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Floristic diversity and conservation significance of forests in and around Chaulabhaja Waterfall: A promising ecotourism site in Similipal Biosphere Reserve, Odisha

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ABSTRACT

Floristic diversity study in ecotourism site is significant from ecological as well as conservation point of view. Chaulabhaja Waterfall, located in Similipal Biosphere Reserve, Mayurbhanj district, Odisha, India, represents a biodiversity-rich beautiful ecotourism site, needs exhaustive floristic inventory. Therefore, comprehensive floristic surveys were carried out resulting in documentation of 210 vascular plant species belonging to 179 genera and 64 families. The dominant families were Fabaceae (25 species), Lamiaceae (12), Euphorbiaceae (12), Malvaceae (11), Apocynaceae (10), and Poaceae (10), with *Ficus* being the most species-rich genus. Growth form analysis revealed the predominance of trees (38%), followed by herbs (29%), shrubs (23%), climbers and lianas (8%). The study also documented pteridophytes like (*Adiantum incisum* Forssk.*, Pteris biaurita* L. and *Hemionitis arifolia* (Burm.f.) T. Moore), reflecting the habitat suitability for survival and growth of fern and fern allies. (*Rauvolfia serpentina* and - *Pterocarpus marsupium* were the two species on conservation concern. Besides, 268 use records of economically important species have been documented. The presence of invasive species (*Ageratum conyzoides* L., *Lantana camara* L., *Mikania micrantha* Kunth.) indicates anthropogenic pressure associated with increasing human. Our findings show the rich floral wealth of Chaulabhaja Waterfall and its adjoining areas with scenic beauty suggesting the site a potential ecotourism destination. Therefore, sustainable conservation and management strategies need to be developed based on maintaining a balance between the ecosystem vis-a-vis tourism. This study provides a baseline data for future monitoring, habitat protection, and conservation-oriented ecotourism planning in the region.

1. Introduction

Floristic inventories are an essential foundation for biodiversity conservation, sustainable utilization, and management of natural ecosystems. They generate baseline data on species composition, habitat preferences, life forms, and ecological relationships, while revealing changes in vegetation structure over time. Such studies help in assessing floristic patterns (McLaughlin, 1994), species invasion (Negi *et al.,* 2024), and the occurrence of endemic and threatened taxa within a defined phytogeographical region (Axelius, 1991). The preparation of floristic checklists, even for relatively small landscapes, is considered a prerequisite for understanding and evaluating the flora of larger areas (Bargali *et al*, 2025). Moreover, the floristic composition of any habitat reflects its ecological character, successional stage, and environmental conditions, thereby informing both scientific research and effective management strategies (Fartyal *et al.*, 2025).

Similipal, located in northern Odisha, is a renowned tropical forest landscapes in India. It is recognized as a

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Tiger Reserve (Government of India, 1973), Biosphere Reserve (UNESCO, 1994) and National Park (Government of Odisha, 2025). The landscape is supporting a wide range of vegetation types such as tropical moist deciduous, semi-evergreen, Sal-dominated forests, and grassland habitats. The area harbours rich floristic diversity, including medicinal plants, wild edibles, orchids, shrubs, climbers, grasses, and several endemic taxa (Saxena and Brahmam, 1989; Misra, 2004; Biswal *et al.,* 2011; Misra *et al,* 2011). Its complex terrain, perennial streams, and varied microhabitats contribute to high species heterogeneity. Besides, Similipal is also a major centre of ecotourism, attracting visitors to waterfalls, forest trails, and wildlife habitats. However, many ecologically sensitive zones within the park are witnessing increased human-attenuated disturbances, often without adequate monitoring or regulation. While certain areas of Similipal have been botanically explored (Mishra *et al.,* 2003; Behera, 2006; Dash and Behera, 2013; Rout *et al.,* 2022), floristic documentation remains incomplete and uneven. Only a few studies have attempted to assess species richness and vegetation patterns in localized landscapes within the park (Raut and Behera, 1997; Sahu *et al.,* 2018; Mohanta *et al.,* 2020, 2022), and many microhabitats remain underexplored. This indicates the need for further floristic inventories to update existing records, detect new occurrences, and identify conservation priorities.

Chaulabhaja Waterfall, located within the Similipal National Park, is one such lesser-known but ecologically significant site. Owing to its secluded location and poor accessibility, it remained relatively undisturbed for decades. In recent years, however, its popularity has grown rapidly through social media and word-of-mouth publicity among trekkers, nature enthusiasts, and adventure groups. Despite being within a protected area, it lacks formal ecotourism infrastructure, visitor management and regulation, and ecological monitoring. As a result, this site is increasingly vulnerable to trampling, littering, vegetation disturbance, and habitat degradation. Therefore, the present study aims to (i) document the flora and vegetation of Chaulabhaja Waterfall and its surrounding region, (ii) analyse the diversity, growth forms and economical importance of plant species, and (iii) identify conservation concerns arising out of emerging ecotourism pressures. This baseline assessment is expected to contribute to future management planning, ecological monitoring, and protection of sensitive microhabitats within Similipal Biosphere Reserve.

2. Materials and methods

2.1. Study area

Chaulabhaja Waterfall ($21^0$51’27.47” N, $86^0$32’48.98” E), is located within the Similipal Biosphere Reserve in Mayurbhanj district of Odisha, India (Fig. 1). The waterfall descends through rugged rocky terrain, surrounded by moist deciduous forest and riparian vegetation, creating a rich mosaic of microhabitats that harbour diverse floral assemblages. The site offers panoramic views of Similipal and lies within an ecological transition zone, linking the protected core zone of Similipal with the surrounding human-influenced landscapes.

Ecologically, the area falls within the Northern Tropical Moist Deciduous Forest type as per the classification of Champion and Seth (1968). The shady ,moist habitats along with the waterfall favour the growth of ferns, climbers, epiphytes, and shade-tolerant herbs, forming unique microhabitats of considerable conservation and ecological significance. The climate of this region is characterized by hot summers (avg. 38^0C to 40^0C), humid monsoons (avg. 1200 mm to 1600 mm), and cool winters (min. 8^0C to 10^0C). With its scenic beauty, rich floral diversity, and ecological value, Chaulabhaja Waterfall has recently emerged as a popular ecotourism and picnic destination.

2.2. Field survey and sample collection

The floristic survey of Chaulabhaja Waterfall and its adjoining areas was conducted between June 2024 to August 2025, covering all major phenological seasons to document maximum plant diversity. Field investigations were carried out at regular intervals to record and collect plant specimens representing different growth forms such as trees, shrubs, herbs, climbers, lianas and epiphytes. The morphological traits of the plant specimens such as shape, size and colour of stem, leaf, flowers, seeds were recorded, which were later used as a source to identify them with the help of the existing floras and literature.

