with human activities being the main culprit), which requires attention. To guarantee its economic and ecological values, more cooperative research both domestic and international is required. Researchers and medical experts continue to be interested in its possible therapeutic properties, making it a valuable natural resource for holistic medicine. Most of the research has been done by researchers thus far. This may be because *Ophiocordyceps* has only lately made its way into the western world and is not readily accessible there. As a result, it has been shunned by default, which has resulted in a lack of awareness, even though it is frequently utilized at home and by local healers in addition to pharmaceutical uses. Nonetheless, *Ophiocordyceps* is becoming increasingly well-liked globally because of its potent therapeutic properties and its aphrodisiac property, especially among those who favor herbal medicines over chemical or synthetic drugs. The many components of *Ophiocordyceps sinensis* are summed up in this overview. With so many benefits, it is essential that every effort be made to make this myco-medical herb available to the medical community worldwide.

Author contributions

AKB: Conception, study and data collection and manuscript preparation. **AT:** Investigations, Formal analysis, conceptualization, manuscript writing.

Data Availability

All data produced or examined in this study are contained within this published article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Bryophytes: Ecosystem Engineers in Soil, Carbon, and Water Cycles

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Abstract
Mosses, liverworts, and hornworts are constituents of bryophytes, the oldest groups of terrestrial plants, i.e., avascular cryptogams. These plants are vital components of ecosystems, as they carry out vivacious ecological functions regardless of their small size and simple thallus organization. In unadorned or disturbed soils, they are the main colonists beside lichens, starting the soil formation through the accumulation of organic matter with the aid of microbial communities. By accumulating carbon in both terrestrial and some aquatic systems, bryophytes subsidize climate upholding and play a key role in the overall carbon cycle. These miniature plants also have a huge impact on hydrological procedures through soil erosion alleviation, surface runoff reduction, and water retention. Away from their roles in ecosystem engineering, these simple plants are becoming more broadly recognized for their ability to reinstate habitat, act as bioindicators of climatic stress, and reinforce ecosystem elasticity in the face of environmental stress. The biology, ecological roles, and physiology of bryophytes are abridged in this review, highlighting their worth for safeguarding tactics, restoration ecology, and climate change alleviation. In the era of global ecological challenges, a better consideration of these earliest land plants can help to manage ecosystems sustainably and reinforce efforts to safeguard biodiversity.

Keywords: Bryophytes, Carbon sequestration, Climate resilience, Ecosystem engineering, Soil formation.

1. Introduction
Derivative from the Greek words 'bryon' (meaning "moss") and 'phyton' (meaning "plant"), bryophytes are non-vascular, poikilohydric plants that belong to some of the most primitive terrestrial flora ancestries. These plants are notable by very simple thallus organization, the total absence of true vascular tissues, and dependency on surface water for hydration, with gametophyte-dominated life cycle. The earliest identified liverwort fossil is from the Upper Devonian of New York, signifying their extensive evolutionary history. Fossil evidence indicates that bryophyte-like organisms colonized land during the Ordovician period (~488–444 Ma). Despite their diminutive size and lucid morphology, bryophytes are crucial to many different ecosystems[1]. They are ecologically tough and able to inhabit cruel and fringe environments due to their great ability for rejuvenation and confrontation to desiccation. By alleviating soils, affecting hydrological fluctuations, and helping in the cycling of nutrients and carbon, these plants function as ecosystem engineers. In both terrestrial and riparian settings, moss mats and liverwort carpets can control microclimatic conditions, buffer temperature changes, and hold onto water. Furthermore, bryophytes frequently develop close relationships with microbial communities, such as cyanobacteria, fungi, and bacteria, which improve nutrient uptake and stress tolerance.

Beyond their ecological significance, bryophytes are significant model systems in plant biology and evolution, providing information on developmental biology, stress physiology, secondary metabolite production, and early land plant adaptations. They are perfect for research on desiccation tolerance, carbon assimilation, and biotechnological applications due to their straightforward morphology, quick life cycle, and genetic tractability. Largely, the ecological, evolutionary, and physiological characteristics of bryophytes emphasize their significance as foundational constituents of terrestrial ecosystems and as key subjects for investigation in plant science, climate resilience, and environmental restoration.

2. Materials and Methods

To summarize the present understanding of bryophytes as ecosystem engineers in the soil, carbon, and water cycles, a thorough literature review was carried out. Keywords including bryophytes, mosses, liverworts, hornworts, ecosystem engineering, soil formation, carbon sequestration, water retention, and microbial interactions were used to search scientific databases, including WoS, Scopus, PubMed, and ScienceDirect. Included were peer-reviewed studies, reviews, and meta-analyses that addressed the ecology, physiology, and functional roles of bryophytes in ecosystems. To highlight bryophyte contributions to soil stabilization, nitrogen cycling, hydrology, microbial relationships, and climate regulation, data from pertinent studies were selected and arranged thematically. The results were incorporated into a conceptual framework that provided a comprehensive knowledge of the ecological and environmental implications of bryophyte characteristics and microbial interactions in relation to ecosystem functioning.

3. Biology and Life Cycle

Bryophytes include three major lineages — mosses (*Bryophyta*), liverworts (*Marchantiophyta*), and hornworts (*Anthocerophyta*) — with mosses being the most species-rich. They undergo alternation of generations in their life cycle, dominated by the haploid gametophyte, which can be thalloid or leafy. Rhizoids substitute for roots, and they lack vascular tissues, relying instead on surface water absorption. They reproduce sexually via oogamy (antheridia and archegonia), producing a sporophyte (foot, seta, capsule) in which meiosis yields haploid spores.

3.1 Distribution and Habitat Diversity

Bryophytes are cosmopolitan, with particularly high species richness in tropical regions[2]. They inhabit diverse microhabitats: soils, rocks, tree trunks, peatlands, urban walls, and even concrete. Their success depends on factors such as moisture availability (critical for reproduction), substrate pH, canopy cover, and elevation[3,4]. Their capacity to desiccate and revive (poikilohydry) allows them to persist in extremely dry or cold environments.

4. Ecosystem Functions

4.1 *Soil Formation*

Bryophytes are pioneer species on bare substrates, influencing pedogenesis through physical, chemical, and biological pathways. Bryophytes contribute to soil formation through a synergistic combination of physical, chemical, and biological processes. Their mats physically stabilize surfaces by trapping particles, reducing erosion, and binding loose sediments through rhizoids and mucilage, which act as a natural glue and create a stable microenvironment that moderates temperature extremes and promotes mineral weathering and microbial activity[5-8]. Chemically, bryophytes secrete organic acids such as oxalic, citric, and malic acids that dissolve minerals and mobilize nutrients, while their cell walls—rich in uronic acids and phenolic compounds—facilitate cation exchange, proton release, and enhanced mineral hydrolysis[11]. They also support diverse microbial consortia, including cyanobacteria, bacteria, and fungi, which further accelerate mineral breakdown through siderophore production and extracellular enzymes[12-14]; additionally, decomposing bryophyte litter contributes humic substances

that stabilize organic matter and drive long-term soil development[15-17]. Biologically, bryophytes host diverse surface- and rhizoid-associated microbes that fix nitrogen, mobilize phosphorus and iron, and enhance nutrient cycling[12, 14], while their persistent litter horizon and bryophyte-based biological soil crusts promote microbial activity[15, 16], soil aggregation, and overall soil stabilization[18]. These systems work synergistically: physical break-up exposes minerals, chemical exudates dissolve them, and microbial groups strengthen and supplement the emerging soil.

4.2 *Carbon Sequestration*

Bryophytes—especially *Sphagnum* mosses—are among the most influential yet often overlooked drivers of global carbon dynamics. Despite making up only 3% of the planet's land area, sphagnum-dominated peatlands can store an astounding 500–600 Gt of carbon[19] due to their consistently wet, acidic, and oxygen-poor conditions, which prevent decomposition and preserve organic matter. In tundra and alpine ecosystems, bryophyte carpets serve as natural insulation layers outside of peatlands, keeping soils cool, delaying microbial degradation, and encouraging long-term carbon retention[20]. Through symbiosis with nitrogen-fixing cyanobacteria, which enrich these systems with biologically available nitrogen and increase moss productivity and carbon sequestration, their ecological influence is further reinforced[21]. Bryophytes are essential to Earth's carbon balance and climate regulation because, in contrast to vascular plants, which store a large amount of their carbon in transient above-ground biomass, they channel a significant portion into stable, long-lived below-ground carbon pools. Though, peatland desiccation owing to climate change or human caused disturbance can reverse their competence from carbon sinks to sources, freeing centuries of stored carbon[22]. Restoration efforts, such as *Sphagnum* farming and peatland rewetting, are being pursued as climate mitigation strategies.

4.3 *Hydrological Regulation*

As organic hydrological sponges, bryophytes stabilize microclimates and buffer water fluxes in a variety of ecosystems. They are able to retain water amounts several times their dry mass[23] thanks to their complex capillary networks, specialized hyaline cells in *Sphagnum*, and dense cushions. They moderate runoff, extend base-flow, and lessen hydrological extremes by capturing and retaining rain, dew, and fog and releasing moisture gradually into the soil and atmosphere[24]. Features unique to a species enhance this function: hummock-shaped while epiphytic mosses in cloud forests collect fog and progressively drip water along trunks and forest floors, maintaining localized humidity[25], sphagnum sustains high water tables in peat lands. Bryophytes also indirectly influence carbon dynamics, plant community succession patterns, and decomposition rates due to their significant impact on soil moisture and microclimate[25].

5. Ecological and Practical Importance

In addition to their ecological benefits, bryophytes are valuable in science, industry, and commerce. As pioneer species, they lay the foundation for subsequent plant communities by starting ecological succession on disturbed or degraded substrates[26]. Their carpets and mats provide vital microhabitats for various microbial assemblages, amphibians, and invertebrates. Bryophytes are useful tools for environmental monitoring because they are sensitive bioindicators that efficiently accumulate pollutants like heavy metals and airborne contaminants. Molecular biology, genetics, and stress physiology all make extensive use of model species such as *Physcomitrium patens*[27]. Bryophytes offer natural solutions for rebuilding ecology by calming soil, controlling water, and aiding in carbon sequestration[28]. Sphagnum peat is still economically significant in horticulture, even though many species of bryophytes are still utilized in traditional medicine and crafts.

6. Future Directions

To fully unlock the ecological and biotechnological potential of bryophytes, future research must advance along several complementary fronts. At the molecular scale, integrating transcriptomic, proteomic, and genetic approaches will be essential for elucidating the mechanisms underlying desiccation tolerance, osmoprotection, and carbon stabilization[29, 30]. Long-term climate-change experiments are equally critical, particularly those examining how bryophyte communities—most notably peatlands—respond to warming, shifting precipitation patterns, wildfires, and permafrost thaw[22]. In addition to developing efficient rebuilding techniques for ecological restoration, applied efforts should concentrate on expanding dissemination methods like cryopreservation, tissue culture, and spore banking31. Advances in remote sensing and tomography, such as drone or NDVI-based mapping to measure moisture and bryophyte cover at landscape scales and micro-CT scanning to visualize internal water routes, provide new tools for biological monitoring[32]. Lastly, more accurate forecasts of their upcoming effects on the world's water and carbon cycles will be possible by integrating comprehensive bryophyte physiological data with ecosystem-level models.

7. Discussion

In addition to being a source of several phytochemicals that are important for medicine[33, 34], bryophytes are a special class of early-diverging land plants that maintain intricate relationships with their associated microbiomes, which are essential to ecosystem function and environmental resilience. The ecological and physiological significance of these relationships is highlighted by recent research, especially in stressful and harsh environments. In their thorough analysis of bryophyte–microorganism relationships, Dangar et al.[35] show how microbial communities maximize nutrient uptake, improve stress tolerance, and support bryophyte survival in a variety of environmental circumstances. In addition, Pandey et al.[36] described the isolation of endophytic bacteria from bryophyte gametophytes in Mount Abu, highlighting the variety and possible functional roles of these microorganisms in plant health and nutrient cycling.

A crucial interface for interactions between bryophytes and microbes has been identified as the rhizoid-associated microbiome. The bryophyte rhizoid-sphere microbiome is highly responsive to water deficit, as demonstrated by Berdaguer et al.[37], suggesting a dynamic microbial contribution to bryophyte resource management and drought tolerance. The production of secondary metabolites in bryophytes and stress physiology are closely related to these interactions. In their review of stress-response pathways, Kulshrestha et al.[38] emphasized the production of specialized metabolites like polyphenols, which both protect against abiotic stress and regulate microbial communities. Furthermore, Liu et al.[39] showed the complex molecular mechanisms underlying resilience by using proteomic and transcriptomic analyses to show that desiccation-tolerant mosses, such as *Racomitrium canescens*, quickly modify gene expression during rehydration. Beyond survival strategies mediated by microbes, bryophytes play an important role in global biogeochemical cycles.

Bryophytes and lichens play a significant role in carbon sequestration, especially in high-latitude ecosystems, according to Porada et al.[40] model of global carbon uptake. Bryophytes that form peatlands, primarily Sphagnum species, are known to play a crucial role in controlling the climate. Leifeld and Menichetti[41] stress that by preserving carbon-rich substrates and reducing CO_2 emissions, peatland restoration—aided by bryophytes—can offer practical natural climate solutions. Understanding and tracking bryophyte cover, which is essential for ecological modeling and conservation tactics, has improved thanks to remote sensing. For high-latitude vegetation monitoring, Assmann et al.[42] showed the value of multispectral sensors and UAV-based NDVI mapping, offering a reliable method to measure

bryophyte distribution at landscape scales. Additionally, ecosystem microclimate regulation is directly impacted by the thermal characteristics of bryophyte mats. Soudzilovskaia et al.[43] showed that dominant species of bryophytes apply sturdy control over soil temperature vacillations through heat transfer characters and water content, proving their impact on local soil microenvironments and ecosystem liveliness balance.

Ultimately, bryophytes have revealed their potential in practical and environmentally friendly management. Singh and Choudhary[44] appraised their utility in phytoremediation, highlighting the capability of bryophytes to bioaccumulate unsafe heavy metals, destroy organic pollutants, and restore polluted habitats, aligning them as capable agents for ecological refurbishment and environmental sustainability. Mutually, these studies emphasize the versatile roles of bryophytes—from facilitating plant–microbe interactions and stress physiology to controlling carbon cycling, environmental remediation, and microclimate. The amalgamation of molecular, ecological, physiological, and remote sensing methods provides a complete frame to recognize bryophyte ecology and clout their ecosystem services in the perspective of worldwide environmental change.

8. Conclusion

Bryophytes, often overlooked because of their diminutive size and simple morphology, are in fact powerful ecosystem engineers that play a critical role in maintaining ecosystem structure and function. Through a combination of physical, chemical, and biological mechanisms, these plants contribute to soil formation, enhance nutrient cycling, sequester substantial amounts of carbon, and regulate hydrological processes, including water retention and runoff control. Their presence supports diverse microbial communities and provides essential habitats for invertebrates and other small organisms, highlighting their broader ecological significance. Conserving and bring back bryophyte-rich habitats not only protects these earliest plants but also offers a innate and cost-effective solution for climate modification and ecosystem resilience. Moreover, bryophytes grant distinctive perceptions into the evolution of land plants, serving as living models for identifying adaptation to land environments. Moving forward, interdisciplinary research combining molecular biology, ecology, physiology, and landscape-level studies will be vital to fully explaining the evolutionary, ecological, and climate-related ability of bryophytes. By connecting fundamental research with applied safeguarding strategies, we can make sure that these small, yet influential plants continue to maintain ecosystems and impact to a more durable biosphere in the face of global environmental change.

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Author contributions

TS –Research concept and design, **AG** – Collection and assembly of data, Data analysis and interpretation, writing the article, **AG, TS** –Critical revision and final approval of the article.

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Not Applicable.

Conflicts of interest

The authors declare that there are no conflicts of interest related to this article. No ethical issues

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Comparative Effects of IAA with BAP and Kinetin on *In Vitro* Callus Induction and Shoot Regeneration in Tomato (*Solanum lycopersicum* L.)

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Abstract

Tomato (*Solanum lycopersicum* L.) is a nutritionally and economically important crop, widely cultivated and recognized as a model plant in genetic and physiological studies. Despite its significance, tomato productivity is limited by biotic and abiotic stresses, underscoring the need for efficient in vitro regeneration systems to facilitate crop improvement. In this study, tomato variety 'Arka Sourabh' was used to evaluate the effect of different hormone combinations on callus induction and shoot organogenesis. Murashige and Skoog (MS) medium was supplemented with indole-3-acetic acid (IAA) in combination with two different cytokinins, 6-benzylaminopurine (BAP) and kinetin (Kn), to evaluate their comparative effects on callus induction and shoot regeneration. The combination of IAA (0.4 mg/L) and kinetin (0.2 mg/L) induced profuse callus formation without subsequent organogenesis, while IAA (2.5 mg/L) with BAP (2.5 mg/L) promoted direct shoot regeneration after 10-15 days of culture initiation. Among the tested treatments, the IAA and BAP combination was found to be most effective for shoot induction from stem nodal cuttings. These results demonstrate that the cytokinin type plays a critical role in determining morphogenic responses in tomato, with BAP favoring shoot differentiation and kinetin enhancing callus proliferation. The optimized protocol can be applied in future studies involving genetic transformation and large-scale clonal propagation of tomato.

Keywords: 6-benzylaminopurine (BAP), callus induction, Indole-3-acetic acid (IAA), In vitro regeneration, Kinetin (Kn), shoot organogenesis, stem nodal cuttings, *Solanum lycopersicum* L.

1. Introduction

Tissue culture is a fundamental technique in plant biotechnology that enables the regeneration of whole plants from small explants under controlled *in vitro* conditions. It is widely used for large-scale clonal propagation, production of disease-free plants, germplasm conservation, and genetic improvement. Among its diverse applications, callus induction and plant regeneration are critical steps, as they create a platform for cellular differentiation, mutagenesis, and gene transfer experiments. The success of tissue culture largely depends on the choice of explant and the balance of plant growth regulators[1], which govern callus formation and subsequent shoot or root development. This technique is applied to a wide range of plant species, including cereals, legumes, vegetables, fruits, and ornamentals. Within this context, tomato (*Solanum lycopersicum* L.) represents one of the most widely studied and commercially significant vegetable crops. It is not only valued for its nutritional composition, being a rich source of vitamins, minerals, antioxidants, and lycopene, but also serves as a model species for genetic and physiological research due to its relatively small genome and well-characterized genetics.

In India and many other countries, tomato occupies a central position in vegetable cultivation, contributing substantially to both the fresh market and processing industries. However, its productivity is frequently challenged by biotic and abiotic stresses, which necessitate the development of efficient regeneration and genetic improvement protocols[2].

Tissue culture-based regeneration systems in tomato are indispensable for the success of transformation, genome editing, and functional studies. The explants, such as cotyledons, hypocotyls, and leaves can be used with varying plant growth hormone combinations to induce callus and promote shoot organogenesis. Therefore, optimizing the hormonal composition of culture media remains a key challenge in tomato tissue culture. Nevertheless, the regeneration response remains highly genotype-dependent and often requires optimization.

In the present study, we aimed to establish a simplified and reproducible regeneration protocol for tomato using a single explant source. Tomato variety 'Arka Sourabh', a pure line variety developed through pure line selection and known for its high yield and disease resistance, was used as the experimental material for tissue culture studies. This streamlined protocol reduces complexity in media preparation and offers a reliable platform for downstream applications such as transgenics and genome editing in tomato improvement programs.

2. Materials and Methods

2.1 Plant Material and Sterilization

Tomato (*Solanum lycopersicum* L.) variety 'Arka Sourabh' was used as the experimental material for tissue culture studies. The seeds were procured from Krishi vigyana Kendra, Taliparamba, Kannur. The seeds were cleansed twice with running water, followed by disinfection using a 0.1% Mercuric chloride solution for 5 minutes. Two drops of Tween 20 were added to the mixture, which was then rinsed thrice with sterilized distilled water. The sanitized seeds were then placed in sterilized petri dishes. The seeds were then inoculated in test tubes containing ½ Murashige and Skoog[3] medium enriched with 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. The medium's pH was adjusted to 5.8 using either 1 N NaOH or 1 N HCl before autoclaving at 121 °C for 15 min. These cultures were kept in dark conditions for three days. Subsequently, the seedlings were exposed to a 16-hour photoperiod. The germinated seedlings served as explants for subsequent tissue culture experiments. Under aseptic conditions, stem nodal cuttings (1-2 cm) were excised from 10-12 days old plants. These explants were then cultured in the media. All cultures were transferred to a growth room for 4-6 weeks.

2.2 Culture Media and Growth Regulators

For callus induction, MS basal medium was supplemented with 0.2 to 1.0 mg/L indole-3-acetic acid (IAA) and 0.2 to 1.0 mg/l kinetin. Shoot induction was performed on MS medium containing 0.5 to 2.5 mg/l IAA and 0.5 to 3.0 mg/L 6-benzylaminopurine (BAP). For root induction, elongated regenerated shoots were excised and transferred to MS medium supplemented with 1.0 mg/L indole-3-butyric acid (IBA), which was found optimal for promoting healthy and profuse root development.

2.3 Statistical Analysis

All experiments were performed in a completely randomized design with three replicates per treatment. The data were subjected to two-way analysis of variance (ANOVA) to evaluate the effects of plant growth regulator concentrations and their interactions. Observations were recorded for each treatment at regular intervals, including explant response, callus formation, shoot regeneration, and root development.

3. Results

3.1 *Callus Induction*

The effect of indole-3-acetic acid (IAA) and kinetin (Kn) concentrations on callus induction

from stem nodal cuttings of tomato is presented in Table 1. Two-way analysis of the treatments revealed significant effects of the hormone combinations on callus formation. The interaction between IAA and kinetin concentrations influenced both the extent and quality of callus production. Lower concentrations favored friable, actively growing callus, whereas higher concentrations resulted in compact, less proliferative callus.

Among the tested combinations, MS medium containing 0.4 mg/L IAA + 0.2 mg/L kinetin yielded the best response, showing the highest mean values for callus induction (Figure 1). Increased levels of both hormones generally reduced callus formation efficiency, producing compact and less viable tissue, indicating the importance of maintaining an optimal hormonal balance. No shoot initiation was observed in these media, suggesting that the tested combinations were more conducive to callogenesis rather than organogenesis. The calli obtained were pale green and friable, though some cultures exhibited browning and necrosis due to excessive phenolic accumulation, which is known to hinder callus growth and viability. Root formation was occasionally observed in some calli (Figure 1c).

Table1.ShowingtheeffectsofIAAandkinetinconcentrationsoncallusinductionfromtomato Stem nodal cuttings

IAA/Kinetin (mg/L)	**IAA (0.2)**	**IAA (0.4)**	**IAA (0.6)**	**IAA (0.8)**	**IAA (1.0)**
Kn**(**0.2**)**	7.40(±2.92)	7.81(±2.97)	7.38(±2.89)	7.34(±2.89)	6.90(±2.81)
Kn**(**0.4**)**	7.21(±2.87)	7.19(±2.86)	7.15(±2.86)	7.15(±2.86)	6.96(±2.81)
Kn**(**0.6**)**	6.81(±2.80)	7.05(±2.84)	7.05(±2.84)	7.04(±2.84)	6.90(±2.81)
Kn**(**0.8**)**	6.78(±2.76)	7.09(±2.84)	6.53(±2.74)	6.54(±2.75)	6.34(±2.71)
Kn**(**1.0**)**	6.20(±2.68)	6.55(±2.75)	6.66(±2.77)	6.15(±2.67)	6.09(±2.66)

Data represent mean ± standard error (SE) of three replicates. Analysis of variance (ANOVA) revealed significant effects of IAA ($F = 248.62$, $p < 0.001$), Kinetin ($F = 11.92$, $p < 0.001$), and their interaction ($F = 41.14$, $p < 0.001$). All hormone concentrations are expressed in mg/L.

Figure1: a) Callogenesis from stem nodal cuttings, **b)** Two-week-old callus, **c)** Root initiation from callus

3.2 *Shoot organogenesis*

The response of stem nodal cuttings on regeneration medium supplemented with varying concentrations of IAA and BAP revealed significant differences in shoot organogenesis. The two-way analysis of the data is presented in Table 2. Visible shoot primordia appeared between 10-15 days after culture initiation (Figure 2a), depending on hormonal composition.

The medium containing 2.5 mg/L IAA + 2.5 mg/L BAP produced the optimum response, where 90% (18 out of 20) of the explants regenerated shoots. Lower BAP levels resulted in fewer shoots, indicating the key role of BAP in promoting shoot morphogenesis in tomato. The

regenerated shoots were healthy, elongated, and suitable for further growth and rooting.

Table 2: Effect of IAA and BAP concentrations on shoot regeneration from stem nodal cuttings of tomato

BAP/IAA (mg/L)	IAA 0.5	IAA 1.0	IAA 1.5	IAA 2.0	IAA 2.5
BAP 0.5	1.50 (±1.22)	2.25 (±1.50)	2.40 (±1.55)	2.80 (±1.67)	3.00 (±1.73)
BAP 1.0	4.20 (±2.05)	4.42 (±.10)	4.78 (±2.19)	5.03 (±2.24)	5.00 (±2.24)
BAP 1.5	5.12 (±2.26)	5.50 (±2.35)	5.40 (±2.32)	5.57 (±2.34)	5.58 (±2.36)
BAP 2.0	5.87 (± 2.42)	5.82 (±2.41)	5.98 (±2.45)	6.90 (±2.63)	6.92 (±2.63)
BAP 2.5	7.10 (±2.66)	7.10 (±2.66)	7.17 (±2.67)	7.20 (±2.68)	7.25 (±2.69)
BAP 3.0	7.00 (±2.65)	6.85 (±2.62)	6.93 (±2.63)	6.02 (±2.45)	5.70 (±2.39)

Data represent mean ± standard error (SE) of three replicates; figures in parentheses indicate square root–transformed means. Analysis of variance (ANOVA) revealed significant effects of BAP ($F = 5562.65$, $p < 0.001$), IAA ($F = 12.35$, $p < 0.001$), and their interaction ($F = 139.51$, $p < 0.001$). All hormone concentrations are expressed in mg/L

The data support the classical concept that a balanced auxin–cytokinin ratio promotes organogenesis, while deviations toward either hormone alone reduce morphogenic potential. The success of 2.5 mg/L IAA + 2.5 mg/L BAP further validates the suitability of stem nodal cuttings as a standard explant source for tomato tissue culture.

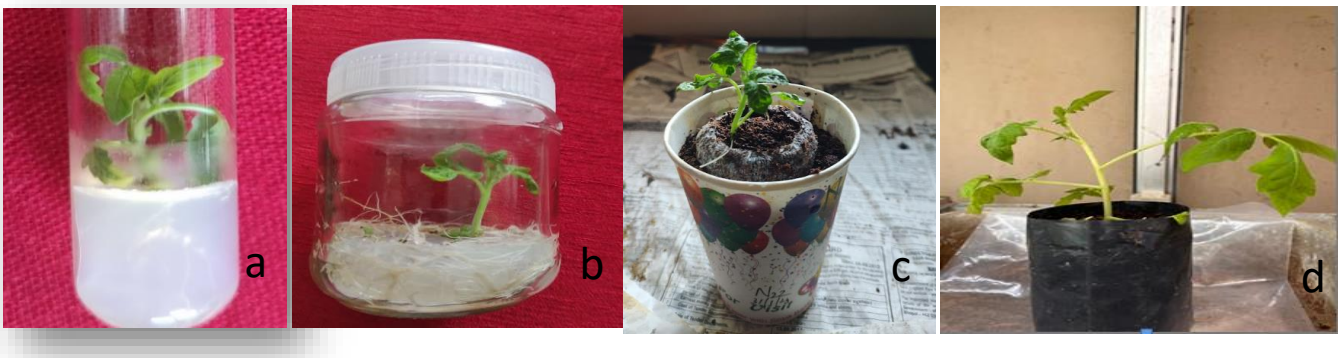

Figure 2: a) Shoot regeneration on medium, **b)** Root formation in regenerated tomato plant, **c)** Acclimatization, and **d)** Hardening

3.3 *Rooting*

Regenerated shoots were successfully rooted when transferred to MS medium supplemented with indole-3-butyric acid (IBA). Maximum rooting (87%) with the highest root number and root length was observed at 1 mg/L IBA (Table .3). Root initiation occurred within 5-7 days and full elongation was visible after one week (Figure 2b). Both lower and higher concentrations resulted in reduced rooting efficiency, indicating that IBA at 1.0 mg/L was optimum for root induction of tomato shoots, ensuring vigorous root development and subsequent acclimatization success.

Table 3: Effect of different IBA concentrations on rooting of tomato shoots.

IBA (mg/L)	% Rooting (Mean ±SD)	Root Number (Mean ± SD)	Root Length (cm, Mean ± SD)
0.5	47 ± 2.5	4.5 ± 0.5	2.0 ± 0.5
0.75	65 ± 3.0	6.0 ± 1.0	2.75 ± 0.25
1.0	87 ± 2.1	9.0 ± 1.0	3.6 ± 0.6
1.25	67 ± 3.5	6.5 ± 0.5	3.25 ± 0.25

Data represent the mean of three replicates with five explants per treatment. SD = standard deviation.

3.4 *Acclimatization and Hardening*

The regenerated tomato plants with well-developed roots were acclimatized in the laboratory using a 1:1:1 mixture of sand, cow dung, and soil. This acclimatization process allowed the plants to gradually adjust from *in vitro* conditions to *ex vitro* environments. Following acclimatization, the plants underwent a hardening phase under controlled environmental conditions, which helped them develop tolerance to external stresses such as variations in temperature, humidity, and light intensity. This phase was crucial for improving their survival rate and ensuring successful establishment under field conditions (Figure 2c-d). Once transferred to the field, the hardened plants exhibited robust growth, with healthy vegetative development.

4. Discussion

The present study demonstrated the differential effects of IAA in combination with kinetin and BAP on the morphogenic responses of tomato stem nodal cuttings. The combination of IAA and kinetin induced profuse callus formation but failed to promote organogenesis, while IAA with BAP effectively triggered direct shoot regeneration.

These findings emphasize that even subtle changes in cytokinin type can redirect the developmental pathway of cultured tissues. Auxin (IAA) primarily stimulates cell division and dedifferentiation, leading to callus formation, whereas BAP, a more active cytokinin than kinetin, promotes cell differentiation and shoot induction. This observation aligns with earlier studies highlighting that BAP plays a crucial role in shoot morphogenesis in tomato[4, 5]. Previous work has also demonstrated that auxin-mediated dedifferentiation is crucial for callogenesis, while an optimum cytokinin concentration promotes tissue proliferation.

The superior response of nodal cuttings corroborates previous reports indicating their high regenerative potential due to the presence of pre-formed meristems[6] Phenolic accumulation and tissue browning observed in some cultures may have limited callus proliferation. The phenolic-induced necrosis is a major barrier to sustained callus growth in tomato tissue cultures. Furthermore, the friable nature of callus obtained at optimal IAA and kinetin concentrations is advantageous for Agrobacterium-mediated transformation, protoplast isolation, and mutagenesis studies.[7]

Overall, the results support the classical concept that the auxin-to-cytokinin balance determines morphogenic outcomes. Kinetin favored callus proliferation, while BAP enhanced shoot induction, validating the decisive role of cytokinin type in morphogenesis. These outcomes agree with the conclusions of Long et. al.[8], Pruski et al.[9], and Raza et al. [10], who also reported that the ratio, concentration, and nature of plant growth regulators critically influence the direction of morphogenic responses.

In addition, recent studies further strengthen these observations. Cytokinin-specific signaling has been shown to reprogram cell fate and accelerate meristem establishment in tomato explants[11], while auxin–cytokinin crosstalk has been identified as a key regulator of shoot induction efficiency in Solanaceae cultures[12]. Moreover, comparative morphogenic analyses consistently demonstrate that BAP outperforms kinetin in promoting shoot organogenesis across diverse tomato genotypes, supporting the superior response observed in the present study. Recent advancements in tomato regeneration research further reinforce these findings: efficient shoot and root formation from cotyledon and protoplast-derived tissues has been achieved through optimized culture systems, confirming that the cytokinin–auxin balance is a central to directing morphogenic transitions[13]. Together, these evidences validate the present results and emphasize the decisive role of cytokinin type and auxin–cytokinin interactions in determining morphogenic outcomes in tomato tissue culture.

5. Conclusion

This study established a simple and reproducible regeneration protocol for tomato using stem nodal cuttings. Optimal callus induction was achieved using IAA with kinetin, whereas IAA combined with BAP promoted efficient shoot organogenesis. IBA (1.0 mg/L) was most

effective for rooting regenerated shoots. These results confirm that variations in the type and combination of growth regulators profoundly affect morphogenic responses in tomato. Overall, the protocol developed here minimizes medium complexity, enhances reproducibility, and provides a reliable platform for downstream applications such as genetic transformation, mutation breeding, and genome editing. By offering high regeneration efficiency with a single explant type, the method can contribute significantly to future tomato crop improvement and biotechnology research.

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All the authors contributed equally to the work and have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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Data Availability

Data supporting this study is available from the corresponding author upon reasonable request.

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Evaluation of chemically characterized *Citrus sinensis* L. essential oil as botanical fungitoxicant against fungal deterioration of stored mustard oilseeds

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The study deals with the bioactive efficacy of *Citrus sinensis* L. essential oil (CSEO) against some storage fungi contaminating stored oilseeds of mustard (*Brassica compestris* L.). The average pH and percent moisture content of collected stored oilseeds of mustard ranged from 5.36 to 5.43 and 9.22 to 10.03%, respectively. Stored oilseeds of mustard were found associated with various storage moulds. During mycological screening of oilseeds, a total of 642 fungal isolates was recovered from three different stored samples. The percent occurrence frequency of sample 3 was found to be the highest (37.85%), whereas sample 1 exhibited the lowest (29.12%). The highest cumulative percent relative density was recorded in *Cladosporium* sp. (21.65%), followed by *Aspergillus niger* (18.06%) and *Aspergillus flavus* (11.37%), while the lowest relative density was found in *Aspergillus nidulans* (1.40%), followed by 1.86% in both *Aspergillus candidus* and *Aspergillus terreus*. The minimum inhibitory concentration (MIC) of CSEO against Aspergillus flavus was recorded at 100 µg/ml. CSEO also exhibited broad-spectrum fungitoxicity and was also comparable to the synthetic fungicide diphenylamine as well as having significant antioxidant activity (IC50 22.82). The chemotype of CSEO was determined by GC/GC-MS analysis, which showed 26 constituents. DL-Limonene was found to be the major component (90.44%), followed by linalyl acetate (2.80%) and β-myrcene (1.71%), whereas other compounds were in traces. The prospects of exploitation of CSEO as an acceptable plant-based additive in qualitative as well as quantitative control of biodeterioration of stored oilseeds have been discussed.

Keywords: *Citrus sinensis*, essential oil, antifungal, *Aspergillus flavus*, DL-Limonene, *Brassica compestris*, oilseeds.

1. Introduction

Oilseeds are among the most important agricultural commodities worldwide, serving as a primary source of edible oils, proteins, nutraceuticals, and industrial raw materials. However, during storage, these oilseeds are highly vulnerable to microbial deterioration, particularly by fungal species such as *Aspergillus*, *Penicillium*, *Fusarium, Cladosporium, Rhizopus, Mucor*, etc.[1,2]. Fungal colonization not only reduces the nutritional and germinative quality of oilseeds but also poses severe health hazards through the production of several mycotoxins[3]. These mycotoxins are known to be carcinogenic, hepatotoxic, and immunosuppressive, thereby representing a serious threat to food safety and global trade[4,5]. Conventional strategies to manage fungal spoilage primarily rely on synthetic fungicides and chemical preservatives.

Despite their effectiveness, the long-term and indiscriminate use of such chemicals is under increasing criticism due to their persistence in the environment, contribution to fungicide resistance, hazardous residues in food and adverse impacts on human health[6]. Consequently, there is a growing need for safer, eco-friendly, and sustainable alternatives to protect stored oilseeds from fungal deterioration.

Essential oils (EOs), volatile secondary metabolites obtained from aromatic plants, have attracted significant attention as promising natural preservatives. They are generally recognized as safe (GRAS), biodegradable, and exhibit broad-spectrum antimicrobial and antifungal properties[7]. The bioactivity of EOs is largely attributed to their chemically diverse constituents, including monoterpenes, sesquiterpenes, alcohols, aldehydes, and phenolic compounds[8]. Several aromatic plant EOs rich in bioactive compounds such as limonene, linalool, citral, ocimine, thymol, menthol, eugenol etc. have been reported to possess potent antifungal, antioxidant, and insecticidal activities[9,10].

The literature on the antifungal activity of plant Eos focusing on their application in stored oilseeds remain relatively limited. Furthermore, reports comprehensively correlate the chemical characterization of EOs with its preservative efficacy under simulated storage conditions[10,11]. Such studies are essential to establish scientific evidence supporting the integration of EOs into sustainable storage practices. The present study was therefore designed to evaluate the chemically characterized *C. sinensis* L. essential oil (CSEO) for its antifungal efficacy against common fungi associated with stored oilseeds for development as a "green preservative" in postharvest management of oilseeds.

2. Materials and Methods

2.1. *Collection of oilseed samples*

Three different yellow mustard seed samples (500g) of about 4-6 months of storage were procured from the local retailers of Gorakhpur, India. The oilseeds were collected in sterilized polythene bags to avoid further contamination and stored at 5°C for further analysis[12].

2.2. *Moisture content and pH determination*

To determine the moisture content**,** a representative portion (≈ 50 g) of the mustard seed samples were weighed accurately and dried in a hot air oven at 100 ± 2 °C for 24 hours until a constant weight is obtained. The percentage of moisture is then calculated using the formula:

$$\text{Moisture content (\%)} = (W_1\text{-}W_2 / W_1) \times 100$$

Where W_1 is the initial weight and W_2 is the final weight after drying.

For pH determination**,** 10 g of each grounded mustard seeds were mixed separately with 100 ml of distilled water (1:10 w/v ratio), shaken for 30 minutes, and allowed to stand for 1 hour. The supernatant suspension is then filtered, and the pH is measured using a calibrated digital pH meter at room temperature[13].

2.3. *Mycobiota analysis of collected mustard seed samples*

Ten grams of each finely ground mustard seed samples were suspended separately in 90ml sterile 0.85% saline solution in an Erlenmeyer flask (250 ml) and homogenized on an electric shaker with a constant speed (120 rpm) for 15 min. Three-fold serial dilutions were prepared separately for each oilseed sample[13]. To inoculate the Petri dishes with 10 ml of freshly prepared potato dextrose agar (PDA) medium, 0.5 ml of the dilution (10^{-3}) was utilized followed by incubation for seven days at 27±2°C. On the third day of incubation, the colony counting process began. Each morphologically distinct mold colony was subcultured on PDA and identified[14,15].

2.4. *Detection of aflatoxigenic potential of isolated Aspergillus flavus*

Ten isolates of *A. flavus* from each mustard samples were randomly selected and tested for their aflatoxigenic potency using SMKY (Sucrose, 200.0 g; Magnesium sulphate, 0.5 g; Potassium nitrate, 0.3 g; Yeast extract, 7.0 g; Distilled water, 1000 ml; pH, 5.6±0.2) as broth nutrient medium[16]. One ml spore suspension (≈10^6 spores ml^{-1}) of each *A. flavus* isolate in 0.1% Tween-80 was inoculated aseptically

to 50 ml SMKY medium and incubated at 27±2 °C for 10 days. After incubation, the content of each flask was filtered (Whatman no. 1). Filtrate of each flask was separately extracted with 40 ml of chloroform in a separating funnel. The chloroform extract was separated and dehydrated with anhydrous sodium sulphate and evaporated till dryness on water bath at 70 °C. The residue left after evaporation was re-dissolved in 1 ml of chloroform and 100 µl of it was spotted on TLC plate (20×20 cm^2 of silica gel). The plate was then developed in Toluene:Isoamylalcohol: Methanol (90:32:2;v/v/v) solvent system[17,18]. The intensity of AFB_1 was observed in an ultraviolet fluorescence analysis cabinet at an excitation wavelength of 360 nm[19]. For quantitative estimation, blue spots of AFB_1 on TLC were scraped out and dissolved in 5 ml cold methanol and centrifuged at 3000 xg for 5 min. Optical density of supernatant was recorded at 360 nm and the amount of AFB_1 was calculated[18].

$$\text{Aflatoxin } B_1 \text{ content } (\mu gL^{-1}) = \frac{D \times M}{E \times l} \times 1000$$

Where, D-absorbance; M-molecular weight of AFB_1 (312); E-molar extinction coefficient of AFB_1 (21,800); *l*-path length (1 cm cell was used).

2.5. *Extraction of Citrus sinensis peel essential oil*

Peels of *Citrus sinensis* (L.) Osbeck fruits were collected from juice shops in Gorakhpur for the extraction of essential oil. Peels (500 g) were thoroughly washed with distilled water and subjected to Clevenger's hydrodistillation apparatus for three hours. The hydrophobic volatile fraction, *i.e.*, *C. sinensis* peel EO (CSEO) was separated followed by dehydration using sodium sulphate and stored in dark clean glass vial at 4-5 °C[18].

2.6. *GC-MS analysis of CSEO*

The CSEO was analyzed through gas chromatography (Perkin Elmer Auto XL GC) equipped with a flame ionization detector. The GC conditions were as follows: column, EQUITY–5 (60m × 0.32mm × 0.25µm) fused silica capillary column; H_2 was the carrier gas; column Head pressure 10 psi; oven temperature program isotherm 2 min. at 70ºC, 3ºC/min. gradient to 250ºC, isotherm 10 min; injection temperature, 250ºC; detector temperature 280ºC. GC-MS analysis was also performed using Perkin Elmer Turbomass GC-MS. The GC conditions were as follows: Injection temperature, 250ºC; column temperature, isothermal at 70ºC for 2 min, then programmed to 250ºC at 37ºC/min and held at this temperature for 10 min; ion source temperature, 250ºC. Helium was used as the carrier gas. The effluent of the GC column was introduced directly into the source of MS. Spectra was obtained in the EI mode with 70ev ionization energy. The compounds were identified by comparison of their relative retention times and the mass spectra with those of authentic reference compounds shown in literature[20].

2.7. *Antifungal and antiaflatoxigenic activity of CSEO*

Minimum inhibitory concentration (MIC) and antiaflatoxigenic efficacy of CSEO was determined against most potent toxigenic isolate *A. flavus* DDUBC2-4 using SMKY broth medium. Different concentrations of the CSEO, *viz*., 20, 40, 60, 80 and 100µg/ml were prepared separately by dissolving their requisite amount in 0.5 ml 5% tween-20 and then mixing it with 49.5 ml of SMKY medium in 150 ml Erlenmeyer flask. The control sets were kept parallel to the treatment sets without CSEO. The flasks were inoculated aseptically with 1 ml spore suspension (≈10^6 spores/ml) of *A. flavus* DDUBC2-4 and incubated at 27±2 °C for 10 days. After incubation, mycelial biomass and aflatoxin B_1 content in broth medium of each flask was determined[17].

2.8. *Fungitoxic spectrum of CSEO*

The spectrum of fungitoxicity of the CSEO was determined at 100 µg/ml by the poisoned food technique using PDA against 11 isolated fungal species *viz*. *Alternaria* sp., *Aspergillus candidus*, *A. fumigatus*, *A. nidulans, A. niger*, *A. terreus*, *Bipolaris* sp., *Cladosporium* sp., *Curvularia lunata*, *Fusarium oxysporum* and *Penicillium* sp. from oilseeds during mycological analysis[13].

2.9. *Comparative efficacy of CSEO with some prevalent fungicides*

A few widely used synthetic fungicides, including benzimidazole (Benomyl), carbendazim 50%WP (Bavistin), diphenylamine (DPA), mencozeb (Dithane M-45), organo-mercurial dust (Agrosan GN) and Sulfur 80%WP (Wettasul-80) were compared to CSEO's fungitoxic effectiveness. 10, 50 and 100 mg/ml were the final concentrations that

were prepared by suspending the necessary amounts of the fungicides in 0.5 ml Tween-20 (5%) followed by 9.5 ml pre-sterilized melted PDA culture medium. Their MICs against toxigenic *A. flavus* DDUBC2-4 was ascertained by usual poisoned-food technique[16].

2.10. Antioxidant activity of CSEO

2.10.1. DPPH radical scavenging assay

To determine the antioxidant activity of CSEO, 50 µl (1:10 dilution in methanol) was applied on TLC plate and developed in ethyl acetate and methanol (1:1). The plate was sprayed with 0.2% DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in methanol and left at room temperature for 30 min. Yellow spot developed due to bleaching of purple color of DPPH reagent, was recorded as positive antioxidant activity of CSEO[21].

2.10.2. Free radical scavenging activity

The magnitude that CSEO bleached the DPPH solution from purple to yellow was utilized to measure their ability to scavenge free radicals. Two-fold concentrations (1.0 to 64.0 µg/ml) of CSEO were prepared separately using 0.004% DPPH solution in methanol (5 ml) and incubated at room temperature for 30 min. Absorbance of the samples were recorded at 517 nm against a blank[21]. The free radical scavenging potential of CSEO was compared with positive control i.e. ascorbic acid.

Free radical scavenging activity (%) = $[(A_{blank} - A_{sample})/ A_{blank}] \times 100$

Where:

A_{blank} - absorbance of the blank (without any test compound); A_{sample} - absorbance of different tested samples

2.11. Statistical analysis

All experiments were conducted in triplicates, and data were expressed as mean ± standard error (SE). Statistical analysis was performed using SPSS software (SPSS 16.0; IBM, NY, USA). Differences between treatments were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A *p*-value of less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1. pH and moisture content

The collected mustard seed samples exhibited slight variation in their physical appearance, moisture content, and pH values, reflecting differences in storage duration and environmental conditions. The pH of crushed oilseed suspensions varied from 5.36±0.04 to 5.43±0.07 (Table 1), showing a slightly acidic nature favorable for fungal colonization. Moisture content of the samples ranged from 9.22±0.34% in sample 2 to 10.03±0.21% in sample 3 (Table 1), indicating lesser storage moisture for oilseeds but sufficient to support fungal growth under prolonged storage. Slightly acidic pH and higher moisture content create favorable conditions for fungal proliferation and mycotoxin production in stored oilseeds, particularly under hot and humid climates. Moisture levels above 8–10% raise seed water activity, promoting the growth of storage fungi such as *Aspergillus*, *Penicillium*, *Fusarium* species etc.[22,23,24]. These storage fungi flourish at slightly lower pH, reduce seed defense activity and enhance fungal enzyme activity, facilitating colonization[1,25]. High temperature and humidity accelerate respiration and lipid peroxidation, further deteriorating seed quality and encouraging mycotoxin synthesis—especially aflatoxins and ochratoxins—by *Aspergillus flavus* and *A. ochraceus*[26,27]. Thus, maintaining optimal moisture content (<7%) and neutral pH during storage is essential to suppress fungal and mycotoxin contamination in oilseeds in tropical conditions.

Table 1: pH and moisture content (%) of collected stored mustard seeds

Mustard seed samples	pH	Moisture content (%)
Sample 1	5.36±0.04[a]	9.56±0.34[a]
Sample 2	5.43±0.07[a]	9.22±0.36[a]
Sample 3	5.41±0.09[a]	10.03±0.21[a]

Values are mean (n = 3) ± SE; P < 0.05. The means followed by same letter in the same column are not significantly different according to One-Way ANOVA and Tukey's multiple comparison tests

3.2. Mycological analysis mustard oilseeds

Mycological examination of the collected mustard oilseeds revealed the occurrence of diverse fungal flora. Sample 1 showed the lowest occurrence frequency (29.12%) while highest (37.85%) in sample 3 (Table 2). The variation in occurrence frequency among samples, suggests differences in storage conditions such as moisture, temperature, and aeration that influenced fungal colonization. A total of 12 identified fungal species belonging to six genera were consistently isolated on Potato Dextrose Agar (PDA). The mycological analysis reflects the susceptibility of oilseeds to colonization by a wide range of storage fungi, particularly under suboptimal storage environments. Collectively, the highest relative density was shown by *Cladosporium* sp. (21.65%) whereas, lowest in *Aspergillus nidulans* (1.40%). The predominance of *Cladosporium* sp. is consistent with its role as a common airborne and surface contaminant thriving under moderate humidity[22,28,29]. The genus *Aspergillus* with six species was dominant during analysis and occupied 41.40% of total fungal isolates. Such dominance by *Aspergillus* indicates their adaptability to oil-rich substrates and warm, humid conditions typically prevailing during storage in tropical climates[25]. The presence of *A. nidulans* with a minimal relative density (1.40%) suggests that not all *Aspergillus* species are equally competitive in the given ecological niche. A total of 9.65% fungal isolates recovered during study were unidentified (Table 2) and point to potential novel or less-characterized fungal species that may require molecular identification for confirmation.