2.3. Plant identification and data analysis

For identification, regional and national floras, including Flora of Orissa (Saxena & Brahmam, 1994-1996), The Botany of Bihar and Orissa (Haines, 1921-25), and relevant recent taxonomic revisions were used. The list of plant species was prepared and were arranged according to the APG IV (2016) classification system for angiosperms. Nomenclature was validated through online databases POWO (https://powo.science.kew.org). Further, the recorded species were analysed for their habit forms (tree, shrub, herb, climber, epiphyte), family-wise distribution, and nativity to assess structural diversity.

Additionally, threatened species were identified based on the IUCN Red List (2023) category and regional literature survey, while economic potential of species was determined using ethnobotanical records (Das and Misra, 1987; Girach

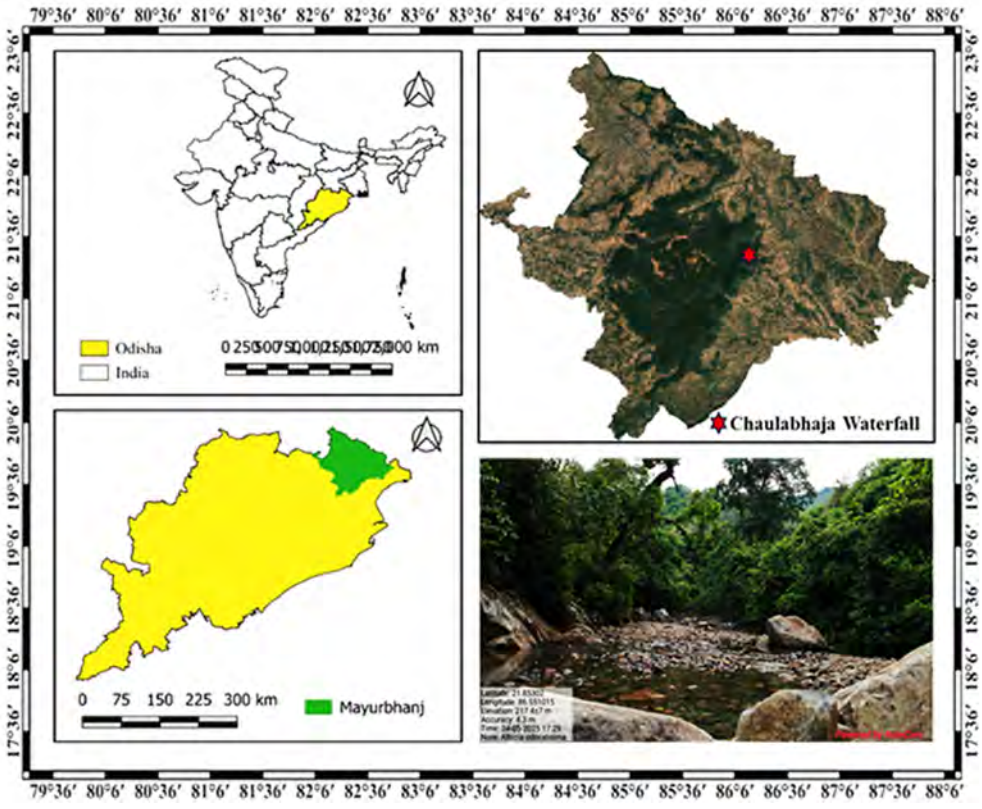

Fig. 1. Map showing the study area

et al., 1999; Pandey *et al.*, 2002; Kumar *et al.*, 2006; Mishra and Bal, 2007; Rout and Thatoi, 2009; Panda, 2014; Dikshit *et al.*, 2016). Likewise, ecotourism pressure in the study area was evaluated through visual observations, documentation of the predominance of invasive species and systematic recording of anthropogenic impacts along trails, resting sites, and waterfall viewpoints.

3. Results

3.1. Floristic composition

A total of 210 vascular plant species belonging to 179 genera and 64 families were recorded from the Chaulabhaja Waterfall and its surrounding region (Table 1). The ten dominant families were Fabaceae (20 genera, 25 species), Lamiaceae (12 genera, 12 species), Euphorbiaceae (8 genera, 12 species), Malvaceae (6 genera, 11 species), Apocynaceae (10 genera, 10 species), Poaceae (10 genera, 10 species), Acanthaceae (8 genera, 9 species), Asteraceae (8 genera, 8 species), Rubiaceae (7 genera, 7 species), and Combretaceae (3 genera, 6 species) (Fig. 2). Among the genera, *Ficus* was the most species-rich genus with six species, followed by *Diospyros, Euphorbia, Ipomoea, Senna, Terminalia,* and *Ziziphus,* each represented by three species. The predominance of Fabaceae, Lamiaceae, and Euphorbiaceae indicates their ecological adaptability to the tropical moist deciduous forest conditions of the area.

3.2. Growth form distribution

The analysis of growth habits revealed that trees constituted the highest proportion of species (38%), followed by herbs (29%), shrubs/undershrubs (23%), climbers and lianas (8%), and palms and parasitic plants (1% each) (Fig. 3). The occurrence of common tree species included *Alangium salvifolium* (L.f.) Wang., *Cleistanthus collinus* (Roxb.) Benth. & Hook. f., *Diospyros malabarica* (Desr.) Kostel., *Holarrhena pubescens* Wall. ex G. Don, *Mallotus philippensis* (Lam.) Müll. Arg., *Macaranga peltata* (Roxb.) Müll. Arg. and *Terminalia elliptica* Willd.

Common herbaceous species were *Achyranthes aspera* L., *Ageratum conyzoides* L., *Eragrostis ciliaris* (L.) R. Br., *Ocimum americanum* L., and *Tridax procumbens* L. Similarly, the shrubs included *Helicteres isora* L. and *Senna hirsuta* (L.) Irwin & Barneby, while climbers such as *Combretum roxburghii* Spreng., *Phanera vahlii* (Wight & Arn.) Benth. and *Symphorema involucratum* Roxb. were more common along forest edges and riparian corridors (Photo Plate 1 and 2).