Table 2: Mycobiota analysis of collected stored mustard seed samples

Isolated Fungi	Mustard Sample 1	Mustard Sample 2	Mustard Sample 3	Total isolates	Relative density (%)
Alternaria sp.	7	6	6	19	**2.95**
Aspergillus candidus	4	2	6	12	**1.86**
Aspergillus flavus	19	24	30	73	**11.37**
Aspergillus fumigatus	14	11	19	44	**6.85**
Aspergillus niger	32	41	43	116	**18.06**
Aspergillus terreus	3	6	3	12	**1.86**
Aspergillus nidulans	2	2	5	9	**1.40**
Bipolaris sp.	9	12	15	36	**5.60**
Cladosporium sp.	39	43	57	139	**21.65**
Culvularialunata	9	11	12	32	**4.98**
Fusarium sp.	13	19	17	49	**7.63**
Penicillium sp.	14	10	15	39	**6.07**
Unidentified	22	25	15	62	**9.65**
Mucorales*	4	2	2		
Total isolates	**187**	**212**	**243**	**642**	
Occurrence frequency (%)	**29.12**	**33.02**	**37.85**		

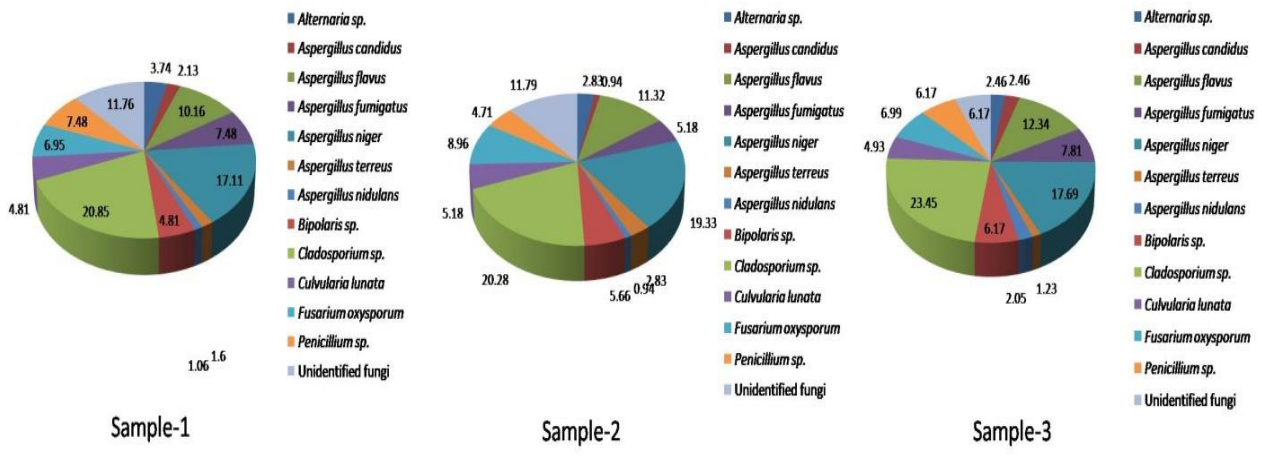

Figure 1. Mycobiota analysis of stored mustard oilseeds

3.3. Detection of aflatoxigenic isolates of A. flavus

Ten isolates of *Aspergillus flavus* were randomly screened for aflatoxin production from each oilseed sample using TLC method revealed a significant toxigenic potential among the fungal populations associated with stored mustard oilseeds. Out of 10 isolates, sample 1, 2 and 3 exhibited 4 (40%), 3 (33.33%) and 5 (50%) toxigenic isolates respectively with intense blue fluorescence under UV light at 365 nm (Table 3). The highest aflatoxin B_1 production (1511.34 µg/L) was reported from isolate *A. flavus* DDUBC2-4 (Table 3) highlights its superior toxigenic capacity and selected as test fungus for further study. The predominance of *A. flavus* as both a frequent and toxigenic species emphasizes its ecological fitness and preference for lipid-rich substrates like mustard seeds, particularly under warm and humid storage conditions conducive to aflatoxin biosynthesis[3,24]. Similar findings have been reported by earlier workers[30,31,32], where, *A. flavus* was identified as a major contaminant in oilseeds with significant aflatoxin production potential.

Table 3: Toxigenicity of *Aspergillus flavus* isolated from selected mustard oil seeds

Mustard sample 1		**Mustard sample 2**		**Mustard sample 3**	
Fungal isolates	AFB_1 (µgL^{-1})	Fungal isolates	AFB_1 (µgL^{-1})	Fungal isolates	AFB_1 (µgL^{-1})
A. flavus DDUBC1-1	-	*A. flavus* DDUBC2-1	1036.18	*A. flavus* DDUBC3-1	-
A. flavus DDUBC1-2	-	*A. flavus* DDUBC2-2	-	*A. flavus* DDUBC3-2	-
A. flavus DDUBC1-3	40.07	*A. flavus* DDUBC2-3	-	*A. flavus* DDUBC3-3	1248.00
A. flavus DDUBC1-4	1162.13	***A. flavus* DDUBC2-4***	**1511.34**	*A. flavus* DDUBC3-4	538.13
A. flavus DDUBC1-5	-	*A. flavus* DDUBC2-5	-	*A. flavus* DDUBC3-5	-
A. flavus DDUBC1-6	-	*A. flavus* DDUBC2-6	1139.23	*A. flavus* DDUBC3-6	612.55
A. flavus DDUBC1-7	618.28	*A. flavus* DDUBC2-7	-	*A. flavus* DDUBC3-7	-
A. flavus DDUBC1-8	1013.28	*A. flavus* DDUBC2-8	-	*A. flavus* DDUBC3-8	417.91
A. flavus DDUBC1-9	-	*A. flavus* DDUBC2-9	-	*A. flavus* DDUBC3-9	641.17
A. flavus DDUBC1-10	-	*A. flavus* DDUBC2-10	-	*A. flavus* DDUBC3-10	-

* Fungal isolate ***A. flavus* DDUBC2-4** from Mustard sample 2exhibited the aflatoxin B1 producing potential

3.4. Chemical characterization of CSEO

EO extracted from *C. sinensis* peels via hydro-distillation yielded 2.4±0.2% (v/w) of very light greenish-yellow aromatic oil indicating efficient recovery of volatile compounds typical of *Citrus* species. Gas Chromatography–Mass Spectrometry (GC–MS) profiling of CSEO revealed the presence of 26phytochemical constituents, accounting for 98.96% of the total composition. DL-Limonene (90.66%) was found as dominant compound followed by linalyl acetate (2.80%) and β-myrcene (1.71%) while, rest other constituents were in traces (Table 4). The predominance of DL-limonene aligns with previous reports describing it as the major monoterpene hydrocarbon responsible for the characteristic citrus aroma and potent antioxidant, antimicrobial, and antifungal activities of CSEO[33,34,35]. The presence of minor constituents such as linalyl acetate and β-myrcene further contributes to the EO's bioactivity and fragrance profile[36]. The dominance of oxygenated monoterpenes and terpenoids suggests that the EO may possess significant biological potential, particularly as a natural preservative or antifungal agent[34].

Table 4: Chemical composition of CSEO

Sr No.	**Retention time (RT)**	**Retention index (RI)**	**Compounds**	**Percentage (%)**
1	9.52	931	α-Pinene	0.34
2	10.87	962	Sabinene	0.37
3	11.05	968	β-Pinene	0.03
4	11.17	972	Methyl heptenone	0.01
5	11.24	992	Octanal	0.42
6	11.35	1020	β-Myrcene	1.71

7	12.05	1028	α-Phyllandrene	0.03
8	12.27	1035	β-Ocimene	0.23
9	12.50	1046	α-Terpinene	0.04
10	**13.20**	**1057**	**DL-Limonene**	**90.66**
11	13.65	1062	Cis-Ocimene	0.03
12	14.55	1068	Caprylic alcohol	0.05
13	15.52	1078	α-Terpinolene	0.18
14	15.78	1128	Nonanal	0.05
15	15.85	1137	Cosmene	0.03
16	15.94	1236	Linalyl acetate	2.80
17	22.80	1253	DL-Carvone	0.07
18	22.52	1267	Z-Citral	0.09
19	17.65	1306	Myrtenyl acetate	0.05
20	18.35	1438	t-Sabinine hydrate	0.37
21	19.67	1484	Decanal	0.02
22	20.77	1574	Isopulegol	0.26
23	21.95	1717	Geranyl formate	0.62
24	23.87	1737	β-citronellol	0.21
25	24.12	1739	6-isopropenyl-3-methyl-2-Cyclohexene-1-one	0.26
26	24.22	1777	Perillaldehyde	0.03
			Total	**98.96**

3.5. Antifungal and antiaflatoxigenic efficacy of CSEO

CSEO showed potent fungitoxicity against *A. flavus* DDUBC2-4 and its minimum inhibitory concentration (MIC) was recorded at 100 mgmL^{-1}. In addition, CSEO was also found efficient to inhibit the AFB_1 production by *A. flavus* DDUBC2-4 and completely checked at 60 mgmL^{-1} (Table 5). A direct relation was found between fungal growth and AFB_1 production i.e. decreases in mycelial biomass resulted in low AFB_1 production and vice versa.

Table 5: Antifungal and antiaflatoxigenic activity of CSEO against *A. flavus* DDUBC2-4

Concentration (mg ml^{-1})	Mycelial Biomass (g)	Aflatoxin B_1 content (µg L^{-1})
Control	0.503±0.026^{c}	1483.670 ± 78.914^{c}
20	0.130±0.014^{b}	305.320 ± 53.431^{b}
40	0.050±0.009^{a}	111.633 ± 22.358^{ab}
60	0.030±0.006^{a}	0.000±0.000^{a}
80	0.021±0.006^{a}	0.000±0.000^{a}
100	0.000±0.000^{a}	0.000±0.000^{a}

Values are mean (n = 3) ± SE; P < 0.05. The means followed by same letter in the same column are not significantly different according to One Way ANOVA and Tukey's comparison tests

The observed inverse relationship between fungal biomass and AFB_1 production supports earlier findings that toxin biosynthesis is growth dependent and can be significantly reduced by disrupting cellular and metabolic processes[25]. One of the most widely recognized mechanisms is disruption of fungal cell membrane and cell wall integrity. Lipophilic EO components such as limonene, thymol, carvacrol, and citral penetrate the lipid bilayer, increasing membrane permeability, causing leakage of vital cellular contents (ions, proteins, nucleic acids), and leading to cell lysis[37,38]. Inhibition of ergosterol biosynthesis, a key structural sterol in fungal membranes, also weakens cell structure and disrupts membrane bound enzyme activity[39]. EOs also interfere with mitochondrial function and energy metabolism, reducing ATP synthesis and impairing fungal growth to inhibit respiratory chain enzymes, leading to oxidative stress and accumulation of reactive oxygen species[39]. The inhibition of mycotoxin biosynthetic pathways is another critical mechanism, certain EO constituents downregulate aflatoxin biosynthetic genes, thereby reducing toxin formation even at sub-inhibitory concentrations[40,41].

3.6. Fungitoxic spectrum of CSEO

CSEO exhibited broad fungitoxic spectrum against some other storage fungi. It completely checked the proliferation of all the tested fungal species at 100 mgml^{-1} (MIC against *A. flavus* DDUBC2-4) except *Alternaria* sp. (86.89±0.92%), *Bipolaris* sp. (83.70±0.94%), *Cladosporium* sp. (74.67±6.58%), *Curvularia lunata* (85.42±0.74%) and *Fusarium oxysporum* (88.70±1.01%) whereas, at 200 mg mL^{-1} (2×MIC against *A. flavus* DDUBC2-4) could not completely inhibit *Alternaria* sp. (94.85±2.59%), *Cladosporium* sp. (89.34±1.07%) and *Curvularia lunata* (94.44±2.78%) (Table 6). The broad-spectrum fungitoxic activity of CSEO against various storage fungi suggests that its bioactive constituents, particularly DL-limonene and linalyl acetate, may interfere with membrane integrity and enzyme systems necessary for fungal growth[8,33]. The partial inhibition of some fungal species even at higher concentrations (2×MIC) reflects variability in fungal susceptibility, likely due to differences in cell wall composition and metabolic adaptation. CSEO as fungitoxicant was evaluated to be less efficacious compared to tested synthetic fungitoxicants except wettasul-80 (> 100 mg mL^{-1}) while MIC of Diphenylamine (100 mg mL^{-1}) against *A. flavus* DDUBC2-4 was found comparable to CSEO (Table 7), highlighting its potential as a natural alternative for controlling fungal contamination and aflatoxin production in stored oilseeds. Overall, the results support the promising role of CSEO as an eco-friendly fungitoxicant with significant antitoxigenic potential.

Table 6: Fungitoxic spectrum of CSEO against some storage fungi

Test Fungi	Percent inhibition	
	MIC (100 mgmL^{-1})	2×MIC (200 mgmL^{-1})
Alternaria sp.	86.89 ± 0.92^{b}	94.85 ± 2.59^{ab}
Aspergillus candidus	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}
Aspergillus fumigatus	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}
Aspergillus nidulans	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}
Aspergillus niger	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}
Aspergillus terreus	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}
*Bipolaris*sp.	83.70 ± 0.94^{ab}	100.00 ± 0.00^{b}
Cladosporium sp.	74.67 ± 6.58^{a}	89.34 ± 1.07^{a}
Curvularialunata	85.42 ± 0.74^{ab}	94.44 ± 2.78^{ab}
Fusarium oxysporium	88.70 ± 1.01^{b}	100.00 ± 0.00^{b}
Penicillium sp.	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}

Values are mean (n = 3) ± SE; P < 0.05. The means followed by same letter in the same column are not significantly different according to One Way ANOVA and Tukey's comparison tests

Table-7: Comparative fungitoxicity of CSEO with some prevalent synthetic fungicide

Fungicides	**MIC against *A. flavus* (mg ml^{-1})**
Benzimidazole **(**Benomyl)	20
Carbendazim 50%WP (Bavistin**)**	40
Diphenylamine (DPL)	100
Mencozeb (Dithane M-45)	40
Organo-mercurial dust (Agrosan GN)	20
Sulfur 80%WP (Wettasul-80)	> 100
CSEO	100

3.7. Antioxidant activity of CSEO

The antioxidant activity of CSEO was evaluated using standard *in vitro* DPPH assays. The results revealed that CSEO exhibited considerable free radical scavenging activity (IC_{50} 22.82 µg mL^{-1}) showed the comparatively lower antioxidant potential against synthetic antioxidant ascorbic acid (IC_{50} 8.64 µg mL^{-1}) as positive control (Figure 2). DL-limonene, the predominant monoterpene constituent of CSEO, and minor compounds like linalyl acetate and β-myrcene are largely responsible for its notable activity. These compounds work together to donate electrons and stabilize reactive oxygen species[33,42,43]. The moderate IC_{50} value suggests that CSEO could serve as a promising natural antioxidant source with potential applications in food preservation and pharmaceutical formulations, offering a safer and eco-friendly alternative to synthetic antioxidants[38].

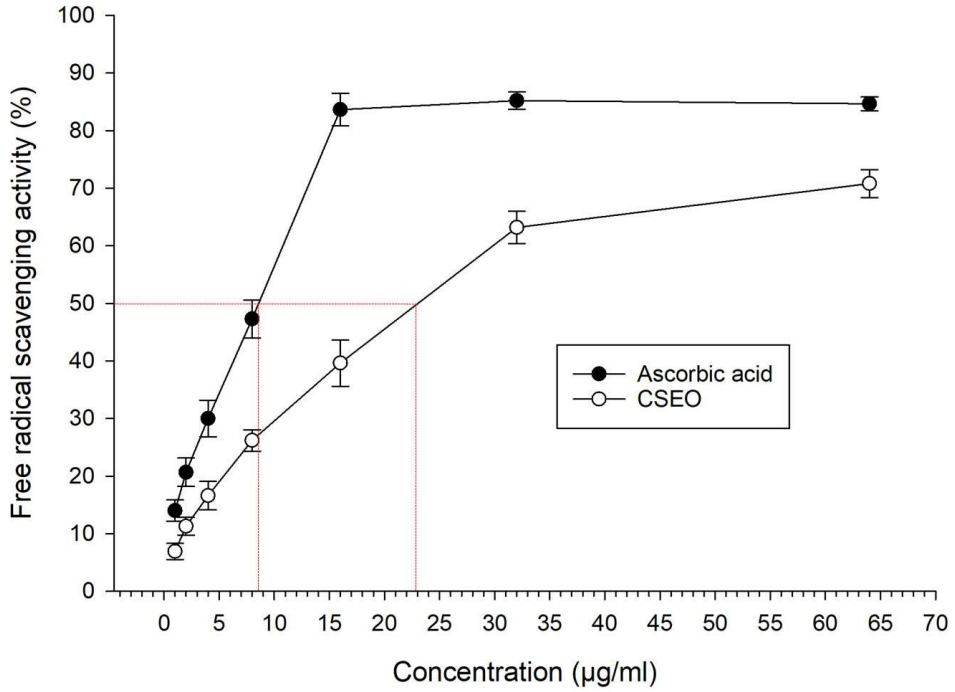

Figure 2. Comparative antioxidant activity of CSEO with ascorbic acid

4. Conclusion

The findings of this study provide a strong basis for the application of CSEO as a natural, multi-functional preservative for oilseeds and other stored commodities. CSEO demonstrated pronounced antifungal, antiaflatoxigenic, and antioxidant activities attributable to its high limonene content, can help minimize postharvest losses and aflatoxin contamination in storage systems. The EO's broad-spectrum fungitoxicity and efficacy comparable to commercial fungicides underscore its potential as an eco-friendly biopreservative for safe storage of mustard oilseeds. These findings support the integration of CSEO into botanical fungicide formulations as sustainable alternatives to synthetic chemicals in postharvest management systems. Future studies should focus on the microencapsulation or vapor-phase application of CSEO to enhance its stability and long-term efficacy under commercial storage conditions.

Author contributions

PP conducted the experiment and wrote the original draft of the paper. **SK** performed mycological analysis and fungal identification. **AK** conceptualized and supervised the whole experimental work as well as editing and corrections in the original draft. All the authors have read and agreed to the final version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Funding

Nothing to declare.

Data Availability

Data supporting this study is available from the corresponding author upon reasonable request.

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Severe Infestation of Killer Mistletoe on *Toona ciliata* in New Forest, Dehradun, Uttarakhand, India

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Abstract
Toona ciliata M. Roem. is a big tree with various economic uses. Mistletoes are partial parasites that attack a number of commercially important tree crops, resulting in the declining quality and quantity of the end product. In the Forest Research Institute, Dehradun, Toon trees are planted as avenue trees at different locations and are infested by mistletoe. The aim of the study was to identify the mistletoe species and to assess the infestation. The mistletoe species was identified as *Scurrula pulverulenta* (Wall.) G. Don. A study reveals varying levels of mistletoe infestation in the branches of Toon trees. In most cases, the infestation is in the initial stages, though some trees showed severe infestation. Suitable management strategies to check infestation are suggested.

Keywords: Infestation, Mistletoe, Toon tree, Genetic Improvement, Management Strategies.

1. Introduction
Toona ciliata M. Roem. is a large deciduous tree with multiple uses. It is commonly known as 'Toon,' Red Cedar, or Indian Mahogany and belongs to the family Meliaceae. It grows up to 25-35 m with a girth of 2 to 3 m, producing a clean bole up to 9-12 m under ideal forest conditions. It is widely spread in Asia between 15° and 25°N in India, Bangladesh, Burma, China, Indonesia, Malaysia, and the Philippines at elevations of 0-1500 m above sea level. In India, it occurs throughout the sub-Himalayan tract and valleys of the outer Himalaya from Jammu eastwards, the plains of Bihar, West Bengal, the Khasi hills, and the valleys of the eastern Ghats in moist localities, the hills of Kurnool, Karnataka, and the Western Ghats[1]. It is usually cultivated as an avenue tree in India.

The species is well known for its versatile timber, which is utilized for construction purposes, shipbuilding, and wood-based goods, like furniture, musical instruments, and carvings[2, 3]. The flowers are used as a pollen and nectar source for honeybees. Economically, the flowers contain nyctanthin, quercetin, and a flavone, which yield a red and yellow dye known as 'Basanti' used to dye cotton and woolen fabrics[4, 5]. The bark is a powerful astringent, a tonic, and an antiperiodic, and it is used to treat dysentery and wounds. The fruit produces aromatic oil[4, 6]. Further, the seeds, leaves, and stems of *T. ciliata* are used in traditional Chinese medicine for the treatment of diarrhea, dysentery, and ringworm[7, 8]. The leaves and young shoots are lopped for cattle fodder in India. Various parts are used medicinally throughout their geographical range.

Mistletoes are partial parasites that infest various tree species[7]. They cause great damage in natural and plantation forests, orchards, and parks throughout the world. They severely damage their host plants in various ways, such as quality of timber, fruit, and oil content, etc.[8].

Materials and Methods

The study was conducted during September 2024 at the Forest Research Institute Campus, Dehradun (Uttarakhand State, India). The average annual rainfall is 2118 mm, of which 80 percent is received from the southwest monsoon during July, August, and September, and the remainder from the retreating monsoon in the cold weather. Except for May and June, relative humidity is well over 40 percent. Maximum temperature reaches up to 44°C and minimum up to 1°C. It has an elevation of 640 m and lies at N latitude 30° 20' 37.7" and E longitude 77° 59' 52.1". *Toona ciliata* is planted in various places at the Forest Research Institute, Dehradun. A survey was conducted for infestation of mistletoes on the *Toona ciliata*. Mistletoe species were systematically studied, and descriptions were recorded. The species was authenticated from the DD Herbarium, Forest Research Institute, Dehradun (DD62161).

Result and Discussion

It was observed that most of the trees of Toon were infested by mistletoes in varying degrees. In most of the species' the infestation was in the initial stage (Fig. 1). However, some of the trees were at a high level of infestation. Mistletoe was identified as *Scurrula pulverulenta* (Wall.) G. Don., which is locally known as 'Banda.'

Figure 1. Infestation of *Scurrula pulverulenta* on branches of *Toona ciliata*

Species Description

It is a stout, woody parasite with dark-grey bark. Leaves opposite, 10-15 cm long, broadly ovate or ovate-oblong, coriaceous, thickly mealy-tomentose when young, base acute or rounded; petiole 1.2-1.7 cm long. Racemes 1.2-6 cm long, axillary, solitary or fascicled. Flowers 2.5 cm long. Pedicels 0.5-0.7 cm long, grey and scurfy outside. Calyx-limb 0. Corolla slender, tubular, and curved; segments 4, linear, and green. Style is very slender. Fruit 0.7 cm long, turbinate, grey, and tomentose. Flowers: September-May, and probably throughout the year.

Various economically important tree species are infested by mistletoes, and reports of such infestations are reported by a number of researchers[9-14]. In the present investigation, infestation of *S. pulverulenta* was observed on branches of Toon trees in various locations. It was observed that infestation was in initial stage, however, in few trees, it was observed in advance stage. In the future, it can pose a serious problem; therefore, it is essential to adopt suitable management strategies to curb the spread of this mistletoe.

Various economically important tree species are infested by mistletoes, and reports of such infestations are reported by a number of researchers[14-18]. In the present investigation, infestation of *S. pulverulenta* was observed on branches of Toon trees in various locations. It was observed that infestation was in the initial stage; however, in a few trees, it was observed in an advanced stage. In the future, it can pose a serious problem; therefore, it is essential to adopt suitable management strategies to curb the spread of this mistletoe.

Conclusion

Various tree species are infested by the killer. Mistletoes and the quality and quantity of utilizable products are seriously deteriorated. Toon is an economically important species of multiple uses. To check the mistletoe infestation, apt management practices should be implemented. Elimination of mistletoes manually may be the best option for curbing infestation. In an advanced stage, it is not possible to remove mistletoe, and eventually the tree will die. Hence, it is suggested that mistletoes should be eliminated in initial stages so that further damage can be checked. A genetic improvement program should be taken for developing superior mistletoe-resistant genotypes, and a new plantation should be raised from such superior material.

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Dose-Dependent Effects of Zinc Oxide Nanoparticles on Growth, Antioxidative Enzymes, and Yield of Pearl Millet

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Abstract

Zinc is an essential micronutrient required for optimal plant growth, metabolism, and yield; however, zinc deficiency remains a major constraint to cereal production under intensive agricultural systems. Recent advances in nanotechnology have highlighted zinc oxide nanoparticles (ZnO-NPs) as a promising alternative to conventional Zn fertilizers due to their enhanced solubility, bioavailability, and targeted delivery. The present study evaluated the dose-dependent effects of ZnO-NPs on growth performance, antioxidative enzymes activity, and yield attributes of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Plants were treated with graded concentrations (0.001, 0.01, 0.1, 1, 2 and 10 ppm) of ZnO-NPs through foliar application, and their effects were compared. Plants exhibited a concentration-dependent response to ZnO nanoparticle application, with significant enhancement in vegetative growth, chlorophyll biosynthesis, and enzymatic antioxidant defense up to 2 ppm. However, at higher concentrations (10 ppm), vegetative growth and chlorophyll biosynthesis declined, indicating the onset of phytotoxic effects. The application of ZnO nanoparticles at 2 ppm was the most effective treatment, significantly enhancing shoot and root biomass, chlorophyll content, tiller production, panicle weight, and seed weight compared to other concentrations. Growth parameters, including plant height, biomass accumulation, and leaf area, were significantly enhanced at optimal concentrations of ZnO nanoparticles (ZnO-NPs). Furthermore, the activities of key antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)-were markedly increased, indicating improved regulation of oxidative stress and enhanced cellular protection. However, higher doses of ZnO nanoparticles (ZnO-NPs) led to a decline in physiological performance, indicating potential phytotoxic effects at excessive concentrations. Yield attributes, such as grain weight and panicle length, were optimized at moderate ZnO-NP application rates. Overall, the findings demonstrate that judicious application of ZnO-NPs can improve growth, antioxidant defense, and yield of pearl millet, highlighting their potential role in sustainable micronutrient management.

Keywords: Antioxidative enzymes, Pearl millet, Foliar application, Sustainable agriculture, Zinc fertilizer, Zinc oxide nanoparticles.

1. Introduction

Zinc deficiency in agricultural soils remains a major constraint to crop productivity and nutritional quality of millets cultivated in semi-arid environments. Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a crucial food for millions of populations in Asia and Africa. This cereal is typically cultivated in areas characterized by low rainfall and is important in economically sensitive areas where food security is a problem due to recurrent droughts and infertile soils[1]. Widespread zinc deficiency in Indian agricultural soils is primarily attributed to intensive cultivation of cereals and legumes without adequate crop rotation or fallow periods, resulting in reduced yield and mineral content[2]. So, pearl millets might help with managing long-term illnesses like diabetes, obesity, heart disease, cancer, and some types of migraines and asthma[3, 4]. According to Kankarwal it can help with different kinds of hunger and make sure that people eat a variety of foods[5].

Zinc has an important role in mitigating nutritional deficiencies and malnutrition prevalent in many underdeveloped countries, including India, which particularly impacts children's health[6]. Pearl millet is grown with a zinc deficient area which is common micronutrient deficiency, despite its hardiness. In addition to lowering the nutritional value of grains and crop productivity, diets low in Zinc can have negative health effects. Many physiological and biochemical functions in plants, including protein synthesis, Photosynthesis, cell transparency, enzyme activation, and membrane system stabilization, synthesis of lipid, protein and carbohydrate metabolism[7, 8]. Zinc also increases water uptake from root and transport to other parts which reduce the stress effects[9, 10, 11, 12]. Often, traditional fertilization approaches such as the application of zinc sulphate are insufficient in Zn deficient soils due to leaching, fixation, and poor root uptake. There is, however, the pioneering field of nanotechnology which looks like it will be helpful in enhancing the nutrient-use efficiency and productivity of crops. Nanoparticles effectively deliver essential nutrients to plants, enhancing absorption and utilization, minimizing losses, and increasing agricultural productivity. Additionally, nanoparticles can stimulate plant growth[13]. They have the potential to enhance crop resilience within sustainable agriculture by reducing traditional chemical uses and adapting to variable climate conditions and environmental challenges[14].

Zinc oxide nanoparticles (ZnO-NPs) are trending because of their advantages over traditional zinc fertilizers, such as greater bioavailability, controlled release of minerals, and high surface area. Zinc oxide nanoparticles interact with the cell wall to create apertures, facilitating their entry and enabling more rapid movement through the apoplastic and symplastic pathways [15]. ZnO-NPs, among the other metal oxides, are most significant nanoparticle which is widely utilized across various industries due to their unique physiochemical characteristics[16, 17]. ZnO-NPs provide plants with a form of zinc that is more soluble and accessible, hence alleviating zinc shortage difficulties, which are mostly caused by the restricted solubility of zinc resources in the soil[18].

Foliar application of ZnO-NPs serves as a more practical method over others because of fast nutrient delivery through the leaf's pores or stomata, rather than through the roots and promotes photosynthesis, enzyme activity, and grain filling[19, 20]. Few studies have demonstrated that foliar application of zinc oxide nanoparticles (ZnO-NPs) not only significantly enhances grain yield and zinc accumulation but also improves growth parameters in millets [21]. The study intends to determine the effect as a foliar supplement in pearl millet cultivation. It should focus on the effect of ZnO nanoparticles on yield and nutritional value, and the plants performance in zinc deficient environments under controlled environment.

2. Material and methods

2.1 Experimental Setup:

Plastic pots (10-inch diameter) were used for the experiment. Each pot was filled with silica sand sourced from Shankargarh, Prayagraj, Uttar Pradesh, India. The sand was sieved to obtain uniform particle sizes ranging from 0.20 to 0.84 mm. Prior to use, the sand was thoroughly washed with water and initially treated with hydrochloric acid. It was then repeatedly rinsed with distilled water and subjected to further chemical treatment using a mixture of 17% hydrochloric acid and 1% oxalic acid to eliminate residual impurities. After treatment, the sand was again extensively rinsed with distilled water and subsequently used for filling the pots[22]. After each acid treatment, the sand was meticulously cleaned with water. Prior to commencing the experiment, the treated sand was leached with a 4 mM calcium nitrate solution, purified using phosphate adsorption and dithizone extraction[22], to reduce its pH to about neutral (around 6.5).

The pots used for plant cultivation were made of high-quality white plastic and were provided with drainage holes at the base. An inverted watch glass was placed over each hole to retain the sand within the container while allowing free drainage of excess nutrient solution and adequate aeration of the root zone.

2.2 Seed Sowing:

Seed which are healthy and uniform looking were surface sterilized with mercuric chloride to eliminate pathogen infections like bacteria, fungal, and viral infections. Seed were soaked with water for 24 h after that seed were grown under glasshouse conditions. The seeds were shown in 10-inch sand pot in depth of 0.5cm. Seedlings started appearing on the 4th day after sowing. Thereafter, they were supplied with distilled water for 48 days without any nutrient supplementation.

2.3 Nutrient Supply:

After the plants reached a certain height, they were supplied with the nutrient medium. For the control (normal) plants, the medium was applied without zinc are 4 mM KNO_3, 4 mM Ca $(NO_3)_2$, 2 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 0.33 µM HBO_3, 0.1 mM Fe EDTA, 10 µM $MnSO_4$, 1 µM $CuSO_4$, 0.1 µM Na MoO_4, 0.1 M NaCl, 0.1 µM $CoSO_4$, and 0.1 µM $NiSO_4$, Fe-EDTA[23]. The amount of DNS applied to the pots varied depending on the growth stage of the plants and prevailing weather conditions. After 48 days, zinc micronutrient (DNS) was not applied, followed by a 30-day treatment period. For this, pre-prepared zinc oxide nanoparticles (ZnO NPs), purchased from CDH with a size range of 90-200 nm, and were used. These nanoparticles were applied at different concentrations: 0.001, 0.01, 0.1, 1, 2, and 10 ppm. These zinc oxide nanoparticles are used on plant for foliar treatment in different pot setup. Foliar treatment was performed twice per week.

2.4 Morphological parameters measurements:

Plants were harvested 45 days after sowing under controlled conditions. Phenotypic parameters, including leaf, root, and shoot lengths, were measured using a scale. Fresh weights of these plant parts were recorded using an electronic balance. For dry weight determination, the samples were dehydrated in an oven at 105°C for 48 hours, after which the dry weights of the leaves, roots, and shoots were measured.

2.5 Chlorophyll Pigment estimation:

Chlorophyll content was determined using the standard method described by Lichtenthaler[24]. Fresh leaf samples of pearl millet (0.1 g) were washed thoroughly with distilled water to remove dust and impurities. The samples were then ground using a mortar and pestle in 10 mL of 80% chilled acetone. The homogenate was centrifuged at 5000 rpm for 10 minutes, and the supernatant was collected. The absorbance of the supernatant was measured using a dual-beam spectrophotometer at wavelengths of 663 nm, 645 nm, 510 nm, and 480 nm, with 80% acetone serving as a blank. The concentrations of chlorophyll **a**, chlorophyll **b**, and total carotenoids were calculated using the standard Lichtenthaler and Wellburn equations.

2.6 Catalase:

Catalase activity was determined using the classical titrimetric method of Euler and Josephson[25]. Briefly, the enzyme extract was incubated with 0.005 N hydrogen peroxide (H_2O_2) in 0.025 M phosphate buffer for a fixed reaction period. The reaction was terminated by adding 2 ml of 2 N H_2SO_4, and the residual H_2O_2 was titrated against 0.1 N $KMnO_4$ until a persistent pink color appeared. The activity of the enzyme was calculated based on the amount of H_2O_2 decomposed during the incubation period.

2.7 Peroxidase:

For peroxidase test, we used Luck's methodology[26]. An experiment was performed to ascertain the reaction temperature at 25°C. The reaction mixture comprises 0.1M $KMnO_4$ at 6.0 buffer pH, 1 ml of 0.5% p-phenylene diamine, and0.01% $KMnO_4$. After reaction mixture is prepared,1 ml of enzyme extract added and allow it to incubate for few minutes to initiate the reaction. Two ml of 4N H_2SO_4 are used to terminate the process. Introduce 2 mL of H_2SO_4 into the blanks before addition of the fresh leaf

enzyme extract and execute the procedure concurrently. Subject the reaction mixture to centrifugation at 4000rpm after a 20-minute cooling period. We measured the color intensity using a spectrophotometer calibrated and using blank reference to 485 nm wavelength.

2.8 Superoxide Dismutase (SOD):

Enzyme was estimated through standard method of Beauchamp and Fridovich[27]. First, we begin by preparing a mixture consisting of a phosphate buffer with a concentration of 0.05 M, 0.013 M methionine, 75 µM NBT, 0.1 mM EDTA, and 2 µM riboflavin. To start the reaction, 100 µL of enzyme were added and exposed to fluorescent light (approximately 4000–5000 lux) for 10 minutes. For control we used mixture without enzyme extract, representing maximum NBT reduction. The reaction was stopped by placing in dark condition, and at 560 nm absorbance taken by using spectrophotometer.

2.9 Ascorbate Peroxidase (APX): Ascorbate peroxidase enzyme was determined by Nakano and Asada upgrade method[28]. The mixture contains 50 mM phosphate buffer, 0.5 mM ascorbate, and 0.1 mM freshly prepared H_2O_2. To start the reaction enzyme added, and absorbance was recorded at 290 nm for few minutes. Enzyme activities were expressed in absorbance per minute.

2.10 Hydrogen Peroxide (H_2O_2): Concentration of hydrogen peroxide analyzed by Brennan and Frenkel[29]. In this fresh leaf of millet was homogenized with acetone and filter with Whatman paper 1, add 2.5 ml H_2O_2, 0.5 ml Titanium tetrachloride and 1 ml ammonium then centrifuge it 10,000 rpm for 5 min. Precipitate solubilized into 5 N H_2SO_4 so that yellow color is found. Read absorbance at 415 nm by spectrophotometer.

2.11 Lipid Peroxidation Assay: We used Heath and Packer modified method to analyze Lipid Peroxidation in Peral millets[30]. First fresh leaf 0.5g was homogenized in 5ml of TCA. Then centrifuge at 10,000rpm for 5 minutes. Now, use 2ml supernatant and 2ml of TBA. Boil for 30 minutes at 95°C and quickly cool on ice. After centrifuge at 10,000rpm for 15 minutes, the absorbance at 532 and 600nm with spectrophotometer is taken.

2.12 Statistical Analysis:

Data was analyzed using SPSS version 27 One-way analysis of variance was analyzed. Mean of Triplicate data were ± standard error (SE) and analyzed using Duncan's new multiple range test at a significance level of ($P \leq 0.05$). Principal component analysis (PCA) and Pearson's correlation analysis apply with version 2025b of Origin Pro analysis software.

3. Result and Discussion

3.1 Effect of ZnO nanoparticle on Morpho - Physiological traits

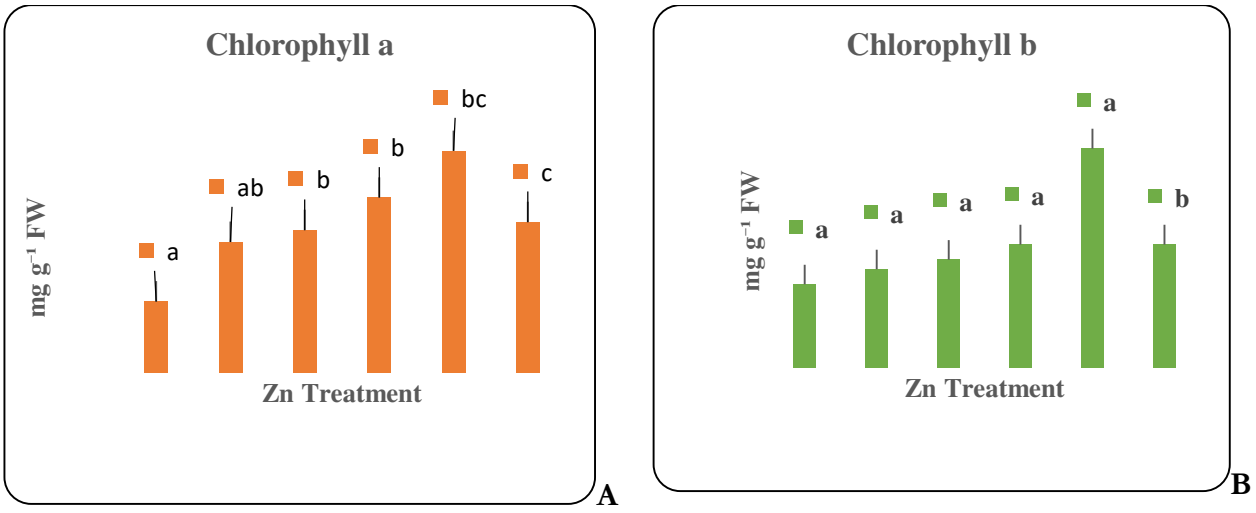
Foliar application of ZnO nanoparticles exhibited a dose-dependent enhancement of growth and biomass parameters in pearl millet. Lower doses (0.001–0.01 ppm) showed only slight enhancement, whereas moderate concentrations, specifically 1 ppm and 2 ppm, led to the most significant increases in plant length, leaf number, and biomass[31,32]. The treatment at a 2-ppm dose exhibited the most pronounced stimulatory effect, leading to increased shoot length as well as higher fresh and dry biomass. Additionally, this experiment demonstrated enhanced photosynthetic efficiency, reflected by an increase in chlorophyll content, which likely contributed to more effective nutrient utilization. However, a decrease in growth was observed at 10 ppm treatment that indicated potential phytotoxic effects at levels surpassing the ideal dosage (Table 1). The results finding indicate that zinc nanoparticles at 1–2 ppm significantly enhance vegetative growth, whereas higher concentrations (≥10 ppm) induce phytotoxic stress, leading to a pronounced decline in physiological performance.

Table 1. Morphological alteration of Pearl millet plant treated with ZnO nanoparticles.

Parameter	0.001ppm	0.01ppm	0.1ppm	1ppm	2ppm	10ppm	SEm ±	CV (%)
Root Length(cm)	5.3 ± 0.202^{a}	5±0.145^{a}	6.6±0.333^{a}	8±0.120^{b}	9±0.233^{c}	5.4±0.305^{c}	0.223	5.9
Shoot Length(cm)	6.333 ±0.509^{a}	9.466±0.658^{ab}	11.966±0.786^{bc}	12.899±0.702^{bc}	14.355±0.587^{bc}	12.03±0.428^{c}	0.611667	9.48
Leaf Fresh Weight(mg)	9.31 ± 0.627^{a}	8.83 ± 0.392^{a}	15.7 ± 1.411^{b}	13.94±1.167^{bc}	20.54±0.591^{cd}	17.033±0.969^{d}	0.8595	10.46
Stem Fresh Weight(mg)	13.706±0.600^{a}	12.433±0.283^{a}	19.986±0.961^{b}	21.566± 0.888^{b}	23.306±0.392^{b}	23.193 ± 0.822^{b}	0.657667	5.98
Root Fresh weight(mg)	12.42±1.173^{a}	8.4±0.599^{a}	15.013±1.071^{a}	17.45±1.493^{a}	14.9±0.609^{a}	18.413±0.666^{a}	0.935167	11.22
Leaf Dry Weight(mg)	1.232±0.144^{a}	1.479±0.107^{a}	2.123±0.196^{ab}	5.106±0.467^{b}	5.98±0.449^{c}	3.513±0.239^{c}	0.267	14.28
Stem Dry Weight (mg)	2.383±0.033^{a}	1.403±0.008^{a}	1.896±0.335^{ab}	7.54 ±0.540^{b}	8.126±0.545^{c}	4.43±0.660^{c}	0.3535	14.25
Root Dry Weight (mg)	2.473±0.082^{a}	1.323±0.069^{ab}	2.256±0.109^{ab}	7.733±0.554^{abc}	6.556±0.326^{bc}	4.256±0.331^{c}	0.245167	10.36
Leaf Number	5.285±0.606^{a}	5.857±0.404^{ab}	7.428±0.719^{bc}	7.857±0.553^{c}	8±0.755^{c}	7.857±0.633^{c}	0.611667	15.03

SEm ±: Standard Error of the Mean; each value is the mean ± SD of triplicate (n = 3); significant at $p < 0.05$ ($p \leq 0.05$)
CV represent Coefficient of Variance standardized measure of data dispersion in %.

3.2 Chlorophyll Content: Significant differences were observed in the photosynthetic pigment content of pearl millet under varying zinc treatments **(Fig. 1).** The levels of chlorophyll in the plants increased with the application of Zn up to 2 ppm, indicating a stimulatory effect. However, further increase in Zn concentration to 10 ppm resulted in a decrease in chlorophyll content, suggesting potential toxicity or inhibitory effects at higher levels [19, 31]. The chlorophyll content increased at the 2 ppm Zn treatment compared to the control, but decreased when the Zn concentration was further increased to 10 ppm. Carotenoid levels were also highest at this concentration, indicating an increased ability for photoprotection[24]. Plants in 10 ppm experienced a marked decrease in pigment levels, suggesting that higher concentrations of Zn inhibited chlorophyll metabolism[33]. At high concentrations, Zn likely induced oxidative stress and inhibited photosynthetic pigment production by increasing ROS, which is widely reported in nanoparticle toxicity studies[34].



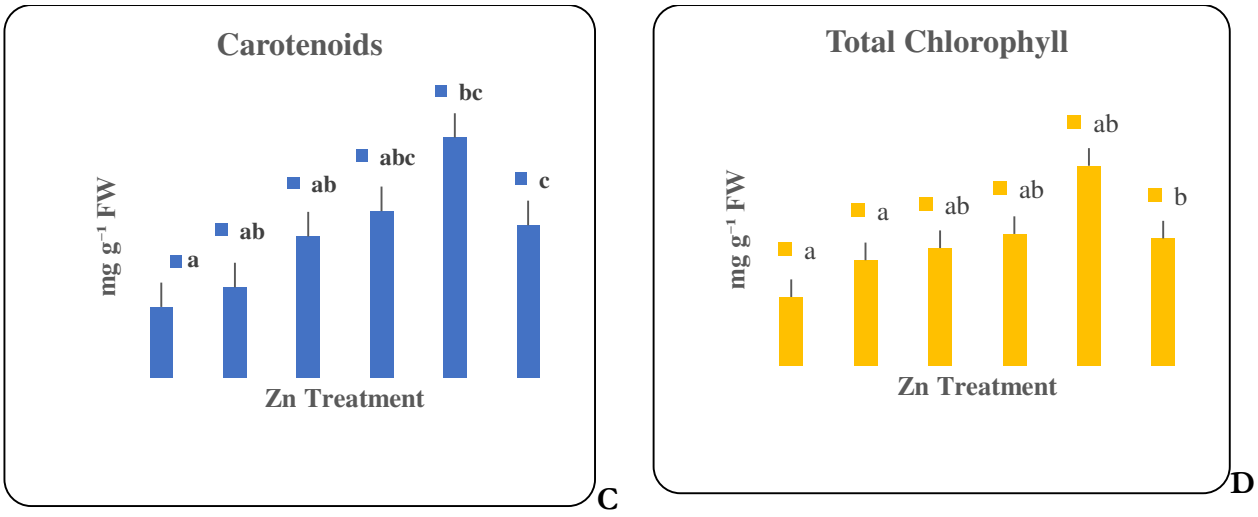


Figure 1. Effect of different Foliar Zinc concentration (0.001-10) on **A-** Chlorophyll a, **B-** Chlorophyll b, **C-** Carotenoid, **D-** Total Chlorophyll; Error show standard error of mean (± SE), Alphabets used (a-c) show significant difference ($p \leq 0.05$; by using Duncan's Multiple Range test in SPSS).

3.3 Antioxidant enzymes activities

3.3.1 Effect of Nanoparticles on Catalase and Peroxidase activity: Catalase and peroxidase activities in millet demonstrated notable variation as influenced by varying dosages of Zn nanoparticles. The activity of catalase showed an upward trend to the highest H_2O_2 decomposition, which occurred at 2 ppm. The activity of the plant transfected with 0.001 ppm showed a minor improvement and 0.01 was observed to be slightly less active. There was a decrease in activity of catalase at 10 ppm, suggesting less activity at a higher concentration of Zn. A similar pattern was observed for peroxidase activity, which increased with low Zn concentrations and plateaued at 2 ppm after the 1 ppm treatment. Minimal activity was recorded at 0.001 and 0.01 ppm Zn. At 10 ppm, peroxidase activity showed a slight decrease following exposure to Zn nanoparticles. This observation indicates that antioxidant activity tends to increase at moderate concentrations of Zn nanoparticles, while it diminishes at higher, potentially excessive concentrations.

3.3.2 Effect of Nanoparticles on SOD and APX activity: Influence of Nanoparticles on SOD and APX. (Fig. 2). The influence of nanoparticles on superoxide dismutase (SOD) and ascorbic acid peroxidase (APX) presented a gradual more enzyme activity that peaked at 2ppm, following a recorded steady increase in 0.001 and 0.01 until 1ppm. A slight decline was observed at 10ppm, suggesting that when high levels of Zn were applied, there was a significant decrease effect on the enzyme. Ascorbic acid peroxidase (APX) activity in millet leaves responded in a dose-dependent fashion following foliar application of ZnO nanoparticles. An incremental enzyme activity was observed at the lowest concentration (0.001ppm) with an increase corresponding with rising concentrations of ZnO nanoparticles until 1. APX activity was highest for 2ppm, demonstrating the greatest activation of the ascorbate-glutathione antioxidant system for this concentration.

3.3.3 Impact of Nanoparticles on H_2O_2 and Lipid Peroxidation: Pearl millet demonstrated a concentration-dependent shift in oxidation indicators after foliar treatment with zinc nanoparticles (ZnONPs). The increase of hydrogen peroxide (H_2O_2) grew constant with escalating concentration of ZnNPs suggesting a greater generation of ROS. At 0.001ppm, the lowest level of H_2O_2 was noted with little increment at 0.1 and 1ppm. The highest accumulation occurred at 2ppm demonstrating significant oxidative stress with notable increases of ZnNPs. However, the significant drop at 10ppm shows that the body's antioxidant defense systems or metabolic changes associated with development may have kicked in when ZnNP levels were too high. A same trend was seen for lipid peroxidation, quantified as malondialdehyde (MDA) concentration. The drop at 10ppm that comes after that shows that ROS production has gone down, either because the cells have become used to stress or because their

metabolism has slowed down. Additionally, the increased levels of H_2O_2 and MDA at medium to high ZnNP doses indicate the activation of oxidative stress and consequent membrane lipid peroxidation.