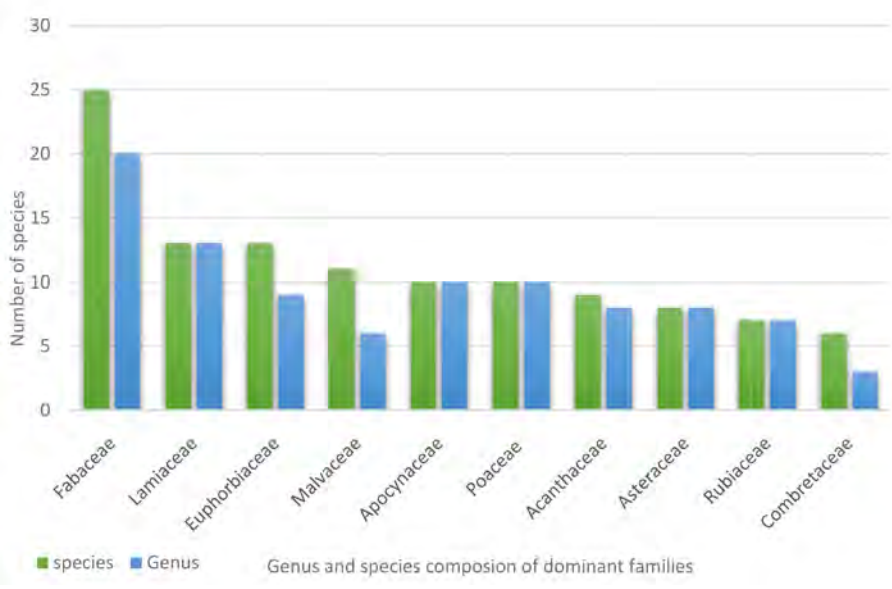

Fig. 2. Histogram showing the distribution of genus & species of dominant families.

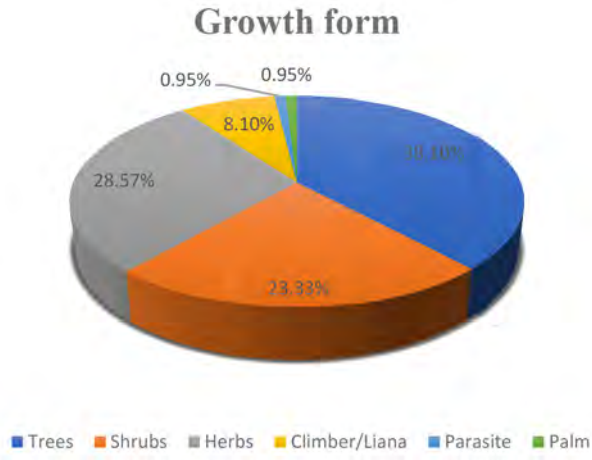

Fig. 3. Pie chart showing the growth form of recorded species

3.3. Economically important species

Chaulabhaja Waterfall has rich wealth of plants having economic potential as culinary, medicine, ornament and for other ethnobotanical uses. The present study represented a total of 268 use records across 6 different categories (Culinary, Edible fruit, Fodder, Medicinal, Ornamental and Timber). Medicinal plants formed the largest group (132 species use records), reflecting the strong ethnomedicinal reliance of local communities on plant-based healing practices. This was followed by species used as fodder (43), ornamental plants (35), culinary resources (20), and timber sources (20). Edible fruit yielding species contributed 18 use records. The predominance of medicinal and fodder species underscores the livelihood dependency on forest resources (Fig. 4).

3.4. Invasive and introduced species

A total of 45 exotic species were recorded belonging to 41 genera and 22 families. Of these, 10 species were recognised as invasive and 35 species as introduced. The presence of invasive species such as *Lantana camara* L., *Mikania micrantha* Kunth, *Chromolaena odorata* (L.) R.M. King & H. Rob., and *Ageratum conyzoides* L. indicates increasing anthropogenic influence around the waterfall, potentially threatening the native flora through habitat alteration and competition.

3.5. Cryptogamic diversity

The upper storey vegetation was often associated with epiphytic ferns, lichens, and bryophytes, particularly

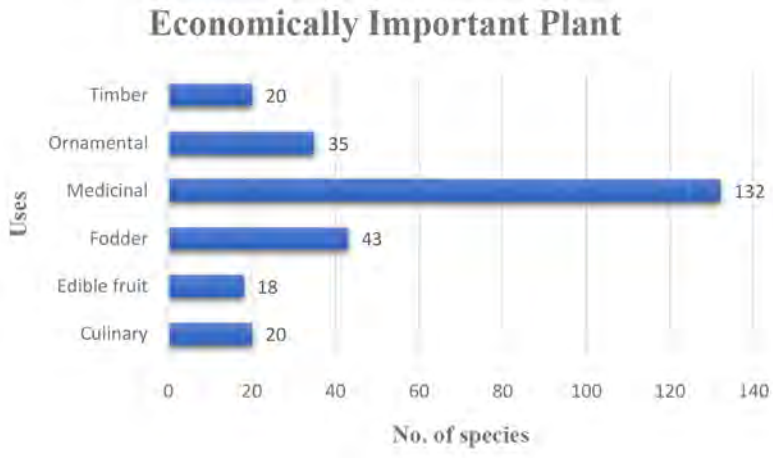

Fig. 4. Number of economically important plant species recorded at Chaulabhaja Waterfall across different use categories.

Table 1

List of species recorded from Chaulabhaja Waterfall and adjacent forests.

Family	Species name	Habit	Common name	Nativity	Uses
Acanthaceae	*Andrographis paniculata* (Burm. f.) Wall. ex Nees	Herb	Bhuinimbo	Native	Medicinal
	Barleria strigosa Willd.	Herb	Saptafena	Native	Ornamental
	Dicliptera bupleuroides Nees	Herb	-	Native	Medicinal
	Justicia adhatoda L.	Shrub	-	Native	Medicinal
	Justicia gendarussa Burm. f.	Shrub	-	Native	Medicinal
	Nicoteba betonica (L.) Lindau	Shrub	-	Native	Medicinal
	Rhinacanthus nasutus (L.) Kurz.	Shrub	Kaurasago	Native	Medicinal
	Ruellia tuberosa L.	Herb	-	Exotic	Ornamental
	Rungia repens (L.) Nees	Herb	-	Native	Culinary, Fodder, Medicinal
Amaranthaceae	*Achyranthus aspera* L.	Herb	Apamarga	Native	Medicinal
	Amaranthus viridis L.	Herb	-	Exotic	Culinary, Fodder
	Ouret lanata (L.) Kuntze	Herb	Pansia	Native	Medicinal
	Ouret sanguinolenta (L.) Kuntze	Herb	Chauladhua	Native	Fodder
Amaryllidaceae	*Crinum asiaticum* L.	Herb	Arsa	Native	Medicinal
Anacardiaceae	*Anacardium occidentale* L.	Tree	Lanka amba	Exotic	Edible fruit
	Mangifera indica L.	Tree	Amba	Exotic	Culinary, Edible fruit
	Spondias pinnata (L.f.) Kurz	Tree	Ambada	Native	Culinary, Edible fruit
Annonaceae	*Annona reticulata* L.	Tree	Ram-phala	Exotic	Edible fruit
Apocynaceae	*Alstoni scholaris* (L.) R. Br	Tree	Khiromula	Native	Medicinal, Ornamental
	Calotropis gigantea (L.) W.T. Aiton	Herb	Arakha	Native	Medicinal
	Cascabela thevetia (L.) Lippold	Tree	Kaniara	Exotic	Ornamental
	Catharanthus roseus (L.) G. Don	Shrub	Sada bihari	Exotic	Medicinal, Ornamental
	Hemidesmus indicus (L.) R. Br.	Climber	Sugandhi	Native	Medicinal

	Holorrhena pubescens Wall. Ex. G. Don	Tree	Kurchi	Native	Medicinal
	Plumeria alba L.	Tree	-	Exotic	Ornamental
	Rauvolfia serpentina (L.) Benth. ex Kurz	Shrub	Patala garuda	Native	Medicinal
	Vallaris solanacea (Roth ex Roem. & Schult.) Kuntze	Liana	-	Native	Medicinal, Ornamental
	Wrightia arborea (Dennst.) Mabb.	Tree	Harido	Native	Medicinal, Ornamental
Araceae	*Colocasia sp.*	Herb	Saru	-	Culinary
Araliaceae	*Heptapleurum venulosum* (Wight & Arn.) Seem.	Liana	-	Native	Medicinal
Arecaceae	*Borassus flabellifer* L.	Palm	Tala	Native	Edible fruit
	Phoenix sylvestris (L.) Roxb.	Palm	Khajuri	Native	Edible fruit
Asparagaceae	*Dracaena roxburghiana* (Schult. & Schult. f.) Byng & Christenh	Herb	-	Native	Ornamental
	Drimia indica (Roxb.) Jessop	Herb	-	Native	Medicinal
Asteraceae	*Acmella radicans* (Jacq.) R.K. Jansen	Herb	-	Exotic	Ornamental
	Ageratum conyzoides L.	Herb	Poksunga	Exotic	Medicinal
	Blumea sp.	Herb	-	-	Ornamental
	Chromolena odorata (L.) R.M. King & H. Rob.	Shrub	Pokasunga	Exotic	Medicinal
	Grangea maderaspatana (L.) Desf.	Herb	Agni kumari	Native	Medicinal
	Mikania micrantha Kunth.	Climber	-	Exotic	Medicinal
	Sphaeranthus indicus L.	Herb	Poka sungo	Native	Medicinal
	Tridax procumbens L.	Herb	Bisalyakarani	Exotic	Medicinal
Bignoniaceae	*Millaingtonia hortensis* L. f.	Tree	Sitahara	Exotic	Medicinal
	Oroxylam indicum (L.) Kurz.	Tree	Bhalu sakti	Native	Medicinal
Boraginaceae	*Ehretia aspera* Willd.	Tree	-	Native	Medicinal
	Heliotropium indicum L.	Herb	Hatisundhi	Exotic	Medicinal
Bromeliaceae	*Ananas comosus* (L.) Merr.	Shrub	Sapuri	Exotic	Edible fruit
Burseraceae	*Protium serratum* (Wall. ex Colebr.) Engl.	Tree	Limbru	Native	Timber
Cannabaceae	*Trema orientale* (L.) Blume	Tree	Jivani	Native	Timber
Cannaceae	*Canna indica* L.	Herb	Sarvajaya	Exotic	Ornamental
Capparaceae	*Capparis zeylanica* L.	Shrub	Sabbi	Native	Ornamental
Cleomaceae	*Cleome viscosa* L.	Herb	-	Native	Medicinal
Combretaceae	*Combretum indicum* (L.) DeFilipps	Shrub	Madhumalati	Native	Ornamental
	Combretum roxburghii Spreng.	Liana	Kala-achindi	Native	Medicinal
	Getonia floribunda Roxb.	Liana	Dubopatli	Native	Medicinal
	Terminalia anogeissiana Gere & Boatwr.	Tree	Dhaura	Native	Medicinal, Timber
	Terminalia bellirica (Gaertn.) Roxb.	Tree	Bahada	Native	Medicinal

	Terminalia elliptica Willd.	Tree	-	Native	Timber
Commelinaceae	*Commelina erecta* L.	Herb	-	Exotic	Fodder
Convolvulaceae	*Ipomoea* sp.	Climber	-	-	Medicinal
	Distimake vitifolius (Burm.f.) Pisuttimarn & Petrongari	Climber	-	Native	Medicinal
	Erycibe paniculata Roxb.	Liana	Joda Koli	Native	Fodder
	Evolvulus nummularius (L.) L.	Herb	-	Exotic	Fodder
	Ipomoea hederifolia L.	Climber	Panikoda	Exotic	Medicinal, Ornamental
	Ipomoea obscura (L.) Ker Gawl.	Climber	-	Native	Medicinal, Ornamental
Cornaceae	*Alangium saviifolium* (L.f.) Wangerian	Tree	Ankula	Native	Fodder
Cucurbitaceae	*Coccinia grandis* (L.) Voigt	Herb	Bana kunduri	Native	Culinary, Fodder, Medicinal
	Diplocyclos palmatus (L.) C.Jeffrey	Climber	Shivlinga	Native	Medicinal
Cyperaceae	*Cyperus platystylis* R.Br.	Herb	-	Native	Fodder
	Cyperus rotundus L.	Herb	-	Native	Fodder
Dilleniaceae	*Dillenia aurea* Sm.	Tree	Rai	Native	Culinary
Dioscoreaceae	*Dioscorea* sp.	Climber	-	-	Culinary
Dipterocarpaceae	*Shorea robusta* Gaertn. f.	Tree	sala	Native	Medicinal, Timber
Ebenaceae	*Diospyros malabarica* (Desr.) Kostel.	Tree	-	Native	Edible fruit, Timber
	Diospyros montana Roxb.	Tree	Bhodrika	Native	Medicinal, Timber
	Diospyros sylvatica Roxb.	Tree	Kanchia	Native	Medicinal, Timber
Euphorbiaceae	*Croton caudatus* Geiseler	Shrub	Furudi	Native	Medicinal
	Croton persimilis Mull. Arg.	Shrub	-	Native	Medicinal
	Euphorbia hirta L.	Herb	Chitakuli	Exotic	Medicinal
	Euphorbia nivulia Buch.-Ham.	Shrub	-	Native	Medicinal, Ornamental
	Euphorbia prostrata Aiton	Herb	-	Exotic	Fodder, Medicinal
	Homonoia riparia Lour.	Shrub	Paani begunia	Native	Medicinal
	Jatropha gossypiifolia L.	Shrub	Kalo	Exotic	Medicinal
	Macaranga peltata (Roxb.) Mull. Arg.	Tree	-	Native	Fodder
	Mallotus nudiflorus (L.) Kulju & Welzen	Tree	Panigambhari	Native	Fodder
	Mallotus philippensis (Lam.) Mull. Arg.	Tree	Sinduri	Native	Fodder, Medicinal
	Riccinus communis L.	Shrub	Jada	Exotic	Medicinal
	Suregada multiflora (A.Juss.) Baill.	Tree	-	Native	Medicinal, Timber
Fabaceae	*Abrus precatorius* L.	Shrub	-	Native	Medicinal
	Acacia auriculiformis A. Cunn. ex Benth.	Tree	-	Exotic	Ornamental, Timber
	Adenanthera pavonina L.	Tree	Kaincha	Native	Ornamental, Timber