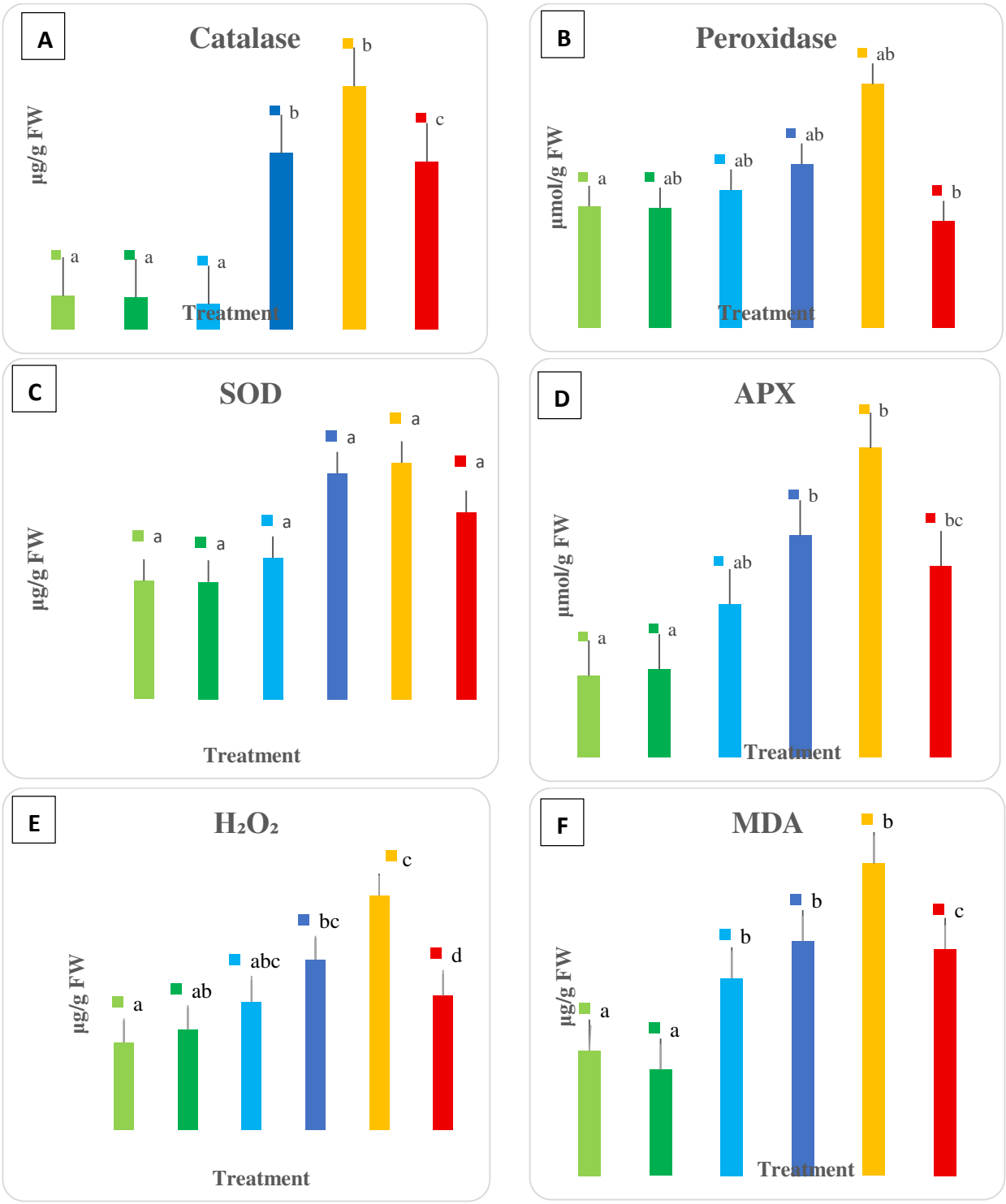


Figure 2. Effect of ZnO nanoparticle on antioxidant enzyme (A) Catalase, (B) Peroxidase, (C) SOD, (D) APX, (E) H_2O_2, (F) Lipid Peroxidase (MDA)

3.4 Yield attributes of Pearl Millet

ZnO nanoparticles markedly enhanced the productive characteristics of pearl millet in a concentration-dependent manner (Table 2). The quantity of tillers, panicle length, panicle and seed weight augmented by rising ZnO-NP concentration up to 2 ppm. At a dosage of 0.01 ppm, a marginal enhancement was seen in the quantity of tillers and panicle weight (0.216 g), with no seed production occurring. A marginal enhancement was seen at 0.1 ppm, when seed production commenced (0.248 g

plant^{-1}). Yield parameters under 1 ppm yielded notable increases in tiller number (7) and seed weight (1.648 g plant^{-1}). The yield parameters (including tillers (10), panicle length (10.8 cm), panicle weight (4.73 gm), and seed weight (3.697 g plant^{-1}) reached their maximum yield at the 2 ppm ZnO-NPs treatment level, suggesting a beneficial stimulatory effect of ZnO-NPs (at moderate concentrations) on reproductive development and subsequent grain filling in pearl millet. In contrast, with the 10-ppm treatment level, a toxicological effect from the nanoparticles may have induced decreased yield attributes due to decreased tillers (6) and decreased seed weight (1.069 g plant^{-1}). Overall, the findings clearly demonstrate that 2 ppm ZnO-NPs is the optimal dose for enhancing productivity in pearl millet, while higher concentrations negatively impact yield.

Table 2. Productive yields of pearl millets under zinc nanoparticle foliar treatments

Treatment	**No. of Tillers**	**Ear Head Length (cm)**	**Panicle Weight (g)**	**Seed Weight (g)**
0.001	3±0.2182^{a}	9.1±0.260^{a}	0.2133±0.0025^{a}	0
0.01	2±0.3779^{a}	10.5±0.297^{ab}	0.22±0.0063^{a}	0
0.1	3±0.218^{a}	13±0.308^{ab}	1.3488±0.0124^{b}	0.248±0.0116^{b}
1	7±0.308^{b}	10.16±0.288^{b}	2.07±0.0107^{c}	1.648±0.0125^{c}
2	10±0.487^{b}	10.8±0.246^{b}	4.73±0.0152^{d}	3.697±0.0127^{d}
10	6±0.308^{c}	10.01±0.218^{c}	0.85±0.0124^{e}	1.069±0.013^{e}

The mean ± SE of three replicates (n = 3) is shown for each value; $p < 0.05$ indicates significance.

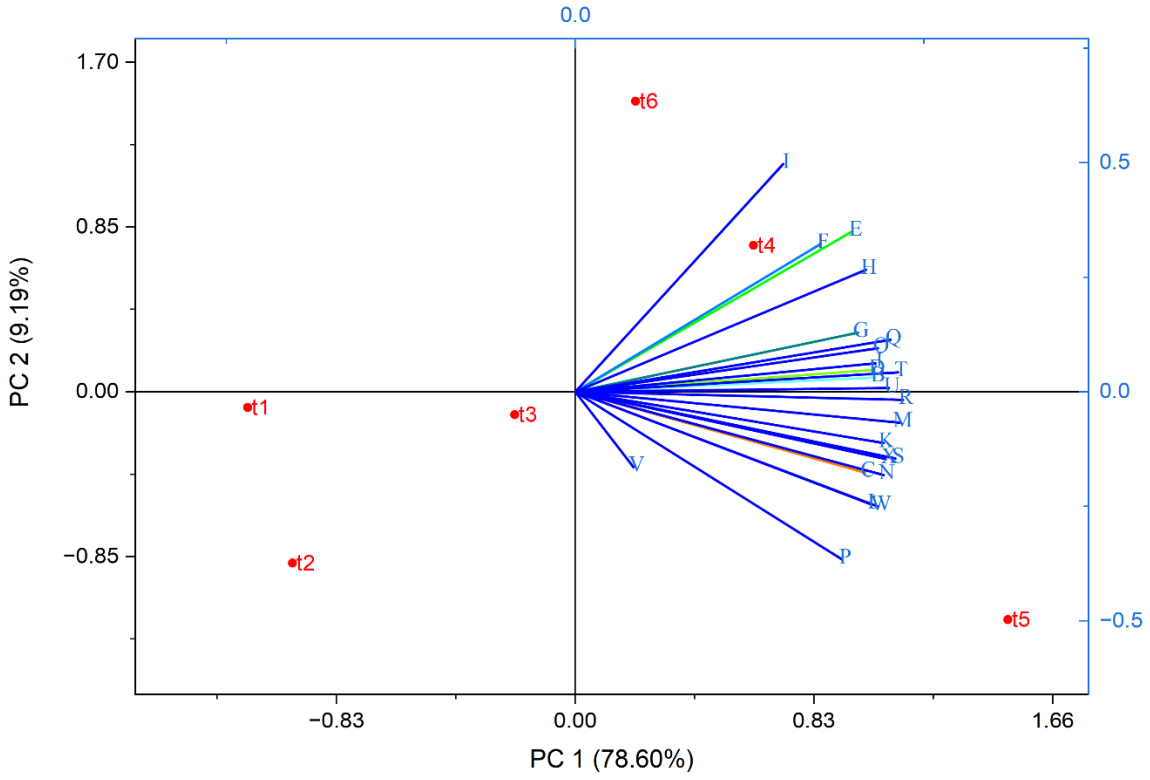


Figure 2. Principal component analysis (PCA) of morpho-physiological characteristics of pearl millet plants exposed to different foliar concentrations of ZnO nanoparticles. Variables included were: **B**-stem length; **C**-root length; **D**-leaf dry weight; **E**-stem dry weight; **F**-root dry weight; **G**-leaf fresh weight; **H**-stem fresh weight; **I**-root fresh weight; **J**-number of leaves; **K**-chlorophyll *a*; **L**-chlorophyll *b*; **M**-carotenoids; **N**-total chlorophyll; **O**-catalase (CAT); **P**-peroxidase (POD); **Q**-superoxide dismutase (SOD); **R**-ascorbate peroxidase (APX); **S**-hydrogen peroxide (H_2O_2); **T**-lipid peroxidation (MDA); **U**-number of tillers; **V**-ear head length; **W**-panicle weight; and **X**-seed weight.

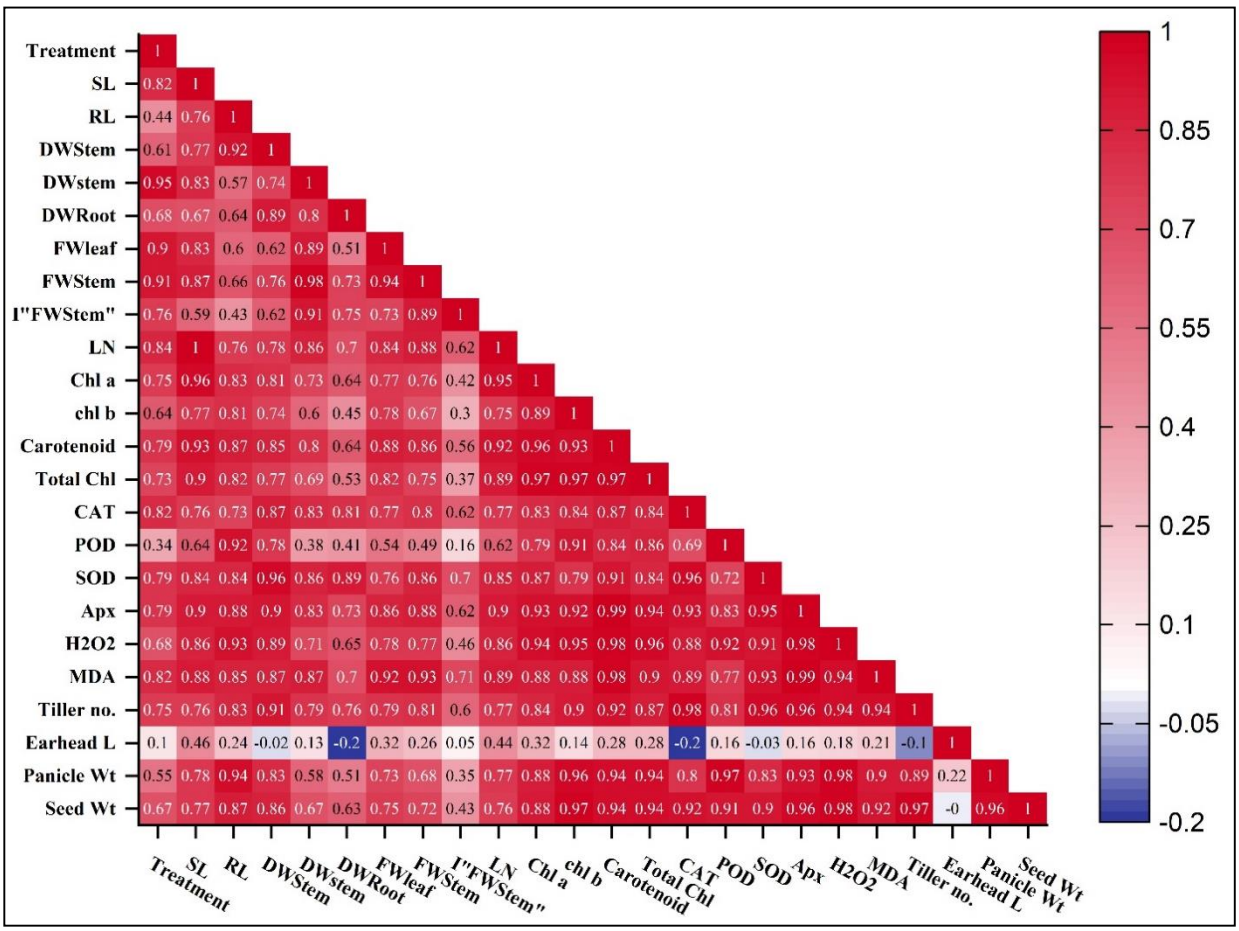


Figure 3. Correlation analysis among morpho-physiological and biochemical characteristics of pearl millet (*Pennisetum glaucum* L.) plants exposed to different foliar concentrations of zinc oxide nanoparticles (ZnO-NPs). Traits include shoot length (SL), root length (RL), stem dry weight (DW), fresh weight (FW), leaf number (LN), chlorophyll content (Chl), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and malondialdehyde (MDA; indicator of lipid peroxidation).

4. Conclusion

The present investigation clearly demonstrates that foliar application of zinc oxide nanoparticles (ZnO-NPs) exerts a pronounced dose-dependent influence on the growth, physiological performance, antioxidant defense system, and yield attributes of pearl millet. Among the tested concentrations, ZnO-NPs at 2 ppm emerged as the most effective treatment, providing an optimal balance between enhanced growth promotion and controlled oxidative stress. This concentration significantly improved vegetative growth parameters, chlorophyll biosynthesis, biomass accumulation, and yield-related traits such as tiller number, panicle weight, and grain weight. However, the concurrent increase in H_2O_2 and MDA at this concentration highlights the importance of closely monitoring stress indicators, particularly under field conditions. These findings demonstrate the considerable potential of nano-fertilization in promoting sustainable agriculture, especially in micronutrient-deficient soils. Nevertheless, the dose-dependent nature of plant responses underscores the necessity for precise optimization to achieve desirable outcomes. The marked upregulation of antioxidant enzymes, including superoxide dismutase, catalase, and peroxidase, at optimal ZnO-NP levels indicates strengthened antioxidative defense and improved cellular protection against reactive oxygen species. Conversely, higher ZnO-NP concentrations (10 ppm) resulted in reduced growth and photosynthetic efficiency, highlighting the onset of phytotoxic effects and underscoring the importance of dose optimization. These findings suggest that while ZnO nanoparticles possess superior bioavailability and efficiency compared to conventional zinc fertilizers, their application must be carefully regulated to avoid adverse effects. Overall, the study underscores the potential of ZnO-NPs as an efficient nano-fertilizer

for improving pearl millet productivity and stress tolerance. Judicious use of ZnO-NPs can contribute to sustainable micronutrient management and enhanced cereal production under zinc-deficient agricultural systems. Further studies on long-term environmental safety and field-scale validation are recommended.

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Conflict of interest: The authors declare that there are no conflicts of financial or personal interest.

Financial interest: The authors declare they have no financial interests.

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Role of *Lemna minor* L. In Phytoremediation and Reduction of Pollutants of Pulp and Paper Mill Effluents

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Abstract

Pulp and paper mill industrial effluents have pollution load in relation to their physicochemical characteristics, exceeding the standard recommended permissible limits set by various agencies. The aquatic macrophytes were found to alter the pH from alkaline to neutral pH in almost every industrial effluent. Changes in pH were dependent mainly upon the duration of the treatment methods adopted. The changes in physicochemical characteristics of paper mill effluent because of phytoremediation by *Lemna minor* L. were elaborated in this experiment. In this study, the TDS, DO, BOD, and COD values of pulp and paper mill effluent were significantly reduced by the application of *Lemna minor* L. The data demonstrated a reduction of TDS, BOD, and COD parameters dependent on concentration and duration. The results of the phytoremediation experiment are very effective for the reduction of pollutants in paper mill effluent. Data indicates that *Lemna minor* L. plants were significant in maintaining the physico-chemical characteristics of the effluent concentration at various exposure durations.

Keywords: Pulp and paper mills effluent, aquatic macrophytes, phytoremediation, BOD, COD, *Lemna minor* L.

1. Introduction

To fulfil the demands for packaging, printing, and hygiene goods, the pulp and paper sector produces about 400 million tons of paper yearly, making it a vital component of the worldwide economy. But it has a big effect on the environment, especially because production procedures like pulping, bleaching, and washing produce wastewater, or effluent. Through water pollution, eutrophication, and biodiversity loss, these effluents endanger aquatic ecosystems, soil quality, and human health if they are not properly handled. The poisoning of the Great Lakes in the middle of the 20th century is only one example of how untreated discharges have traditionally caused significant environmental harm in areas with considerable paper manufacturing, such as North America, Europe, and Asia[1]. Innovative and environmentally friendly treatment techniques are crucial as environmental sustainability gains international attention. Bioremediation, particularly using aquatic plants like *Lemna minor* L. (common duckweed), offers a promising solution for reducing pollutant concentrations in pulp and paper mill effluents. The environmental impact of the pulp and paper business includes pollution of the air, water, and solid waste. Water pollution is especially bad because of the high-water consumption (up to 60 cubic meters per ton of paper produced) and the effluents that are released thereafter, which are packed with pollutants[1].

These discharges, which come from chemical or mechanical pulping, bleaching with chlorine, and washing operations, can lower oxygen levels, kill fish, and add harmful materials to the food chain[2]. Effluent restrictions have been established by stricter rules, such as those enforced by the U.S. Environmental Protection Agency under the Clean Water Act, although compliance is still difficult, particularly in poor nations where enforcement is lax[1]. Advanced treatment techniques are required because of the sheer volume and tenacity of these effluents, which worsen pollution and water scarcity worldwide.

The complicated effluents from pulp and paper mills are distinguished by high levels of chemical and biochemical oxygen demand (BOD and COD), which signify substantial organic loads that lower the dissolved oxygen in receiving waters[3]. COD frequently surpasses 1,000 mg/L, much over permissible discharge limits, while BOD values can vary from 200 to 1,000 mg/L. Lignin and its derivatives, which are complex aromatic polymers found in wood and give it a black hue while preventing decomposition, are important pollutants. Aquatic life is at risk from these lignin compounds because they are mutagenic and androgenic[4]. Chlorine bleaching produces chlorinated organic compounds, which are bio-accumulative and carcinogenic and include furans, dioxins, chloroform, and adsorbable organic halides[1] (AOX). Heavy metals (such as Mercury, Lead, and Cadmium), sulphates, chlorides, and nutrients like nitrogen and phosphorus are examples of inorganic pollutants that cause eutrophication and toxicity[5]. Effluents may become more acidic because of gaseous pollutants like sulphur dioxide and hydrogen sulphide dissolving in them[6]. Fatty acids, resin acids, and phenols increase toxicity even more, leading to long-term consequences such as lung problems in people and endocrine disruption in animals[7]. Research shows that even at 2 % concentrations, untreated wastewater may kill fish[2]. Furthermore, lignin's dark coloring hinders light penetration in aquatic bodies, which damages photosynthesis and modifies ecosystems[4].

Physical, chemical, and biological procedures are the traditional ways of treating pulp and paper effluents. While secondary treatment uses activated sludge or anaerobic digestion to lower BOD and COD, primary treatment uses screening and sedimentation to remove suspended solids[3]. Specific contaminants like color and AOX are the focus of tertiary treatments such as chemical precipitation and advanced oxidation[8]. These approaches do have some serious disadvantages, though. Chemical and physical processes use a lot of energy and result in secondary wastes that need to be disposed of further, including sludge that contains heavy metals[9]. Only partial decolorization and detoxification are achieved by biological treatments when dealing with resistant substances like lignin and chlorinated organic[10]. These techniques are unsustainable for smaller mills because of their high operating costs, which are estimated to be between 10 and 20 percent of output costs, and the requirement for professional maintenance[9]. Alternative methods are required since effluents frequently do not satisfy regulatory levels, even after treatment.

Bioremediation is an economical and environmentally beneficial option that uses natural biological processes to break down or sequester contaminants. Enzymes, plants, or microbes are used to change dangerous chemicals into less dangerous ones. Bioremediation uses plant uptake or microbial degradation to target ligno-cellulosic wastes and chlorinated chemicals in pulp and paper effluents[11]. Bacillus strains and fungi like *Phanerochaete chrysosporium* have demonstrated the ability to decolorize wastewater by enzymatic degradation[12]. But because of its ease of use and other advantages, such as producing biomass for feed or bioenergy, plant-based bioremediation, also known as phytoremediation, is especially beneficial[13].

Using plants to absorb, collect, or break down pollutants in soil, water, or the air is known as phytoremediation. Because of their quick growth, large biomass output, and ability to withstand

contaminated conditions, aquatic plants are perfect for treating wastewater. BOD, COD, and nutrient levels have been shown to decrease in constructed wetlands that use plants such as water hyacinth, *Eichhornia crassipes* L[14]. Among the mechanisms are rhizo-filtration (root adsorption), phyto-extraction (uptake into plant tissues), and phyto-stabilization (immobilization). Phytoremediation provides a complete solution for pulp and paper effluents by addressing both organic and inorganic contaminants. It is appropriate for sustainable wastewater management because of its advantages, which include low energy needs, aesthetic value in treatment systems, and resource recovery potential[13].

Lemna minor L.

Lemna minor L., the common duckweed, is a member of the family and order Aerales. The Lemnaceae family includes plant species that have an oval or circular shape with a leaf surface area of no more than a few square millimeters[15]. It is found on floating surface or immersed in water bodies[16]. Due to fast growth rate of duckweed, it is well suited for waste treatment purposes, as it is relatively easy for maintenance and operates such as system[17]. Duckweed plants obtained by treating water are collected from the water surface itself; duckweed grown in sewage water or livestock waste (wastewater) is not poisonous and can be used as fish and cattle feed, or as crop fertilizer[18]. For more certainty, it can be kept in safe water for a certain term or cleaned with UV-rays or ozone gas after drying[19]. Duckweed can survive from pH of 5 to 9 conditions but grow best at the range between pH 6.5 to 7.5 range, with the growth generally controlled by temperature and sunlight exceeding nutrient concentrations in the water[20]. The most suitable humidity (moisture) content of fresh duckweed growth is 95 %[21]. Plant cultivation and growth do not require a significant initial population in water bodies, because even a small amount will be enough for its quick reproduction and multiplication[22]. The most common method for rapid growth of duckweed is ensuring the water surface is calm with small to no current flow; in case the water moves too much, the plant growth will slow down[23]. If desired, cultivation can be done separately in a rectangular container at least 5 inches deep, 18 inches long, and 12 inches wide[24]. Duckweed has a great capacity to absorb nutrients, making it efficient in removing them from water and its application for treating sewage and untreated water a highly effective, commercially feasible, natural and simple method[25]. In fact, the plant has been successfully used for domestic and industrial wastewater tertiary treatment for over a decade[26].

The physiological and biochemical adaptations of *Lemna minor* L. are what give it its bioremediation effectiveness. Enzymes like peroxidases and catalase detoxify organics, and their strong root system improves rhizo-filtration by adsorbing dissolved contaminants. Its accumulation rates are higher than those of many species, and it can withstand organics, agrochemicals, and heavy metals. It promotes microbial breakdown in the rhizosphere of pulp and paper effluents, where bacterial populations supported by root exudates degrade resistant substances like lignin and phenols[27]. Eutrophication is lessened by nutrient intake, which lowers phosphate and nitrogen. Partially decomposed or volatilized contaminants are chlorinated. According to studies, exposure to diluted effluents (12.5 to 75 %) can reduce color by 50 %, COD by 60 %, and BOD by up to 70 % in a matter of days[28]. Additionally, the plant has an 80 to 90 % efficiency rate for removing phenolics and dyes from methylene blue analogues.