	Albizia odoratissima (L.f.) Benth.	Tree	Kala sirisa	Native	Ornamental, Timber
	Bauhinia purpurea L.	Tree	-	Native	Culinary, Ornamental
	Bauhinia sp.	Tree	Kanchana	-	Culinary
	Butea monosperma (Lam.) kuntze	Tree	-	Native	Medicinal
	Butea superba Roxb. ex Willd.	Liana	Palasa	Native	Medicinal
	Cassia fistula L.	Tree	Sunari	Native	Medicinal
	Delonix regia (Bojer ex Hook.) Ref.	Tree	-	Exotic	Ornamental
	Entada rheedei Spreng.	Liana	Osta	Native	Medicinal
	Millettia extensa (Benth.) Benth. ex Baker	Shrub	Gurendi	Native	Medicinal
	Mimosa pudica L.	Herb	Lajakuli	Exotic	Fodder, Medicinal
	Mimosa rubicaulis subsp. Himalayana (Gamble) H. Ohashi	Shrub	-	Native	Medicinal
	Phanera vahlii (Wight & Arn.) Benth.	Liana	Siali	Native	Medicinal
	Pleurolobus gangeticus (L.) J.St.-Hil. ex H.Ohashi & K.Ohashi	Shrub	-	Native	Medicinal
	Pongamia pinnata (L.) Pierrea	Tree	Karanja	Native	Medicinal
	Pterocarpus marsupium Roxb.	Tree	-	Native	Timber
	Samanea saman (Jacq.) Merr.	Tree	Chakunda	Exotic	Ornamental, Timber
	Senegalia torta (Roxb.) Maslin, Seigler & Ebinger	Shrub	-	Native	Medicinal
	Senna hirsuta (L.) H.S. Irwin & Barneby	Shrub	Bada chakunda	Exotic	Mediicnal
	Senna obtusifolia (L.) H.S. Irwin & Barneby	Shrub	-	Exotic	Fodder, Medicinal
	Senna occidentalis (L.) Link	Shrub	Chakunda	Exotic	Medicinal
	Tamarindus indica L.	Tree	Tentuli	Exotic	Culinary
	Xylia xylocarpa (Roxb.) W. Theob.	Tree	-	Native	Medicinal
Hypoxidaceae	*Curculigo orchioides* Gaertn.	Herb	Talamuli	Native	Medicinal
Lamiaceae	*Anisomeles indica* (L.) Kuntze	Shrub	-	Native	Mediicnal
	Callicarpa tomentosa (L.) L.	Shrub	Bodopatri	Native	Medicinal
	Clerodendrum infortunatum L.	Shrub	-	Native	Ornamental
	Coleus strobilifer (Roxb.) A.J.Paton	Herb	-	Native	Medicinal, Fodder
	Gmelina arborea Roxb. ex Sm.	Tree	Gambhari	Native	Timber
	Leucas montana (Roth) Spreng.	Herb	-	Native	Medicinal
	Mesosphaerum suaveolens (L.) Kuntze	Shrub	-	Exotic	Medicinal
	Ocimum americanum L.	Herb	Banatulasi	Native	Medicinal
	Premna barbata Wall. ex Schauer	Tree	-	Native	Fodder, Medicinal
	Symphorema involucratum Roxb.	Shrub	-	Native	Medicinal
	Tectona grandis L.f.	Tree	Saguan	Native	Timber
	Vitex negundo L.	Shrub	Begunia	Native	Medicinal

Lecythidaceae	*Careya arborea* Roxb.	Tree	Kumbhi	Native	Ornamemtal, Timber
Loganiaceae	*Strychnos nux-vomica* L.	Tree	Kochila	Native	Medicinal
Loranthaceae	*Dendrophthoe falcata* (L.f.) Ettingsh.	Parasite	Madang	Native	Ornamental
Lythraceae	*Lagerstroemia speciosa* (L.) Martyn	Tree	-	Native	Ornamental
Malvaceae	*Abutilon indicum* (L.) Sweet	Herb	Pedipedika	Native	Medicinal
	Abutilon persicum (Burm.f.) Merr.	Shrub	-	Native	Medicinal
	Corchorus aestuans L.	Herb	-	Native	Fodder
	Grewia asiatica L.	Tree	-	Native	Medicinal
	Grewia tiliifolia Vahl	Tree	-	Native	Medicinal
	Helicteres isora L.	Shrub	-	Native	Medicinal
	Pterospermum acerifolium (L.) Willd.	Tree	Kanaka champa	Native	Medicinal
	Pterospermum xylocarpum (Gaertn.) Oken	Tree	-	Native	Medicinal
	Sida cordata (Burm. f.) Borss.Waalk.	Herb	Bajramuli	Native	Fodder
	Sterculia foetida L.	Tree	-	Native	Ornamental
	Urena lobata L.	Shrub	Rakta pheni	Native	Fodder
Melastomataceae	*Memecylon umbellatum* Burm.f.	Tree	-	Native	Medicinal, Timber
Meliaceae	*Azadirachta indica* A. Juss.	Tree	Nimba	Exotic	Culinary, Medicinal
	Cipadessa baccifera (Roxb. ex Roth) Miq.	Shrub	Pittamari	Native	Medicinal
Menispermaceae	*Tiliacora acuminata* (Lam.) Miers	Liana	-	Native	Medicinal
	Tinospora cordifolia (Willd.) Hook.f. & Thomson	Shrub	Guluchi	Native	Medicinal
Molluginaceae	*Trigastrotheca pentaphylla* (L.) Thulin	Herb	Pita gohun	Native	Fodder, Medicinal
Moraceae	*Artocarpus heterophyllus* Lam.	Tree	Panasa	Native	Culinary, Edible fruit
	Broussonetia papyrifera(L.) L'Hér. ex Vent.	Tree	Tutkuli	Native	Edible fruit
	Ficus benghalensis L.	Tree	Bara gacha	Native	Culinary, Edible fruit
	Ficus exasperata Vahl	Tree	-	Native	Medicinal
	Ficus hispida L.f.	Tree	Dimiri	Native	Fodder, Medicinal
	Ficus microcarpa L.f.	Tree	-	Native	Fodder, Medicinal
	Ficus recemosa L.	Tree	Dimiri	Native	Culinary, Medicinal
	Ficus religiosa L.	Tree	Ashwastha	Native	Fodder, Medicinal, Ornamental
	Streblus asper Lour.	Tree	Sahada	Native	Medicinal
	Taxotrophis taxoides (B. Heyne ex Roth) Chew ex E.M. Gardner	Shrub	Phutkuri	Native	Edible fruit
Moringaceae	*Moringa oleifera* Lam.	Tree	Sajana	Native	Culinary, Medicinal
Musaceae	*Musa* sp.	Herb	Kadali	-	Culinary, Fodder
Myrtaceae	*Syzygium cumini* (L.) Skeels	Tree	Jamu	Native	Edible fruit
Nyctaginaceae	*Boerhavia diffusa* L.	Herb	Atikapudi sag	Native	Culinary, Fodder, Medicinal