Sustainable development objectives are met by *Lemna minor* L. based bioremediation, which produces value-added products at a cheap cost (savings of 50 to 70 % compared to chemical treatments) and lowers the carbon footprint of the sector. Scaling this strategy might improve water quality and promote circular economies in areas like China and India, where paper mills pollute rivers[29]. Future developments might include hybrid systems that include bacteria and plants, as well as genetic engineering for

improved pollution tolerance. Such technologies are essential as climate change exacerbates water stress.

2. MATERIAL AND METHODS

2.1 Study Area:

For the experiment, plants of *Lemna minor* L. were collected from a pond near the IET campus of Dr. R.M.L. Awadh University in Ayodhya. The effluent was collected aseptically in a sterile plastic container from a pulp and paper mill located in Darshannagar, Ayodhya, U.P. (India). It was transported on ice to the laboratory and stored at 4° C until further use.

2.2 Experimental Design:

In April 2025, samples of effluents were collected from the discharge point of Yash Paper Mill in Ayodhya (Faizabad). The effluent samples were stored in plastic containers at 4° C until further experimentation. *Lemna minor* L. plants were gathered from a nearby natural pond and were thoroughly washed with running tap water, followed by rinsing by distilled water, to eliminate any surface contamination. The experiment was conducted by using plastic tubs with a capacity of 10 liters each. One tub was filled with 5 liters of distilled water, while the other contained 5 liters of the Yash Paper Mill effluent. *Lemna minor* L. plants were then immersed in each tub (Fig. 1). The plants were allowed to grow, and different pollution load parameters were analyzed at intervals of 15 days over a period of 40 days.

At each specified interval, 50 mL samples were withdrawn from both tubs for analysis of various physicochemical parameters. The volume of effluent lost during sampling was replenished by adding an equivalent amount of distilled water to each tube. The analysis of the selected pollution parameters in the effluent was completed at intervals of 0, 10, 20, 30, and 40 days from the start of the experiment, using standard methods.

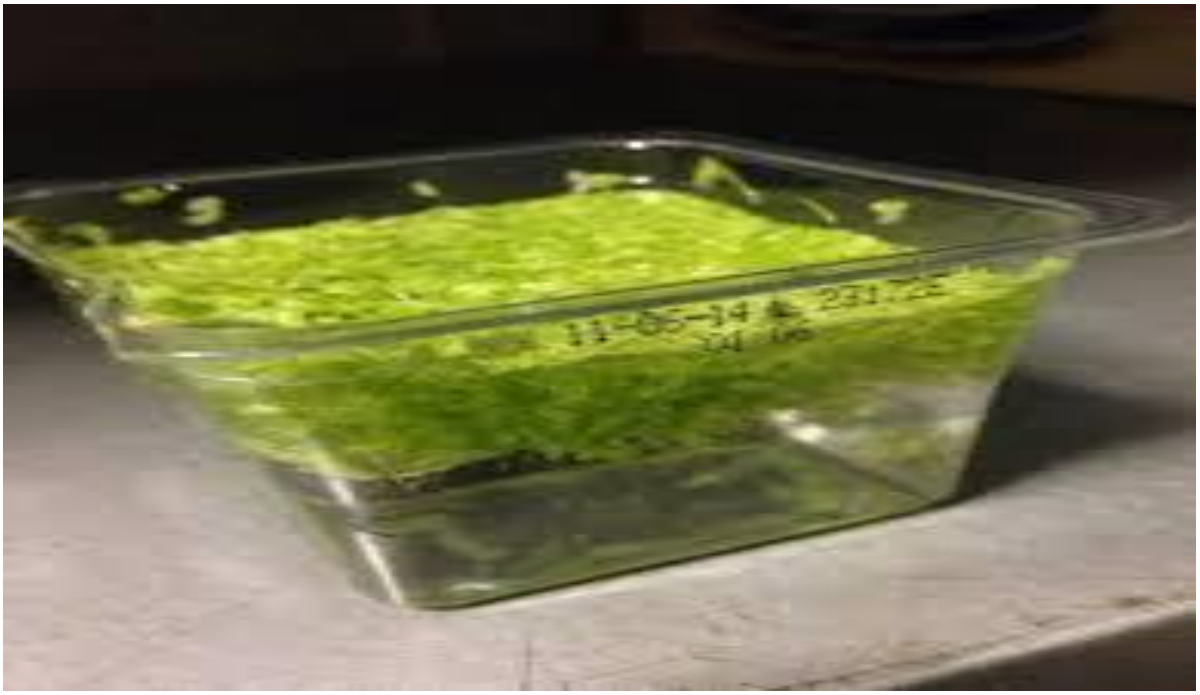

Figure 1: Use of the aquatic plant *Lemna minor* L. for the reduction of pollutants in pulp and paper mill effluent in laboratory

2.3 Dissolved oxygen (DO):

Oxygen present in the sample oxidizes the divalent manganese to its higher valency, which precipitates as brown-hydrated oxides after the addition of NaOH and KI. Upon acidification, manganese reverts to the divalent state and liberates iodine from KI equivalent to the DO content in the sample. The DO is calculated by the azide modification of Winkler's method.

$$\text{Calculation-DO(mg/L)} = \frac{ml\ of\ titrant \times normality \times 8 \times 1000}{V}$$

2.4 Biological oxygen demand (BOD):

It is defined as the amount of oxygen required by bacteria in decomposing organic material in a

sample under aerobic conditions at 200 over a period of 5 days.

$C_6H_{12}O_6 + 6O_2 \longrightarrow CO_2 + H_2O$ + Microorganism

We are calculating carbonaceous BOD by the dilution method. In the first step, we calculate the initial DO of the sample, and the DO after 5 days of incubation.

Calculation - BOD (mg/L) = $\frac{(D1-D2)}{P}$

2.5 Chemical oxygen demand (COD):

The COD test determines the oxygen required for chemical oxidation of most organic matter and oxidizable organic substances with the COD test are determined by the reflux digestion method.

Calculation -COD (mg/L) = $\frac{(A-B\times N\times 8\times 1000)}{ml\ of\ sample}$

where 'A' is the volume of Ferrous Ammonium Sulphate (FAS) for the blank, 'B' is FAS for the sample, 'N' is the normality of FAS, 'V' is the sample volume, and '8000' converts units to mg/L. This measures oxygen needed for oxidation, determined by titrating unreacted dichromate after refluxing the sample with an excess oxidant.

2.6 Total dissolved solids (TDS):

Many solids are found dissolved in natural water, the common carbonate, bicarbonate, chloride, sulphate, phosphate, etc. In other words, TDS is simply the sum of the cations and anions concentration expressed in mg/L.

Calculation -TDS (mg/l) = $\frac{(W2-W1)\times 1000\times 1000}{ml\ of\ sample}$

2.7 Total suspended solids (TSS):

TSS applies to the dry weight of the material that is removed from the measured volume of water sample by filtration through a standard filter.

Calculation –TSS (mg/L)= $\frac{(W2-W1)\times 1000\times 1000}{ml\ of\ sample}$

2.8 Alkalinity:

Alkalinity is a measure of the water's ability to absorb hydrogen ions without a significant pH change. The alkalinity of a sample can be estimated by titrating with standard sulphuric acid. Titration to pH 8.3 or decolorization of phenolphthalein indicator will indicate complete neutralization of OH and ½ of CO_3, while to pH 4.5 or a sharp change from yellow to pink of methyl orange indicator will indicate total alkalinity.

Calculation -Alkalinity (mg/L) = $\frac{A\times N\times 50\times 1000}{ml\ of\ sample}$

2.9 pH:

It is the negative logarithm of hydrogen ion concentration, more precisely, hydrogen ion activity. The pH changes of the effluent in the due course of growth, decolorization, and de-chlorination were measured for each set of experiments using a pH meter.

3. Result and Discussion

The efficiency of *Lemna minor* L. in scavenging contaminant indicates that the presence of such macrophytes is an important element for contaminant removal in wastewater. Hydrophytes can supply required oxygen by oxygen leakage from the roots into the rhizosphere to accelerate aerobic degradation of organic compounds in wetlands. This assumption was confirmed in the present study, since the accumulation of heavy metals was higher in plants than in water. Phytoremediation can be classified as phyto-extraction, phyto-degradation, phyto-stabilization, phyto-stimulation, phyto-volatilization, and rhizo-filtration. Rhizofiltration, also referred to as phyto-filtration, is based on hydroponically grown plants that are most efficient in removing heavy metals from water. Phyto-extraction was considered to have poor role in metal extraction, but it should be promoted.

3.1 Effects on pH:

The pH value of any sample basically depends on the nature of the sample, i.e., the acidity and basic nature of the aqueous solution. The pH measurement is useful in effluent treatment to find the design, types, and efficiency of the solution. Discharged from the effluent treatment plant has both acidic and alkaline characteristics of effluents. The untreated pulp and paper mill effluent pH value was 7.4; the final treated effluent pH was found to be 7.8 (Fig. 2). So, the pH value was basic in nature and increased due to the microbial activities[3, 30].

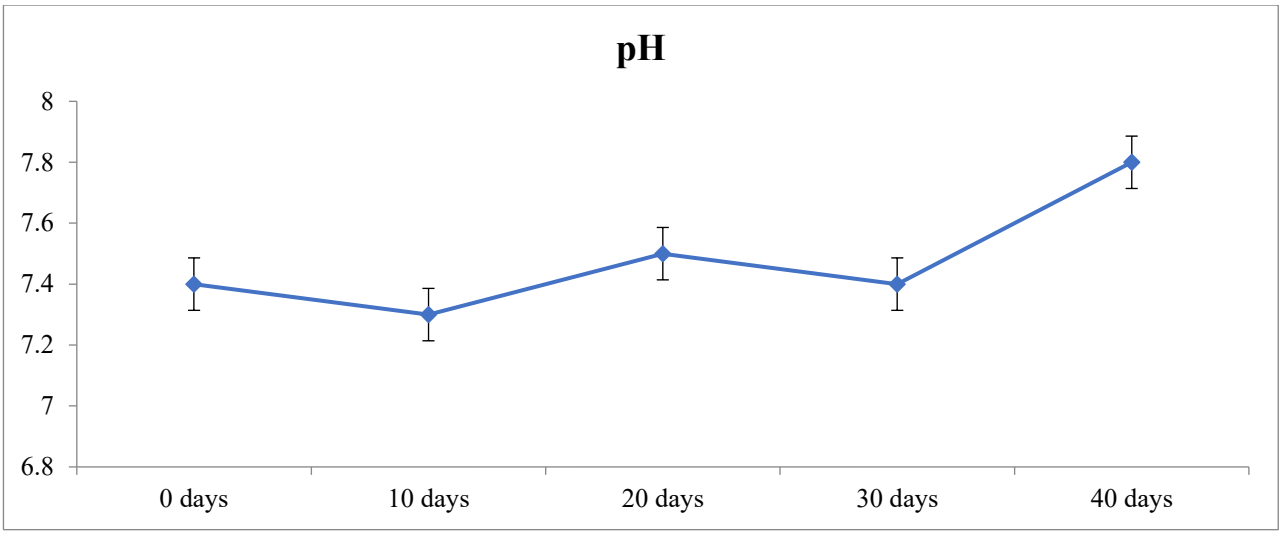

Figure 2: Graph showing the pH reduction and then again slightly rise in pH after 30 days of treatment of paper mill effluent by *Lemna minor* L.

3.2 Effects on BOD:

Biological oxygen demand (BOD) is the demand of oxygen required by microbes to degrade organic matter. It is a good parameter of water quality to assess the water quality of any sample. In the present investigation, the BOD of untreated effluent was 98.8 mg/l, and after 40 days of treatment of this effluent by *Lemna minor*, the BOD value reduced to 64.4 mg/l (Fig. 3). It shows a 65.16 % reduction. This reduction may be due to *Lemna minor,* which utilizes organic matter for its metabolic activity[31].

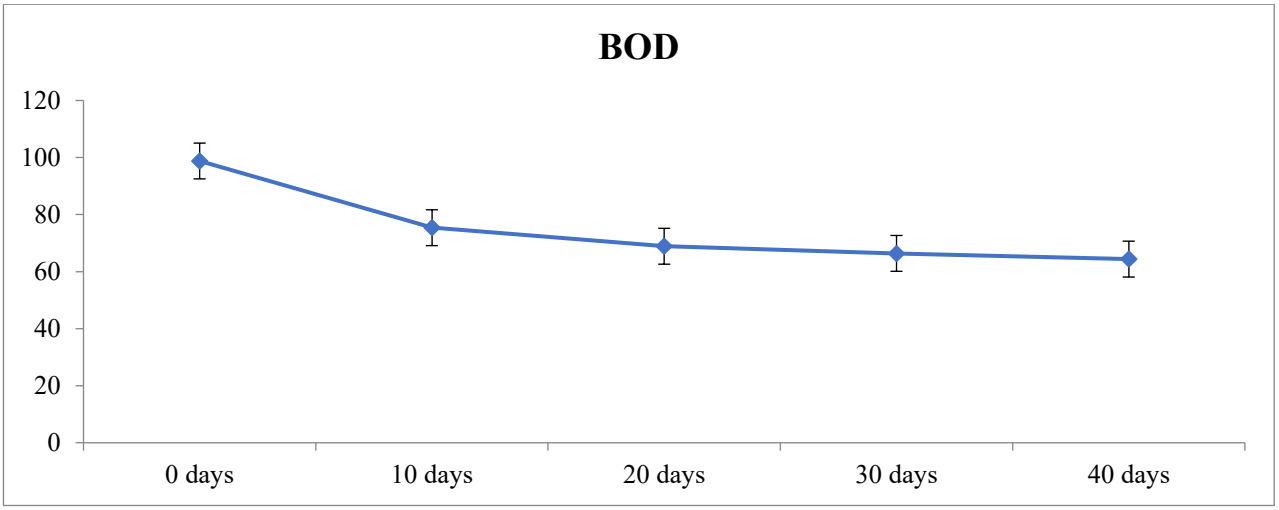

Figure 3: Graph showing the BOD reduction in paper mill effluent after treatment with *Lemna minor* L.

3.3 Effects on Total suspended solids (TSS):

The undissolved matter present in water or wastewater is usually referred to as suspended solids. In the present investigation, the TSS of untreated effluent was 35.6 mg/l, and after treatment of this effluent by *Lemna minor* L., the TSS value reduced to 25.8 mg/l (Fig. 4). It shows a 72.3 % reduction. This reduction may be due to the photosynthetic activities of water plants by smothering benthic organisms[32, 33].

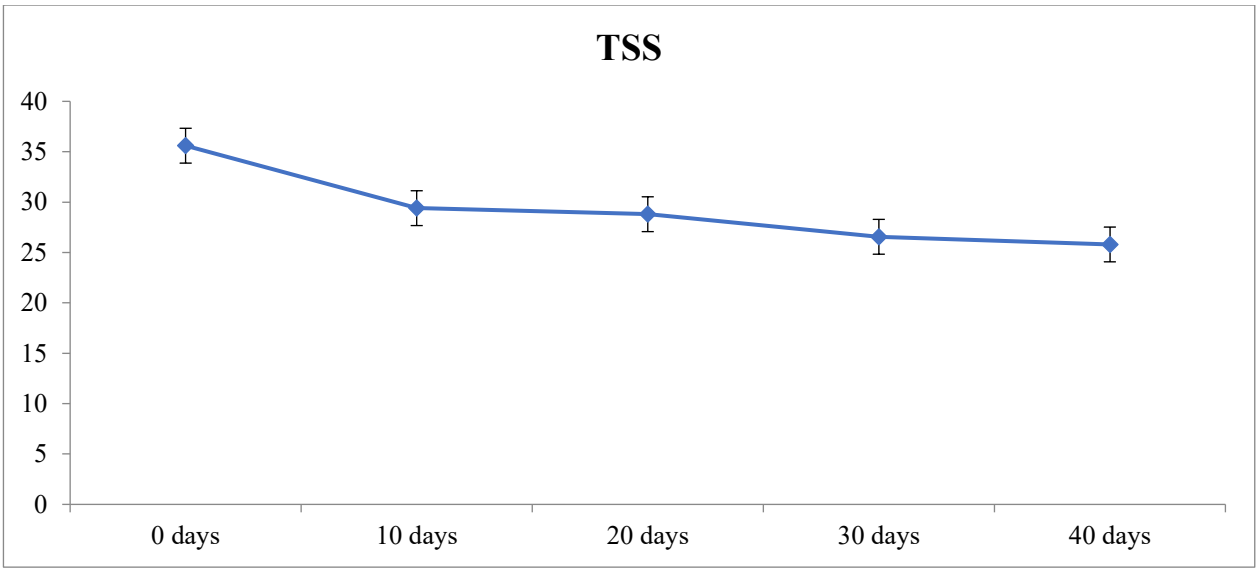

Figure 4: Graph showing the TSS reduction in paper mill effluent after treatment with *Lemna minor* L.

3.4 Effects on Total dissolved solids (TDS):

TDS is a measure of the combined content of all inorganic and organic substances contained in a liquid in molecular, ionized, or micro-granular (Colloidal solution) suspended form. In the present investigation, the TDS of untreated effluent was 1450 mg/l, and after treatment of this effluent by *Lemna minor*, the TDS value reduced to 1090 mg/l. It shows a 75.17 % reduction (Fig. 5). This reduction may be due to the decrease in the concentration of suspended solids[32, 34].

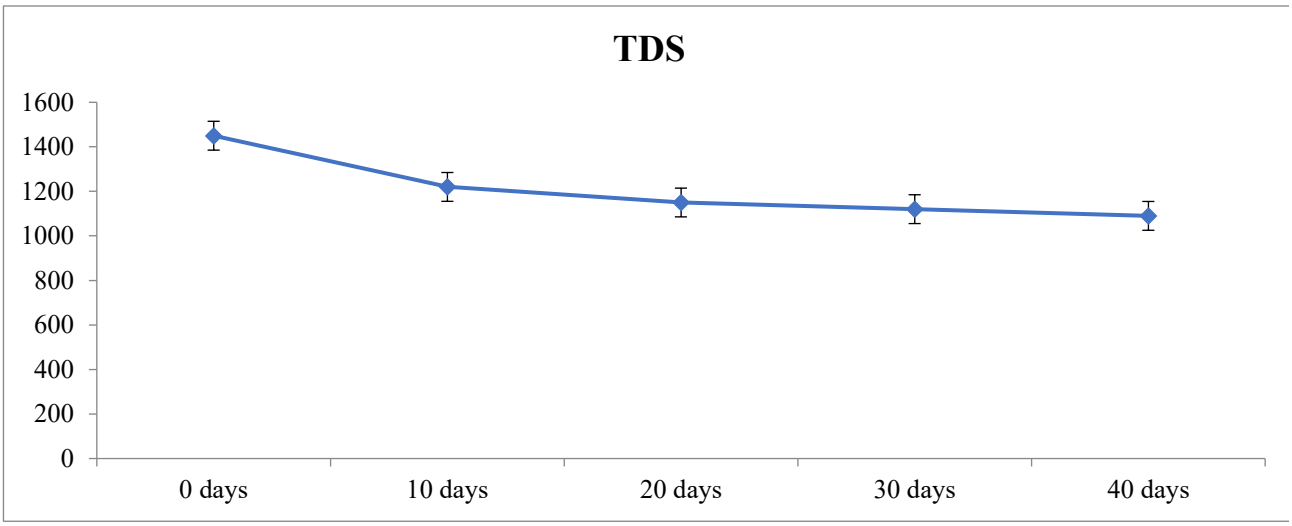

Figure 5: Graph showing the TDS reduction in paper mill effluent after treatment with *Lemna minor* L.

3.5 Effects on Dissolved oxygen (DO):

DO analysis measures the amount of Gaseous oxygen dissolved in an aqueous solution. It is a good parameter of water quality. In the present investigation, the DO of untreated effluent was 2.9 mg/l, and after treatment of this effluent by *Lemna minor*, the DO value increased up to 6.5 mg/l. It shows a 232.14 % reduction (Fig. 6). This increase may be due to the *Lemna minor*, which oxygen gets into water by diffusion from the surrounding air through aeration and a waste product of photosynthesis[34].

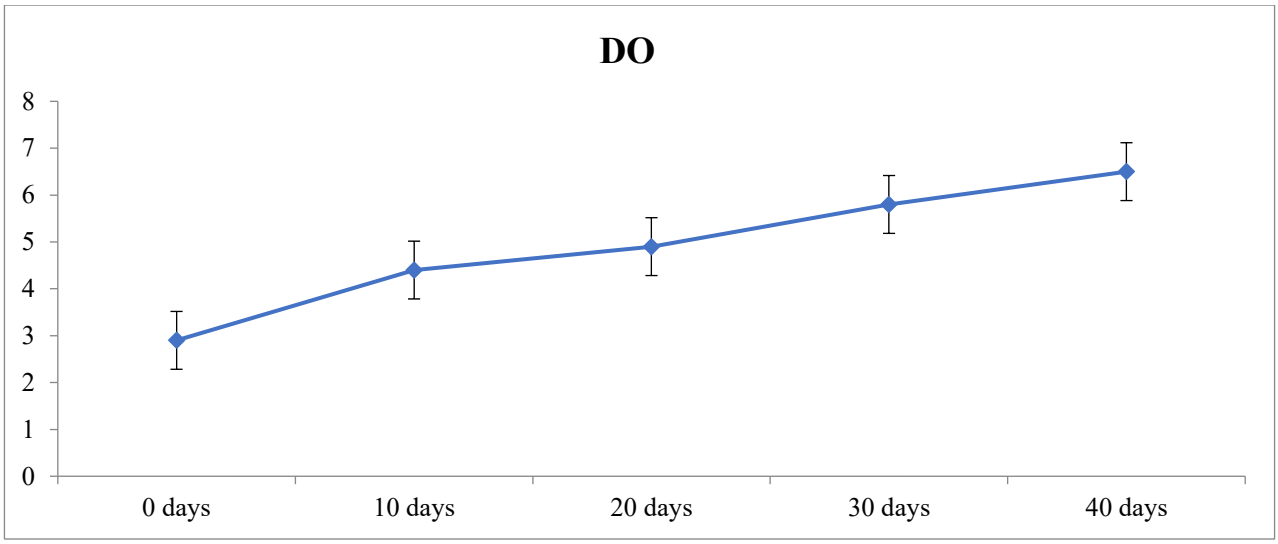

Figure 6: Graph showing the rise in DO activity in paper mill effluent after 20 to 40 days of treatment with *Lemna minor* L.

3.6 Effects on Alkalinity:

Alkalinity is a quantitative capacity of an aqueous solution to neutralize the acidity of any substance. In the present investigation, the untreated effluent was 425.6 mg/l, and after treatment by *Lemna minor*, the alkalinity value increased to 458.2 mg/l due to the increase of pH of paper mill effluent (Fig. 7). It shows a 7.11 % increase as compared to before and after treatment by *Lemna minor*. Alkalinity was highly dependent on presence of our extensive use of sodium hydroxide ions in the pulp and paper industry[35]

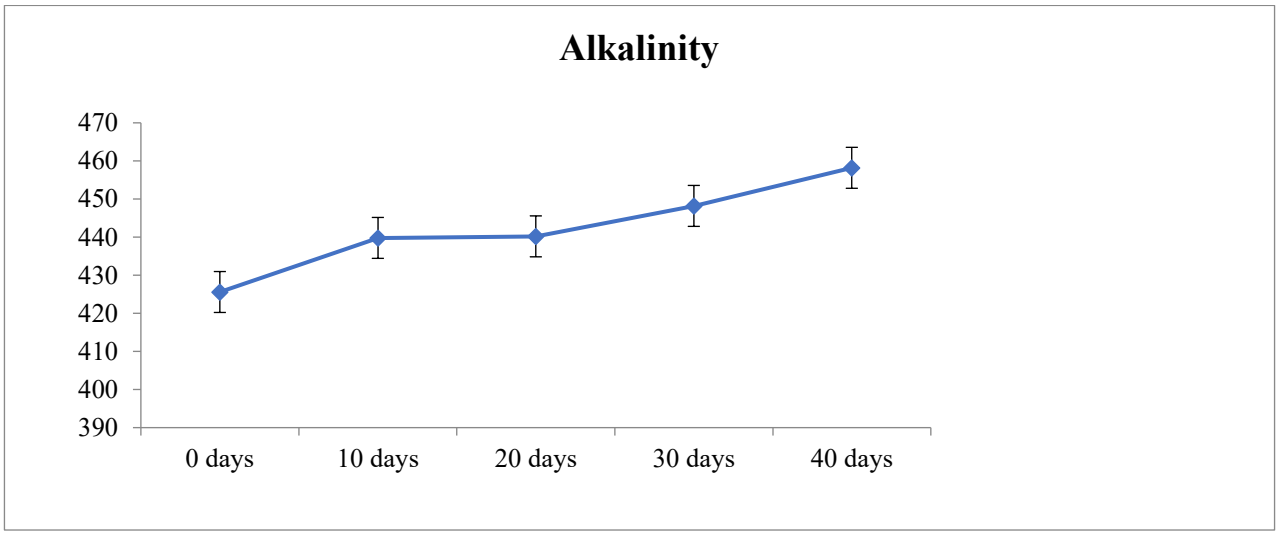

Figure 7: Graph showing the slight rise in alkalinity during beginning of 10 days treatment in treated paper mill effluent by *Lemna minor* L. and it again occurred after 20 to 30 days of treatment.