Oleaceae	*Jasminum scandens* (Retz.) Vahl	Shrub	Bana malli	Native	Ornamental
Orchidaceae	*Dendrobium aphyllum* (Roxb.) C.E.C. Fisch.	Herb	-	-	Ornamental
	Vanda sp.	Herb	-	-	Ornamental
	Vanda tessellata (Roxb.) Hook. ex G. Don	Herb	-	Native	Ornamental
Orobanchaceae	*Aeginetia indica* L.	Parasite	-	Native	Medicinal
Oxalidaceae	*Averrhoa carambola* L.	Tree	-	Exotic	Culinary
Phyllanthaceae	*Bridelia retusa* (L.) A.Juss.	Tree	Panikasi	Native	Medicinal
	Cleistanthus collinus (Roxb.) Benth. & Hook. f.	Tree	Karada	Native	
	Cleistanthus patulus (Roxb.) Müll.Arg.	Tree	-	Native	Medicinal
	Flueggea virosa (Roxb. ex Willd.) Royle	Shrub	-	Native	Medicinal
	Phyllanthus vitis-idaea (Burm.f.) J. Koenig ex Roxb.	Shrub	-	Native	Fodder, Medicinal
Plantaginaceae	*Mecardonia procumbens* (Mill.) Small	Herb	-	Exotic	Fodder, Medicinal
	Scoparia dulcis L.	Herb	-	Exotic	Medicinal
Poaceae	*Alloteropsis cimicina* (L.) Stapf	Herb	-	Native	Fodder
	Bamboo sp.	Shrub	Bunsa	-	Fodder, Timber
	Cenchrus pedicellatus (Trin.) Morrone	Herb	-	Native	Fodder
	Cynodon dactylon (L.) Pers.	Herb	Dooba ghasa	Native	Fodder, Medicinal
	Dactyloctenium aegyptium (L.) Willd.	Herb	-	Native	Fodder
	Dichanthium annulatum (Forssk.) Stapf	Herb	-	Native	Fodder
	Eragrostis ciliaris (L.) R.Br.	Herb	-	Native	Fodder
	Ischaemum ciliare Retz.	Herb	-	Native	Fodder
	Paspalum scrobiculatum L.	Herb	-	Native	Fodder
	Setaria pumila (Poir.) Roem. & Schult.	Herb	-	Native	Fodder
Pteridaceae	*Adiantum incisum* Forssk.	Herb	-	Native	Medicinal
	Hemionitis arifolia (Burm.f.) T.Moore	Herb	-	Native	Medicinal
	Pteris biaurita L.	Herb	-	Exotic	Medicinal
Rhamnaceae	*Ziziphus jujuba* Mill.	Tree	Barakoli	Exotic	Edible fruit, Fodder
	Ziziphus oenoplia (L.) Mill.	Shrub	Kantaikoli	Native	Edible fruit, Fodder
	Ziziphus rugosa Lam.	Shrub	Kotaikoli	Native	Fodder
Rubiaceae	*Adina cordifolia (Roxb.) Hook.f. & Benth.*	Tree	-	Native	Medicinal
	Gardenia latifolia Aiton	Tree	-	Native	Medicinal, Ornamental
	Meyna spinosa Roxb. Ex. Link	Tree	-	Native	Medicinal
	Mitracarpus hirtus (L.) Dc.	Herb	-	Exotic	Medicinal

	Paederia foetida L.	Shrub	Pasaruni	Exotic	Medicinal
	Psydrax dicoccos Gaertn.	Tree	-	Native	Medicinal
	Spermacoce articularis L.f.	Herb	Jibkata	Native	Medicinal
Rutaceae	*Aegle marmelos* (L.) Correa	Tree	Bela	Native	Edible fruit, Medicinal
	Citrus sp.	Tree	Lembu	-	Medicinal
	Murraya paniculata (L.) Jack	Tree	Kamini	Native	Medicinal, Ornamental
Sapindaceae	*Schleichera oleosa* (Lour.) Oken	Tree	Kusuma	Native	Edible fruit
	Lepisanthes tetraphylla (Vahl) Radlk.	Tree	-	Native	Medicinal
Sapotaceae	*Madhuca longifolia* (L.) J.F. Macbr.	Tree	Mahula	Native	Edible fruit, Fodder, Medicinal
Solanaceae	*Datura stramonium* L.	Shrub	Datura	Exotic	Medicinal, Ornamental
Ulmaceae	*Holoptelea integrifolia* (Roxb.) Planch.	Tree	-	Native	Medicinal, Timber
Verbenaceae	*Lantana camara* L.	Shrub	Nagaboiri	Exotic	Medicinal
	Lippiajavanica (Burm.f.) Spreng.	Shrub	-	Exotic	Medicinal
	Stachytarpheta jamaicensis (L.) Vahl	Shrub	-	Exotic	Culinary, Medicinal
Vitaceae	*Ampelocissus latifolia* (Roxb.) Planch.	Shrub	-	Native	Medicinal
	Leea indica (Burm.f.) Merr.	Shrub	-	Native	Medicinal

Photo plate 1. Photographs of recorded species found in Chaulabhaja Waterfall and its vicinity.

Photo plate 2. Photographs of recorded species found in Chaulabhaja Waterfall and adjacent forests.

along shaded and moist rock surfaces. Three species of pteridophytes were identified and recorded, such as *Adiantum incisum* Forssk., *Pteris biaurita* L. and *Hemionitis arifolia* (Burm.f.) T. Moore.

3.6. Conservation concern and ecotourism

Two species of conservation concern were identified in the study area, namely *Rauvolfia serpentina* (L.) Benth. ex Kurz (Endangered) and *Pterocarpus marsupium* Roxb. (Near Threatened), as per the IUCN Red List category (2023). The presence of these species indicates that the site provides suitable habitats for sensitive taxa. Observations also revealed signs of emerging ecotourism, such as trampling along trails, littering near resting and viewing points, minor vegetation clearing, and small campfire remains. While ecotourism presents opportunities for local livelihood development and environmental education, these activities may simultaneously create pressures on the habitat, including disturbance to native flora, facilitation of invasive species, and extraction of plant resources. These findings suggest that sustainable management practices are needed to balance the benefits of ecotourism with the conservation of Chaulabhaja's floral diversity.

4. Discussion

The present study recorded 210 vascular plant species across 64 families and 179 genera, indicating a highly diverse flora of Chaulabhaja Waterfall and its adjoining forests. This richness is 16.74% of the flora of Similipal National Park. Comparison with previous studies such as work of Mishra *et al.* (2012) on Similipal Biosphere Reserve, Odisha; Sahu *et al.,* 2012 on Malyagiri Hill, Odisha; Sahoo *et al.,* 2017 on Nayagarh forest division, Odisha; Rout *et al,* 2022 on Similipal Biosphere Reserve, Odisha; Pal *et al.,* 2022 on Panchalingeswar Hill, Odisha, the study found considerable proportion of documented species overlaps with earlier floristic surveys of Similipal and surrounding forest regions of Odisha. The dominance of Fabaceae, Lamiaceae, and Euphorbiaceae reflects their adaptability to the local tropical moist deciduous forest conditions, where Fabaceae family has been reported to be one of the most diverse family in many tropical regions.The genera such as *Ficus, Diospyros, Euphorbia, Ipomoea* and *Terminalia* contribute to structural complexity and provide essential resources for fauna. The observed diversity of growth forms, with trees constituting 38%, supports a multilayered forest structure, while herbs (29%) and shrubs (23%) maintain understorey richness, facilitating nutrient cycling and microhabitat diversity. Unlike several studies from Eastern India (Sahu *et al.,* 2013; Pal *et al.,* 2022), where herbs constitute the highest proportion of growth forms, the present study recorded a higher percentage of trees, indicating a deviation from the common pattern.

Regarding the predominance of economically important plants, particularly medicinal (132 records) and fodder species (43 records), underscores the significant reliance of local communities on forest resources for subsistence and traditional healthcare. This pattern is consistent with ethnobotanical studies in Odisha and other parts of India, where forests often provide critical livelihood and cultural services (Girach *et al.,* 1999; Pandey *et al.,* 2002; Kumar *et al.,* 2006; Rout and Thatoi, 2009; Panda, 2014; Dikshit *et al.,* 2016). The presence of timber, ornamental, culinary, and fruit-yielding species further highlights the multiple use values of this forest ecosystem.

The signs of ecotourism-related disturbance observed in the study area indicate a shift from low-impact visitation to sustained anthropogenic stress, which has direct implications for biodiversity conservation. In many forested and riparian landscapes, even unregulated foot traffic, littering, and opportunistic clearing around trails can alter microhabitats, reduce seedling establishment, and accelerate the declination of sensitive species (Kaushik *et al.,* 2024). When such pressures occur in habitats that still support threatened taxa, the risk of long-term ecological degradation increases substantially. Similar trends have been reported across ecotourism hotspots in the Western Ghats and Himalayas, where increasing visitor access has facilitated vegetation trampling, removal of understorey biomass, and competition for space with native flora (Mandal and Joshi, 2014). The situation is further complicated when invasive or disturbance-tolerant species gain footholds in areas subjected to recurrent human movement. If ecotourism activities continue without regulation, the remaining populations of conservation-priority species may experience progressive habitat compression, reduced regeneration windows and increased edge effects. Therefore, integrating ecotourism management with habitat protection strategies is critical, not to discourage visitation, but to prevent the gradual erosion of ecological integrity in biologically significant sites.

Although this study provides the first comprehensive account of the floral diversity of the Chaulabhaja Waterfall landscape, certain limitations must be acknowledged. The survey was conducted over a limited temporal window and did not capture seasonal variations in phenology, especially for ephemeral herbs, geophytes, and aquatic or semi-aquatic taxa that emerge briefly during monsoon or post-monsoon periods. The study also relied primarily on qualitative measures rather than quantitative measures of density, frequency, or regeneration status, which could have helped

in assessing community structure and ecological dominance more precisely. Additionally, permanent plots were not established, preventing long-term monitoring of species dynamics, recruitment, and disturbance impacts. Some microhabitats such as rocky crevices, riparian edges, and canopy strata, were difficult to access and may have led to under-representation of specialized or conservation concern species. Finally, the influence of tourism intensity, grazing pressure, and invasive species was inferred from field observations rather than quantified using standardized ecological indices. These limitations highlight the need for more seasonally stratified, long-term, and quantitatively robust assessments to fully understand species stability, conservation priorities, and management needs in this emerging ecotourism zone.

5. Conclusion

The present study highlights Chaulabhaja Waterfall as a biologically significant site supporting conservation-priority species along with a diverse assemblage of economic potential species and native flora. Observed signs of ecotourism including trampling, littering, and localized vegetation clearance, indicated emerging anthropogenic pressures that may compromise regeneration and facilitate invasive species spread. These findings emphasize the dual challenge of promoting ecotourism while safeguarding sensitive plant communities and maintaining ecosystem integrity. Sustainable management strategies, such as zoning of visitor areas, construction of eco-friendly pathways, active and biological control of invasive species, monitoring of threatened taxa, and community engagement, are essential to balance human use with conservation objectives. Implementing such measures can ensure the long-term preservation of Chaulabhaja's floral diversity while supporting environmentally responsible ecotourism and local livelihoods.