3.7 Effects on chemical oxygen demand (COD):

Chemical Oxygen Demand is the measure of the amount of oxygen required to break down both organic and inorganic matter. In the present investigation, the untreated effluent was 544 mg/l, and after treatment of this effluent by *Lemna minor*, the COD value reduced to 474.4 mg/l. It shows 87.2 % (Fig 8). This reduction may be due to the destruction of organic substances as well as the self-purification capacity of the water body[31].

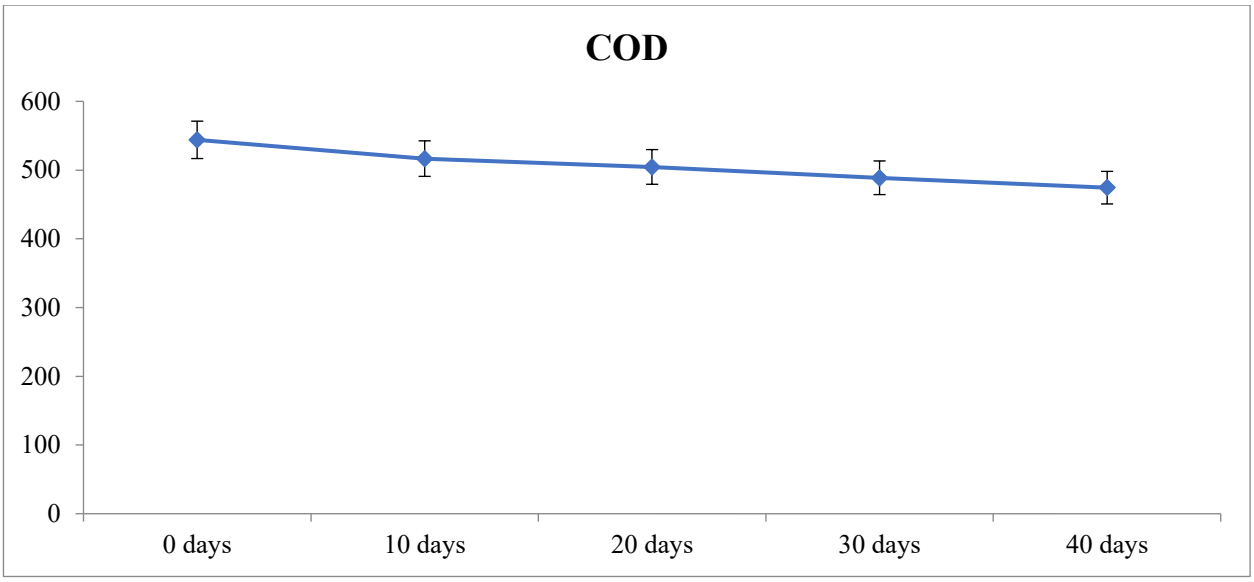

Figure 8: Graph showing the reduction in COD of paper mill effluent treated with *Lemna minor* L.

4. Conclusion

Nowadays, water contamination caused by industrial effluent having heavy metals is a major problem worldwide. Both wastewater and un-sufficiently treated industrial water contribute continuously to degrade the environment. In contrast to organic contaminants, heavy metals persist and are likely to accumulate in the environment through food chains etc. and causing different types of damages. To overcome their effects, conventional remediation technologies, such as chemical precipitation, reverse osmosis, ion-exchange, and solvent extraction, have disadvantages, including incomplete metal removal, being quite expensive, and the generation of toxic sludge, which requires disposal. Hence, 'Phytoremediation' has proved to be a viable option to purify water contaminated with heavy/trace elements since it is cost-effective and has a positive impact on the environment. This is an alternate technology in which small-scale wastewater treatment can be achieved. With increasing time, the concentration of the pollutants decreases. However, beyond attainment, *Lemna minor* L. ceases to contribute towards pollution removal. The variation in parameters caused by phyto-remediation of industrial effluents cannot exceed a finite limit and a maximum on the first day of the experiment. So, the use of such plants in the treatment of industrial effluents having heavy metals as contaminants is a safe and cost-effective technique for sustainable prospects.

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Authors' Contributions: RK and SV conducted the experiment and wrote the original draft of the paper. AKS conceptualized and supervised the project, while PK handled the editing and corrections and helped in writing original draft. Additionally, RV, SV, and PK were involved in the various investigation of the study. All authors have read and agreed to the final version of the manuscript.

Data Availability Statement: All data generated is included in this article.

Conflict of interest: The authors declare that there is no conflict of interest regarding this research work.

Ethical Statement: This study involved plant materials and environmental samples only. No experiments were conducted on human participants or live animals. All experimental procedures complied with relevant institutional, national, and international guidelines for plant research and environmental safety. The authors confirm that no ethical approval was required for this study.

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Morpho-taxonomic and phylogenetic characterization of *Artomyces pyxidatus* (Auriscalpiaceae) from the western Himalayas, India

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Abstract

In this present communication, *Artomyces pyxidatus*, belonging to the family Auriscalpiaceae, is described from the state of Uttarakhand as a new record for the region with molecular phylogeny. It was found in association with *Quercus leucotrichophora* and is characterized by being gregarious, repeatedly branched, smooth, and whitish to pale yellowish at first and sometimes darkening to pale tan or brown; it tastes peppery-acrid. *Artomyces pyxidatus* is presented here as a new record for the Uttarakhand mycobiota, and its identity is based on detailed morphology, anatomy, and nrITS-based phylogeny.

Keywords: *Artomyces pyxidatus*, Uttarakhand, new record, nrITS, Taxonomy, Phylogeny

1. Introduction

Earlier classifications grouped coral fungi into a single taxonomic category, based on the assumption that all coralloid and clavarioid forms shared a close evolutionary relationship[1, 2]. Subsequent molecular phylogenetic studies have demonstrated that these fungi are not monophyletic; instead, coralloid and clavarioid taxa are distributed among multiple orders within the Agaricomycotina[3]. To address this heterogeneity, the genus *Artomyces* included to clavarioid species characterized by simple to pyxidately branched basidiomata, the presence of hymenial gloeocystidia and gloeoplerous hyphae, and small, hyaline, amyloid basidiospores[4, 5]. Molecular phylogenetic analyses of the Russulales by Miller et al.[6] subsequently placed *Artomyces* within the Auriscalpiaceae, where it forms a distinct lineage sister to other genera in the family, including *Auriscalpium, Lentinellus, Gloiodon*, and *Dentipratulum*. Lickey et al.[7] conducted the only detailed phylogenetic and taxonomic analysis of *Artomyces* to date. Their findings corroborated Jülich's view[5] that *Artomyces* represents a distinct genus, clearly separable from *Clavicorona* Doty. In that study, seven new species were formally introduced, one species was transferred from *Clavicorona* through a new combination based on *Clavicorona microspora* Qiu X. Wu & R.H. Petersen, and two additional taxa were provisionally recognized but left unnamed. In summary, Lickey et al.[7] (2003) recognized a total of 15 species in the genus *Artomyces*, using an integrative approach that combined morphological characters, nuclear ribosomal internal transcribed spacer (nrITS) sequence data, and mating compatibility studies. During a fungal foray in Mandal area of Goesphwar, district Chamoli in Uttarakhand, we encountered a clavarioid basidiomycete in the genus that was fruiting on highly decayed wood of *Quercus leuchotrichophora* Sm. This clavarioid fungus, *Artomyces pyxidatus* (Pers.) Jülich was reported for the first time from India. In this present communication, we are presenting this species with micromorphological characteristics along with its phylogenetic analysis.

2. Materials and Methods

2.1 Morphological study

Various macro-morphological features were recorded from fresh fruiting bodies in the field along with the habitat and associated host plants. Macro-chemical test reactions with 10% KOH and 50% ammonia solution were noted down. Colour codes follow Kornerup and Wanscher[8]. Photographs of the fresh basidiomes were captured with a Canon EOS1300D. Micro-morphological characteristics were observed from free-hand sections of dried materials. Sections were mounted in a mixture of 5% KOH, 1% Phloxine, and 1% Congo red, then observed under a compound microscope (Olympus BX43). Line drawings were made with the help of Camera lucida attached to a microscope at 1000x magnification. Microphotographs of various elements were taken using a digital camera attached to an Olympus CX33 compound microscope. A total of 50 basidiospores from each of the specimens were observed. Basidiospore measurements are represented as minimum-mean-maximum length × minimum-mean-maximum width and Q = length/width of basidiospores.

2.2 DNA extraction, PCR amplification, and sequencing

Nuclear genomic DNA was isolated from 100 mg of dried fruit bodies using a fungal genomic DNA Mini Kit (RGCB Thiruvananthapuram). The ITS region of the nuclear ribosomal DNA gene was amplified using primer pairs ITS1 and ITS4[9]. PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X Phire PCR buffer 0.2 mM each dNTPs, 1 µl DNA, 0.2 µl Phire Hotstart II DNA polymerase enzyme, 0.1 mg/ml BSA and 3% DMSO, 0.5 M Betaine, 5 pM of forward and reverse primers. PCR amplification was carried out in a PCR thermal cycler programmed for 2 min at 96°C, followed by 30 cycles of 30 sec at 96°C, 40 sec at 50°C, and a final stage of 4 min at 60°C. The PCR products were purified with QIAquick Gel Extraction Kit (QIAGEN, Germany) and then subjected to Sanger sequencing in an automated DNA sequencer using the same primers. The obtained sequences were then submitted to GenBank (https://www.ncbi.nlm.nih.gov/) and accession numbers of two collections are given in Figure 1.

2.3 Phylogenetic analysis

Phylogenetic analysis was performed using the nrITS dataset comprising our newly generated two sequences along with the sequences retrieved from BLAST searches[10] as well as previously published phylogenies[11, 12]. In this study, a dataset of 56 nrITS sequences of *Artomyces* species including our sequences were used to analyze the data. The nrITS dataset was aligned with MAFFT v.7[13]. Maximum likelihood (ML) phylogenetic analysis inferred from nrITS sequences was performed using MEGA-X software[14]. One-thousand bootstrap (BS) replicates were analyzed to obtain nodal support values. Bootstrap support values (>50%) obtained from ML analysis are shown above or below the branches at nodes. The species of *Lentinus* were used as an out-group for the present phylogenetic analysis.

3. Results

3.1 Phylogeny

The Indian collections are placed within the *Artomyces pyxidatus* clade suggesting their identity as *A. pyxidatus,* SC/PU/24 (which is also evident in the BLAST search and are also well supported by 100% bootstrap values). Our nrITS phylogenetic analysis showed that sequences derived from the Indian collections of *A. pyxidatus* (SC/PU/24) nested within the clade consisting of the sequence of *A. pyxidatus* (PP392806; KR673584) collection from China and Korea, respectively with support value of (MLbs = 100%). Our phylogenetic analysis strongly suggests that the Indian collections are conspecific with Asian *A. pyxidatus* (Fig. 1).

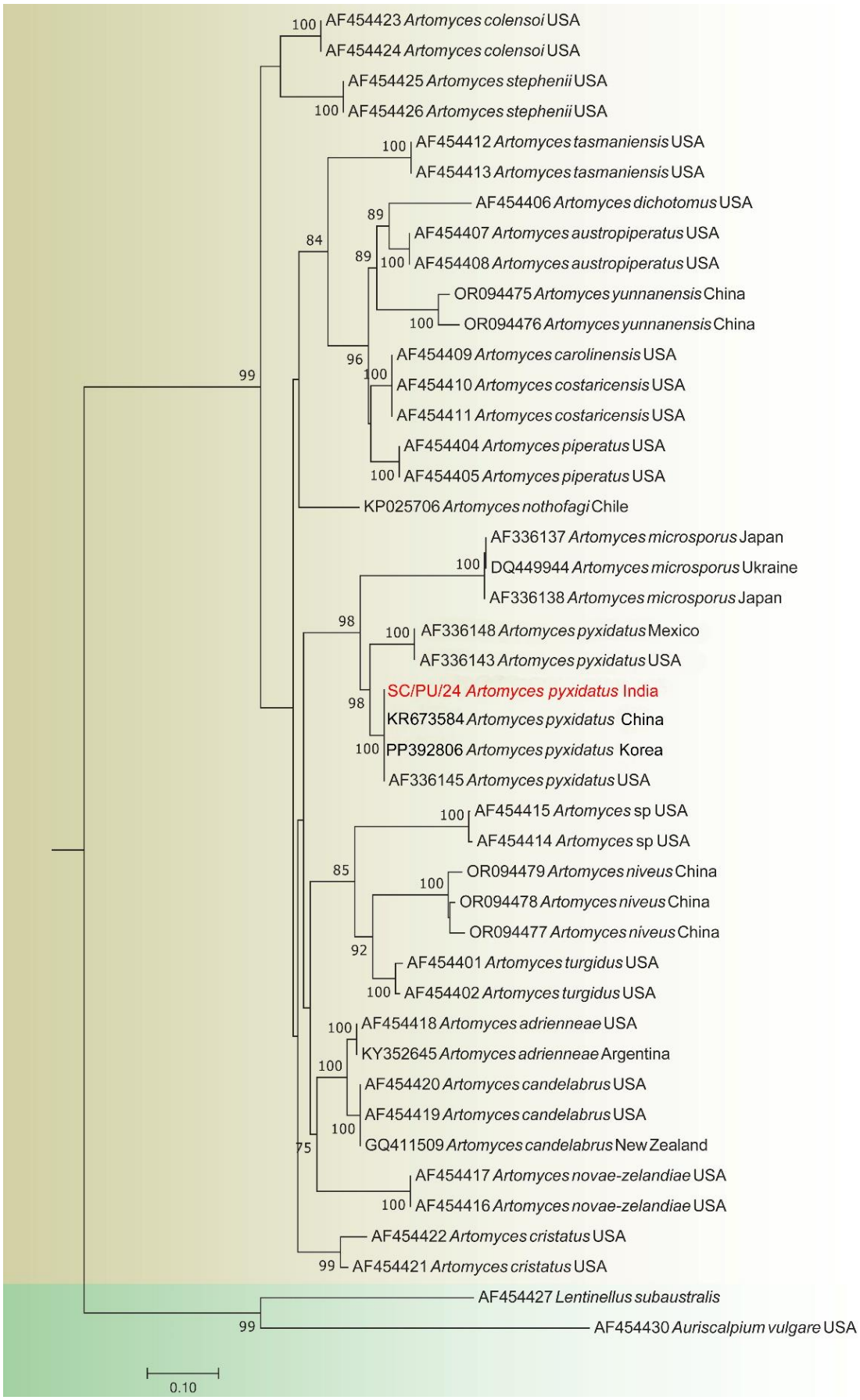

Figure 1. Maximum Likelihood phylogenetic tree inferred from ITS-rDNA sequence data using GTR+GAMMA model of nucleotide evolution constructed in RAxML v.2.0.10. Branches are labelled with ML bootstrap support values (≥50 %). Sequence derived from Indian collection of ***Artomyces pyxidatus*** is shown as bold in the tree

3.2 Taxonomy

***Artomyces pyxidatus* (Pers.) Jülich** (Figs. 2, 3)

Artomyces pyxidatus (Pers.) Jülich (1982) (Syn: *Clavaria coronata* Schwein., 1832; *Clavaria petersii* Berk. & M. A. Curtis, 1873; *Clavaria pyxidata* Pers., 1794; *Clavaria pyxidata* var. *asperospora* S. G. M. Fawc., 1938; *Clavaria pyxidata* var. *pyxidata* Pers., 1794; *Clavicorona coronata* (Schwein.) Doty, 1947; *Clavicorona pyxidata* (Pers.) Doty, 1947; *Clavicorona pyxidata* var. *asperospora* (S. G. M. Fawc.) Lickey et al., 2003; *Clavicorona pyxidata* var. *pyxidata* (Pers.) Doty, 1947; *Merisma pyxidatum* (Pers.) Spreng., 1827)

Description: Basidiomata lignicolous, single or in small groups, reaching up to 80 mm in height and about 25 mm in width at the apex. Typically branched in ranks of 2-5, coronate with pointed tips. Apices coronate-cristate to cuspidate, pale yellow (2B3) to light brown (5A2), becoming dark brown (5A4) on drying. A distinct white basal mycelium is present at the base. Odor peppery, Taste acrid.

Context composed of two hyphal types: generative hyphae with thin walls, usually encrusted with yellow-brown deposits, measuring 3.0–12.0 µm in diameter and bearing clamp connections; and gloeoplerous hyphae, abundant in the trama, aseptate, 3.4–7.5 µm wide, which terminate in the hymenium as gloeocystidia. Gloeocystidia are frequent, predominantly cylindrical to clavate, occasionally fusiform, flexuous, or subcapitate to capitate, 4–7.5 µm wide, consistently constricted within the subhymenium. Basidia are mostly four-sterigmate narrowly clavate to cylindrical, with conspicuous basal clamp connections, measuring 19.5–22.3 × 4.7–5.5 µm. Basidiospores measure 3.0–4.1 × 2.5–3 µm with Q values of 1.0–1.4 (Qm = 1.3), are globose to subglobose, smooth, hyaline, and strongly amyloid in Melzer's reagent, containing a single large guttule.

Habit and Habitat: Gregarious, on wood log of *Quercus leucotrichophora* Sm.

Specimen examined: INDIA. Uttarakhand: district Chamoli, Gopeshwar, Mandal 30°27'20.84"N 79°16'38.24"E, elev. 1900m, 04 July 2024, Shikha Choudhary and Priyanka Uniyal (SC/PU/24).

Distribution: This species has been reported from China and USA[5, 15] and now also from Himalayan district of Chamoli, India.

Notes: *Artomyces pyxidatus* is a species with wide geographical distribution with diverse hosts and chiefly characterized by the presence of almost white, profusely branched basidiomata with cuspidate tips and elongated basidiospores[7]. Present Indian specimen is in conformity with *Artomyces pyxidatus* based on its morphological and microscopical similarities which is also supported by the results of phylogeny depicted in the phylogram.

Discussion

The present record of *Artomyces pyxidatus* from the Chamoli district of Uttarakhand represents a significant extension of its known geographical range to the western Himalaya. The Indian specimen closely matches published descriptions of *A. pyxidatus* in both macro- and micromorphological characters.

Ecologically, the occurrence of *A. pyxidatus* on decaying wood of *Quercus leucotrichophora* at about 1900 m elevation is consistent with its known preference for hardwood substrates in temperate forest ecosystems, while also adding a new host record. The close morphological agreement, supported by phylogenetic placement, validates the identification and underscores the wide ecological amplitude of the species.

Conclusion

The present study provides the first confirmed record of *Artomyces pyxidatus* from the Western Himalayan region of India. Detailed macro- and micromorphological observations, coupled with phylogenetic support, firmly establish the identity of the species. This new regional record significantly extends the known distribution of *A. pyxidatus* and emphasizes the underexplored diversity of clavarioid fungi in the western Himalaya. Continued systematic surveys integrating morphological and molecular data are essential to improve our understanding of fungal diversity, biogeography, and host associations in this ecologically important region.

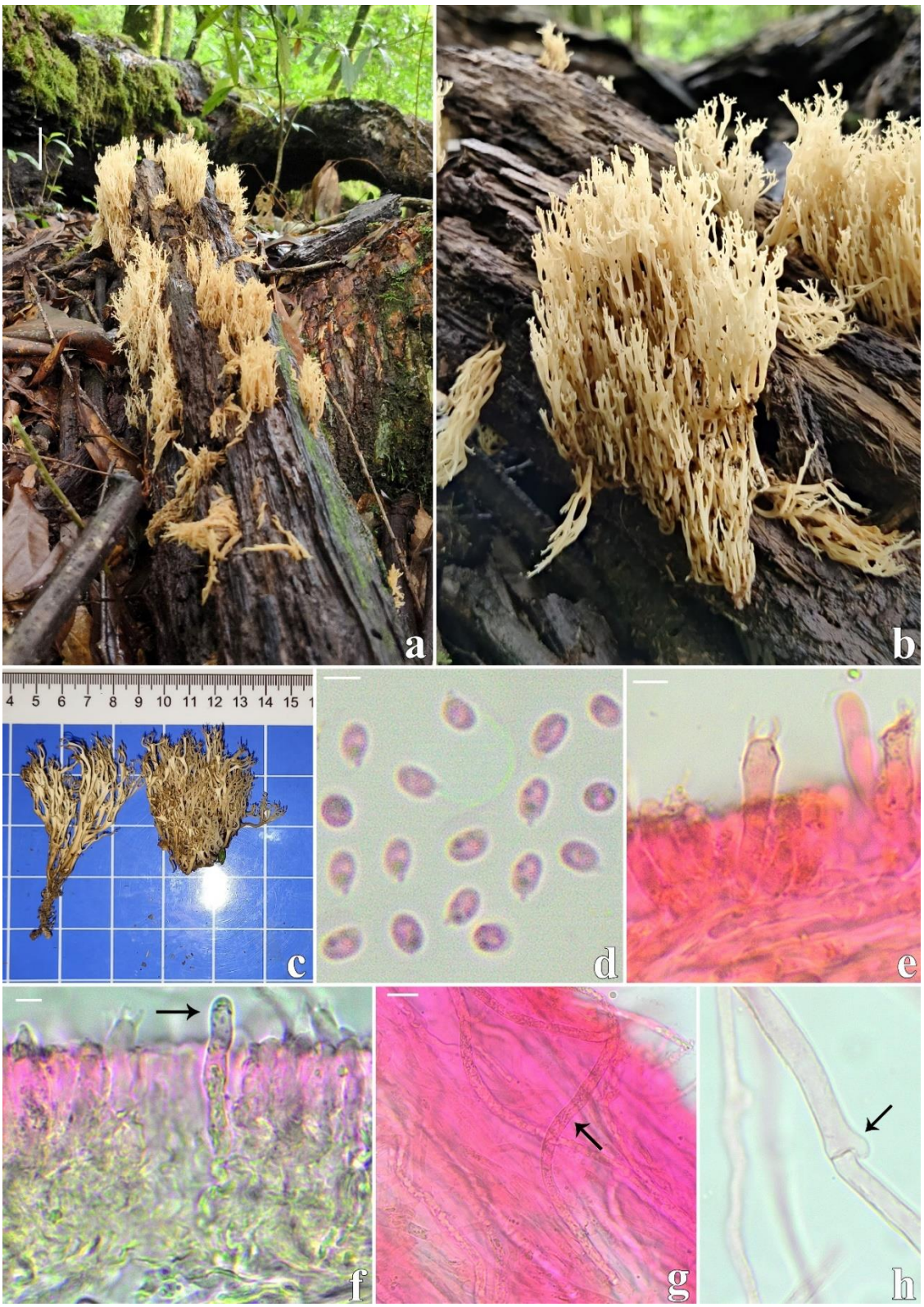

Figure 2. ***Artomyces pyxidatus*.: a–c.** Fresh basidiomata in the field and basecamp; d. Basidiospores; **e.** Basidia; **f.** Gloeocystidia; **g.** Gloeopleurous hyphae; **h.** Contextual hyphae showing clamp connection.Scale bars: **a =** 10 mm, **d–h =** 10 µm

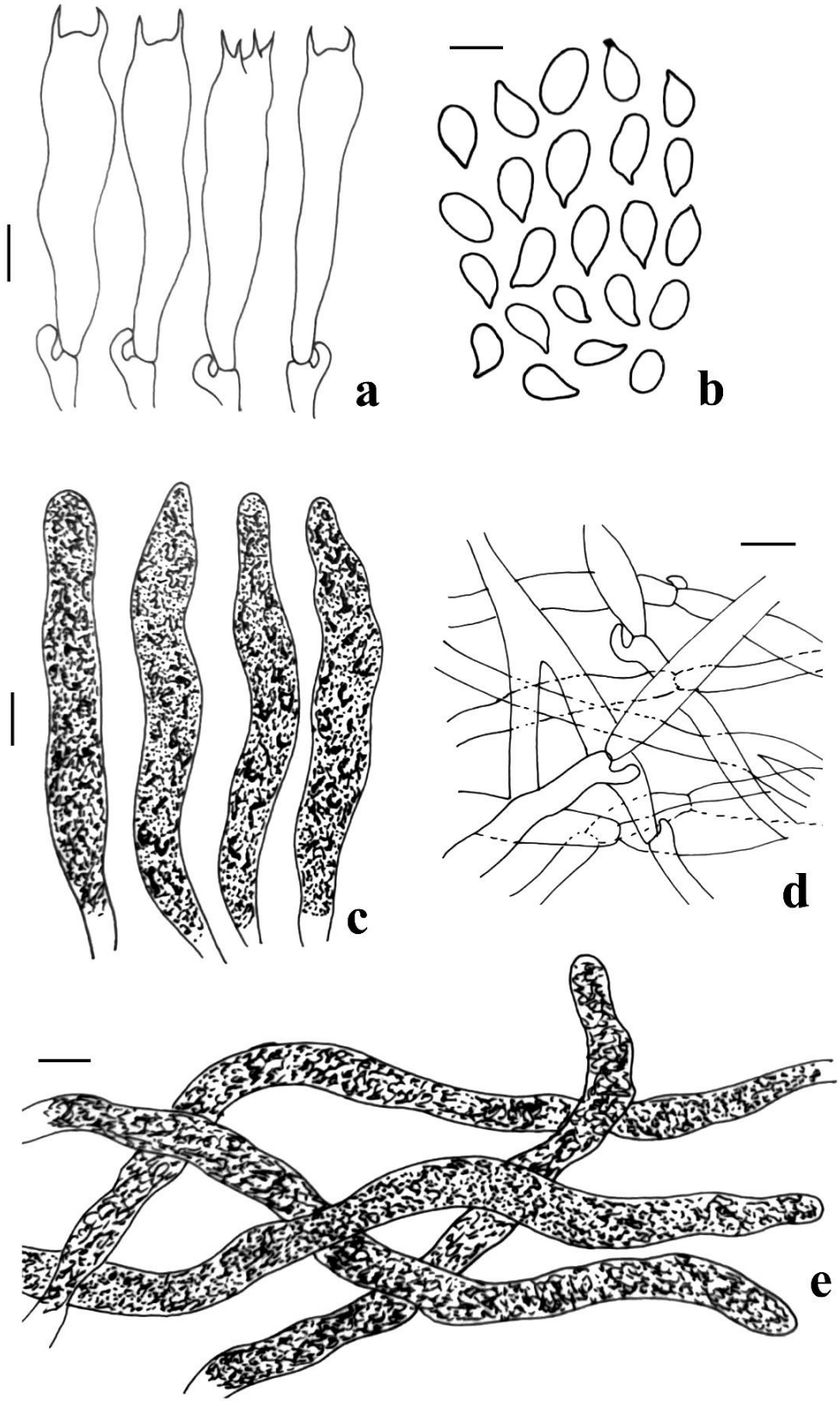

Figure 3. Line drawings of *Artomyces pyxidatus*.: a. Basidia; **b.** Basidiospores; **c.** Gloeocystidia; **d.** Contextual hyphae showing clamp connection; **e.** Gloeopleurous hyphae. Scale bars: **a–e =** 10µm

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Authors' Contributions

SC conducted the experiment and wrote the original draft of the paper. PU conceptualized and supervised the project, while YPS handled the editing and corrections and helped in writing original draft. All authors have read and agreed to the final version of the manuscript.

Data Availability Statement

All data generated is included in this article.

Conflict of interest

The authors declare that there is no conflict of interest regarding this research work.

Ethical Statement

This study involved plant materials only. No experiments were conducted on human participants or live animals. All experimental procedures complied with relevant institutional, national, and international guidelines for plant research and environmental safety. The authors confirm that no ethical approval was required for this study.

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Cultivation of "Badri Berry" (*Hippophae salicifolia* D. Don) for multiple profit with special reference to natural demographic surveys of Uttarakhand Himalayas

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Abstract
Sea buckthorn, the "Badri Berry" (*Hippophae salicifolia* D. Don) of Uttarakhand, is an important medicinal plant that is grown naturally in extremely cold conditions in cold desert areas of this Himalayan state. The plant has high medicinal importance but was unknown to the general public till the recent past. With general awareness of its modern traits recently, it has gained global attention due to its nutritional and therapeutic properties. It is one of the most important underutilized plants at high altitudes in the cold desert region of the Himalayas, including J&K, Himachal, and Uttarakhand. It has immense industrial, medicinal, cosmetic, and pharmaceutical importance and has a lot of potential as a bio-resource for land reclamation because of its ability to bind soil, provide rapid surface cover, fix nitrogen, and endure cold and drought. However, research on sea buckthorn is still limited due to recent awareness among people in general. This brief review and research data summarize the demographic status, economic potential, multiple uses, and properties, consequently, which may lead to its large-scale conservation, cultivation, and utilization within the state and country. Sea buckthorn, which will give momentum to recognize it as an exceptional plant in the state of Uttarakhand.

Keywords: Sea buckthorn, Badri Berry, *Hippophae salicifolia*, Pharmaceutical, Super fruit, Demographic Status, Nutritional properties, Soil Conservation.

1. Introduction
Sea buckthorn is locally known as 'Chuk,' 'Ames,' and 'Ameel' in Uttarakhand. The second author of this article proved it as "Badri Berry" on the basis of the ancient literature of Skanda Purana and Srimad Bhagavatam. Hence, further in this paper, we will mention sea buckthorn (*Hippophae salicifolia* D. Don) as "Badri Berry." Badri berry is a hardy, spiny, and thorny shrub thriving in Uttarakhand's high altitudes. It is famous for its tiny, bright orange-yellow berries, which are very rich in vitamin C and antioxidants. Yellow berries are used traditionally for health and now for their juices, oils, and supplements. Multiple uses of the plant make it a valuable "green gold" for local livelihoods and ecological balances for the whole Himalayas.

The natural variety of sea buckthorn, i.e., "Badri Berry" of the Uttarakhand Himalayas, is scientifically known as *Hippophae salicifolia*, while in Himachal and Jammu Kashmir, it's another species, *H. rhamnoides*, which is common. The berries of *H. rhamnoides* are bright orange in color, and the berries of *H. salicifolia* are bright yellow. It is a dioecious and deciduous shrub belonging to the family Elaeagnaceae. It is widely recognized as a "multipurpose wonder plant" due to its rich nutritional profile, extensive health benefits, and significant ecological value in cold-temperate regions of the Himalayas, especially in Uttarakhand.

Hippophae salicifolia D. Don, known as willow-leaved sea buckthorn, is a significant wild species in the Uttarakhand Himalayas, thriving in riverine areas (for example Alaknanda and Yamunotri) from 1500 to 3500 m, valued for its nutritious fruit and soil stabilization, with key habitats in Uttarkashi, Chamoli, and Pithoragarh, often forming dense stands on sandy/gravelly slopes. Local names include 'Chuk' or 'Ameel,' and studies highlight its importance for bio-fencing, fuel, fodder, and medicine, with research focusing on its genetic diversity and ecological roles[1].

Figure 1. 'Badri berry' (*H. salicifolia*) plant with fruits

Sea buckthorn is a resilient, nutrient-dense shrub known for its bright orange/yellow berries profoundly rich in vitamins (C, E, and B12), antioxidants, and healthy fatty acids (omega 3, 6, 7, and 9). It has been used for centuries in traditional medicine (Ayurveda and Tibetan) for glowing skin, skin protection from cold, digestion, inflammation, and many more ailments. It is a 'super fruit' gaining modern use in juices, supplements, and cosmetics and as a restorative plant for harsh environments, valued for its potential health benefits and unique nutritional profile. It is exceptionally rich in a diverse array of nutrients and bioactive compounds, which vary depending on the plant part, origin, and processing method. It is often called a "natural vitamin treasure house" due to the presence of nearly 200 components with potential nutritional and medicinal value[2].

In Uttarakhand, the Herbal Research and Development Institute, Mandal-Gopeshwar, is working on multiplication and conservation through cultivation of suitable varieties of this plant in farmers' fields. Promotion of farmers for its cultivation and nursery development activities is being done for its mass-scale cultivation and multiple profits, which may uplift the farmers' socio-economic status in the near future.

2. Cultivation and Management

The plant of 'Badri berry' has significant and abundant wild growth in high hills of Pithoragarh, Rudraprayag, Chamoli, and Uttarkashi districts. The plant thrives in cold, high-altitude deserts, tolerating harsh conditions but needing well-drained soil. It grows on hill slopes in well-drained, sandy soil having pH 6.5-7.5, ensuring at least one or more male plants in 12 to 15 female plants for good fruiting and spacing them adequately 2 to 3 m apart for air[3].

In Jammu & Kashmir, Ladakh aims to be the Sea Buckthorn Capital with vast natural reserves, though production needs modernization. Uttarakhand and Himachal Pradesh also have significant wild growth and government institutions; universities and non-governmental organizations are working on this plant on a project-based management basis.

Large-scale 'Badri berry' cultivation requires scientific farming in high-altitude cold regions, as it is very difficult to grow, care for, and harvest the crop in cold desert areas. At present basic need of large-scale cultivation of 'Badri berry' is

planned management of progressive farmers' awareness, large scale planting material production and sustainable cultivation of available planting material of this 'golden crop'.

In Uttarakhand, the Herbal Research and Development Institute (HRDI), Mandal-Gopeshwar, has previously worked on planting material, fruit juice extraction, jam, and herbal tea production with the assistance of financial support from the National Medicinal Plant Board (NMPB), Government of India, from 2016 to 2018. Under this project HRDI established a high-altitude nursery at Munsiyari, Pithoragarh, and managed it as the quality planting material production site of 'Badri berry' in 2018. Approximately 200 plants of *H. salicifolia* were raised in this nursery in 2018, out of which 91 plants survived well in the nursery, and flowering and fruiting have taken place in 10 to 15 plants since 2024. It shows that it takes 5 to 7 years for flowering and fruiting after cultivation of healthy samplings.

3. Demographic Surveys & Methodology

In view of data recording on demographic structure, fruit, dry fruit, seed, and juice extraction of this 'Badri berry,' various surveys were conducted in different years and seasons by the institute. Harshil, Har-Ki-Dun & Gangotri valleys in Uttarkashi, Badrinath hills & Mana valley in Chamoli, and Darma & Vyas valley and Munsiyari hills in Pithoragarh districts were surveyed during the project period in 2016 and 2017 and after the project period in 2025 as follows:

During the Project Period in 2016 & 2017: - Surveyed for juice extraction and data collection of 'Badri berry' in Darma & Vyas valley in Oct-Dec 2016 and 2017.

After Project Period in 2023: - Surveyed for juice extraction and data collection of plants in Mori, Osala, Gangad villages, and Har-ki-Dun valley in Oct 2023.

After Project Period in 2025: - Surveyed for juice extraction and data collection of plants in Joshimath and Badrinath hills in Nov 2025 and Munsiyari and Khaliyatop hills in Dec 2025.

During the surveys, local people known to this species were contacted and asked questions for data collection on multiple uses of fruit juice and plant parts, natural availability around their habitation, area distribution, and expected area around the regions. On average, 15 to 20 local people of each valley/village were asked questions for data collection on juice preparations, plant part use, distribution, growth behavior pattern of the plant, and available data were recorded for this species in different parts of Uttarakhand. Fruits were collected from different sizes (smallest to largest) of fruiting trees, and juice was extracted from the collected fruits. Fresh fruit dried in sunlight for 20-25 days and weighed. After extraction of juice from fresh fruits, the remaining seeds/residue were dried and weighed for data recording on seed availability, fresh fruit weight, dry fruit weight, and per-unit juice extraction.

4. Results and Discussions

4.1 *Area and Distribution*

During the different surveys at different intervals, data was recorded on the basis of local inhabitants except in the Niti and Yamunotri valleys. Data recorded on flowering and fruiting, average height of fruiting trees in different locations, approximate distributions of the species in the region, and altitudinal range of occurrence, which is shown in Table-1. During the surveys it was observed that only a maximum of sixty percent of people were aware of the species and its importance, but in tribal areas like the Mana, Darma, Vyas, and Chaudas valleys, one hundred percent awareness was found about the species and its valuable fruit juice extraction. It was observed during interrogation of the tribal community in these areas that the tribal community traditionally obtained and used the juice of the 'Badri berry.' The majority of old people of the tribal community used to boil the juice up to half the quantity and keep it for use for a long time, up to 20 years. It is seen in the field that boiled juice becomes black in color and is never perishable.

Survey works were not conducted by authors for Gaurikund in Rudraprayag district and Niti valley in Chamoli district, and available data were cited from the existing literature to have complete data on sea buckthorn distribution within the state.

Table 1. Growth behavior pattern and approximate distribution of 'Badri berry' in cold alpine areas of Uttarakhand Himalayas

Sl	District	Area Visited/ Villages Surveyed	Growing Altitude Range msl	Recorded Average Plant Height m	Flowering Period	Fruiting Period	Approximate distribution area (ha)
1.	Uttarkashi	Sankari, Taluka, Dhatmir, Osla and Gangad (Har-di-Dun Valley)	1500- 3500	3-7	June-July	Aug-Sept	300
		Gangotri, Mukhwa, Harshil, Bagoli, Dharali, Jhala, Sukhhi, Purali, Barsu and Raithal (Gangotri-Harshil Valley)	1700-4000	3-10	June-July	Aug-Sept	800
		Kharsali, Janki Chatti, Hanuman Chatti, Phool Chatti, Syanachatti and Bhairo Ghati (Ymunotri Valley)	1500-3700	2-8	June-July	Aug-Sept	1000
***2.**	Rudraprayag	Gaurikund, Sonprayag, Trijuginarayan along Mandankini River (Kedar Valley)	1800-2700	3-9	June-July	Aug-Sept	**300***
3.	Chamoli	Pandukeshwar, Govindghat, Hanuman Chatti and Mana Village (Mana-Badri Valley)	2700-3800	2-8	June-July	Aug-Sept	1200
		Malari, Gamshali, Bampa, Farkya, Lata, Tolma and Niti (Niti Valley)	2200-3500	3-9	June-July	Aug-Sept	**200** *
4.	Pithoragarh	Munsiyari, Darkot, Sarmoli, Laspa and Khaliya Top (Munsiyari Valley)	2200-3300	3-8	May-June	July-Aug	400
		Dugtu, Daatu, Baling, Sella and Nagling (Darma Valley), Garrijang, Budhi, Gunji, Nabi, Rongkang, Napalchu and Kuti (Vyas Valley) and Sridang and Sirkha (Chaudas Valley)	1500-4000	2-10	June-July	Aug-Sept	1000
Approximate distribution area of sea buckthorn i.e. 'Badri berry' in Uttarakhand Himalaya							5200
*Distribution data source of Kedar Valley and Niti Valley[4]							

It is recorded that approximate wild distribution of the highly important species in the state is about five thousand two hundred hectares, which shows sufficient planting material is available in the nature for enhancement of rapid conservation programs in farmer's field.

Naturally in cold desert/alpine areas of Uttarakhand Himalaya, 'Badri berry' plants are available from 1500 m to 4000 m elevation range. Plant height was recorded from 2 m minimum to 10 m maximum. It is observed that in extremely dry hill slopes plant populations are found sparsely distributed here and there. This may be due to plant growth checked by extreme dry conditions and less availability of soil moisture, while in shady and damp areas population growth found in herd of about 100 to 200 plants and height of plant found 7 to 10m. Plant growth and more height gain may be due to the availability of sufficient moisture for growth enhancement of the plant. It is already reported in literature that generally *H. salicifolia* is a shrub that could grow up to a tree size (4–10 m) at 1500–3200 m above mean sea level and is limited in its biogeographical distribution to the Himalayas[5, 6]

Indigenously, the ethno-botanical uses of *H. salicifolia* by the regional people of the Central Himalaya include animal feed, cosmetics, food, fuel, medicine, veterinary care, and bio-fencing, etc.[7, 8].

Generally, it was observed that the flowering period of the plant is June-July throughout the state, but it was recorded as May-June in the Munsiyari nursery in the cultivated nursery area of sea buckthorn plants. Fruit ripening of these cultivated plants was also observed two months early in August-September, while ripening of fruit in the wild is recorded from Oct to Dec in the naturally grown regions. The height of the Munsiyari nursery is 2300 m; therefore, there is a slightly higher temperature in comparison to the natural cold desert habitat of the plant, which may affect the flowering and fruiting of the plants. Early flowering and early ripening of fruit may be due to higher temperatures in cultivated areas in the Munsiyari nursery. These plants in the Munsiyari nursery were raised in 2018, and flowering and fruiting started during May to Sept in 2024. This shows that if 'Badri berry' is cultivated on farmers' land, flowering, fruiting, and ripening of fruits may take place early, from the end of August to September.

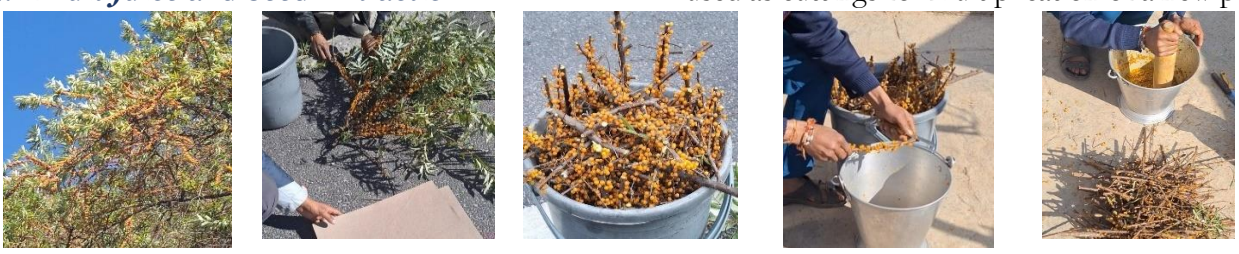
Fruits with small fruiting stems were collected from Har-ki-Dun, Harshil Valley, Badrinath Hills & Mana Valley, and Munsiyari & Vyas Valley from September to December in the years 2022, 2023, and 2025 to find out the data on juice extraction, seed availability per unit, and cuttings available per unit from wild collection, which is shown in Table 2. Tiny stems with fresh fruit and leaves were collected from the source in nature. Fresh fruits were separated from the tiny stems and collected in a fresh pot, weighed, and juice extracted. After extraction of juice, the weight of residue is taken and recorded, and the remaining tiny stems are cut down into 6-inch pieces for raising new plants as cuttings in nurseries.

It is observed that planting material in the form of seeds and cuttings may be gotten from the wild, by which no adverse impact on its natural population may occur. It is also seen that plucking of ripened fruit and extraction of juice from them left residue and small stem cuttings. In the remaining residue, seeds are available, which may be used as seed for multiplication, and the small stem after removing the fruit may be used as cuttings for multiplication of a new plant.

Table 2. Juice extraction, seed availability per unit and cutting available per unit from wild collection of 'Badri berry' from different locations

S.N	District	Sample collection area	Weight of fresh fruit Kg	Quantity of juice extracted kg	Weight of fresh residue after juice extraction kg	Weight of dry residue after juice extraction kg	Seed counts in dry residue	Available 6 inch cutting
1.	Uttarkashi	Sukhi Harshil	4 kg	1.1	2.95	0.750	18000	776
2.	Chamoli	Badrinath hills	5 kg	1.2	3.25	0.812	23000	975
3.	Pithoragarh	Munsiyari Nursery	3 kg	0.770	2.25	0.66	14100	580
		Darma Valley	4 kg	1.2	2.90	0.760	18500	796
Total Average			4 kg	1.06 kg	2.83 kg	0.745 kg	18400	3137
Per Kg Average			**1 Kg**	**0.265 kg**	**0.709**	**0.186**	**4600**	**784**
Average Percentage			**100 %**	**26.5 %**	**70.90 %**	**18.6 %**	**-**	**-**

4.2 *Fruit Juice and Seed Extraction*



Fruiting tree | Fruit collection | Fresh fruit with stem | Fruit separation from stem | Manual juice extraction

Figure 2: Fruit Collection of 'Badri berry' (*H. salicifolia*)

During the study fresh fruits with small stems were collected from the wild for extraction of juice manually, and average per-unit data on juice quantity, fresh residue quantity after juice extraction, dried residue quantity, seed counts in dried residue, and available stem cuttings for raising new plants were recorded.

It is observed that an average of 26.5 percent pulp juice may be extracted manually from one kg of fresh fruit, which may be enhanced if juice may be extracted mechanically. After extraction of juice from fresh fruits manually, 70.90 percent remaining residue is calculated, which included seed and fruit bark and other contents like small stem parts. When it dried, fresh remaining residue after extraction of juice, the average dried weight was 18.6 percent of fresh residue.

Based on the study, it may be concluded that from 1 kg of fresh fruit, 265 gm of juice may be obtained manually with 709 gm of fresh residue bearing seeds, which remains 186 gm after sun drying. It may also be concluded that in 1 kg of fresh fruits, approximately 4600 seeds are available, and from the stem part of 1 kg of fresh fruit, around 700 to 800 cuttings of 6 inches for new planting material may be obtained for raising in a nursery.

As per several studies, the reported moisture content of berries comes under the range of 84.9 to 97.6% for the sea buckthorn, and Uttarakhand's 'Badri berry' yields significant juice, with berries containing 60-80% juice, rich in nutrients.[9]. In the present study juice was extracted 26.5% in manual efforts; it may increase up to 60-80% mechanically. Therefore, it may be concluded that farmers who are involved in the cultivation of 'Badri berry' in Uttarakhand may extract 25 to 30 percent juice manually from one kg of fruits (Fig. 3).

4.3 *Dry Fruit and Seed Extraction*

During the study, whole fruit of 'Badri berry' is dried without juice extraction. It is taken from Badrinath hills and Munsiyari locations for dry fruit quality and quantity observation and seed count in dry fruits, and data were recorded, which are shown in Table-3.

Table 3. Dry fruit weight and seed count in 'Badri berry'

S.No.	District	Sample Collection Area	Weight of fresh fruit	Weight of dry fruits	Seed counts in dry fruits
1.	Pithoragarh	Munsiyari hills	1.0 kg	200 gm	4578
2.	Chamoli	Badrinath hills	0.20 kg	050 gm	918
Total			1.20 kg	250 gm	5496
Total Average per kg			**1.0 kg**	**208 gm**	**4580**

Fresh Fruit | Semi-dried fruit | Dry fruit | Dry residue after extraction of juice

Figure 3. Different stages of fruits of 'Badri berry' (*H. salicifolia*)

In Uttarakhand, dried 'Badri berry' fruit is a traditional super-food among tribal people, used for its potent vitamin C and antioxidants to boost immunity, fight colds/coughs, and heal skin ailments like cuts and burns, while they also use fresh juices and boiled juices traditionally. In view of exploring the difference in fresh and dry fruit weight, the data were recorded after drying of fresh fruits in sunlight for 20 to 25 days, and it was observed that 200 to 300 gm of dry fruits were obtained from one kg of fresh fruits.

As reported in the citation, 84.9 to 97.6% moisture content is available within the berries, so the weight of dry fruit is directly correlated to

the dryness of the fruit, and generally it ranges from 200 to 300 gm per kg of fresh fruit.

5. Conclusions

'Badri berry' in Uttarakhand offers significant ecological benefits, such as soil conservation and land reclamation, alongside extensive nutritional and medicinal advantages due to its rich composition of vitamins, antioxidants, and essential fatty acids. All parts of the plant are useful for the society, as fruits may be used for making juice, jam, jelly, pickle, and dry fruit; leaves may be used in herbal tea and other herbal preparations; and the plant has soil-binding capacity with nitrogen-fixing ability. Therefore, it is high time to promote conservation of the 'Badri berry' through cultivation for upliftment of the society of Uttarakhand.

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Author contributions

DSB: Conception, study and data collection and manuscript preparation. **AT:** Investigations, Formal analysis, conceptualization, manuscript writing.

Data Availability

All data included in this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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