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Enhancing seed germination through green synthesized iron oxide nanoparticles from *Oscillatoria princeps* Vaucher ex Gomont isolated in the rice fields of Mayurbhanj district of Odisha

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ABSTRACT

The work was conducted with the green synthesis and optimization of iron oxide nanoparticles (IONPs) using *Oscillatoria princeps* Vaucher ex Gomont extract as strong reducing and capping agents to surmount the disadvantages of physical and chemical methods of nanomaterials synthesis. The IONPs synthesis was confirmed by UV-visible spectroscopy by observing the peak at 280-300nm. Optimization of IONPs synthesis was performed based on three major independent variables- precursor molar concentration, pH and temperature. The optimal synthesis was achieved at a precursor concentration of 0.05 M, at pH 10, and a temperature of 60°C. The impact of IONPs and ionic iron-$FeCl_3$ + $FeSO_4$ (0.05M) on seed germination and seedling growth was evaluated in two crops viz. mustard (*Brassica rapa*) and rice (*Oryza sativa*). For both the crop species, IONPs showed a hormetic response. At a concentration of 5 mg/ml IONPs, the lengths of shoots and roots reached their maximum. In case of *B. rapa* there was a rise of 15% in shoot length and 36% in root length while for *O. sativa*, shoot growth increased by 34% and root length reduced by 14% over the control. Conversely, at 10mg/ml shoot length reduced significantly for both the species while root length increased only in case of *B. rapa* as compared to the control. In the Vigor Index analysis, 5 mg/ml IONPs resulted in the highest VIGOR of 425.36 in case of *B. rapa* and 802.66 for *O. sativa* respectively. The ionic iron- $FeCl_3$ + $FeSO_4$ (0.05M) completely inhibited the growth resulting no VIGOR at all in *B. rapa*.

1. Introduction

Nanoparticles, defined as materials with at least one dimension less than 100 nm, possess unique physicochemical properties including high surface area, reactivity, and quantum effects (Strambeanu *et al.,* 2015). These characteristics allow them in cross disciplinary applications (Mahmoudi *et al.,* 2011; Sharma *et al.,* 2014). With the intention of enhancing productivity of crops while reducing environmental effect, nanoparticles are being investigated extensively for application in agriculture as nano-fertilizers, growth promoters, and biocontrol agents (Yadav *et al.,* 2023).

Microalgae and seaweeds are rich source of bioactive compounds including proteins, polysaccharides, pigments etc. that serve as stabilizing and reducing agents for the synthesis of nanoparticles (Sharma *et al.,* 2012). The use of algal extracts in nanomaterial fabrication presents a cost-effective, eco-friendly, and scalable alternative to the conventional methods of their chemical and physical synthesis (Khan *et al.,* 2022; Ying *et al.,* 2022).

Among various types of nanoparticles, metal oxide nanoparticles have garnered significant interest due to less toxicity and easy separation after treatment. Despite the fact that iron (Fe) is vital for all living things, its deficiency

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affects a variety of crops including their yield quality and nutritional properties (Li *et al.,* 2019; Zuo & Zhang, 2011). In plants, iron is essential for many processes, such as respiration, synthesis of chlorophyll, electron transport, and enzymatic reactions (Mimmo *et al.,* 2014). The green synthesized IONPs using algal extracts offers a dual advantage—providing iron in a bioavailable form and the utilization of sustainable synthesis routes (Win *et al.,* 2021).

As seed germination is a critical phase in the plant life cycle, directly influencing crop establishment and productivity, enhancement of seed germination using biogenic IONPs is an emerging strategy to support early plant development. Therefore, the present study was aimed to synthesize IONPs using cyanobacteria *Oscillatoria princeps* Vaucher ex Gomont to evaluate their effect on the germination of two economically important crops: mustard (*Brassica rapa*) and rice (*Oryza sativa*) in laboratory conditions.

2. Materials and methods

2.1. Preparation of algal extract and IONPs synthesis

Cyanobacterium, *Oscillatoria princeps* Vaucher ex Gomont was utilized from the algal repository of Plant Ecology Laboratory, Department of Botany, Gauhati University after isolation from the rice field soils of Mayurbhanj $21^0$55’48.09” N & $86^0$46’16.482”E of Odisha, India. Preparation of algal extract and IONPs synthesis was done following Mittal *et al.* (2023) with slight modification. To achieve the purpose, the algal biomass was dried and ground to fine powder using mortar and pestle. Dried algal powder (0.1gm) was mixed in 100 ml ethanol and heated for 60 min at 55^0C. After that, the mixture was allowed to cool followed by filtering to get algal extract. Ferric chloride ($FeCl_3$) anhydrous and Ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) solutions were mixed with continuous stirring in 2:1 ratio for 10 min at 550 to get iron salt solution. To synthesize IONPs, both were mixed at 3:7 ratio respectively and stirred for 1 hour. Once, the synthesis process was completed, the IONPs were extracted and washed using deionized water following repeated centrifugation.

2.2. Characterization of IONPs

The optical properties and nanoparticle formation were confirmed using a UV–VIS Spectrophotometer. The absorbance spectrum of the nanoparticles was recorded in the wavelength range of 200–800 nm.

2.3. Optimization of IONPs synthesis

Optimization of IONPs synthesis was performed based on three major independent variables: precursor molar concentration, pH and temperature.

Table 1

Experimental levels of the different independent variables

Independent variables	Levels			
Molar Concentration	0.01M	0.02M	0.05M	0.1M
pH	4	6	8	10
Temperature	Room temperature	40 ^{0}C	50 ^{0}C	60 ^{0}C

2.4 In vitro seed germination test

Seeds of rice (*Oryza sativa*) and mustard (*Brassica rapa*) were collected from RARS (Assam Agricultural University) Shillongani, Nagaon (Assam). The seed treatments were then done following the standard protocol as proposed by Win *et al.*, (2021) with slight modification. The collected seeds were sterilized by washing thoroughly with 70% ethanol, 0.1% mercuric chloride for 3 minutes followed by rinsing with deionized water. Then, 20 numbers of aseptic seeds of each crop were soaked in test tubes containing IONPs at different concentrations (1 mg/ml, 5 mg/ml, 10 mg/ml) and with $FeCl_3$ + $FeSO_4.7H_2O$ (0.05 M) composite solution. Another 20 seeds of both the crops were sterilized and soaked in the test tubes with deionized water as control set. After that, all the seeds were shifted to respective plates with two layers of wet filter papers and incubated for 7 days at 25^0C with a 16:8 (light: dark period). The experiment was conducted in triplicates and ten seedlings were randomly selected from each triplicate to have their shoot and root lengths measured from both treated and control sets. Vigor index (VIGOR)was then calculated by the following formula

Vigor index (I) = Germination % ×Seedling length (Root + Shoot)

2.5. Statistical analysis

Data were analyzed using MS Excel 2019 and ORIGIN Software

3. Results and discussion

3.1. Visual representations

After mixing algal extract with iron salt solution, synthesis of IONPs was primarily distinguished when color changed from pale orange to dark brown as shown in Fig. 01. This aligns with the previous studies of Win *et al.*, (2021), Laid *et al.*, (2021) and Mittal *et al*, (2023).

3.2. Characterization with UV-Vis Spectrophotometer

Using UV-Vis spectrophotometer, the colloidal solution of IONPs was analyzed and an absorbance peak was found at 280-300nm which corresponds to the characteristic surface plasmon resonance absorption band of IONPs (Laid *et al.*, 202; El-Kassas *et al.*, 2015; Kaleem *et al.*, 2024).

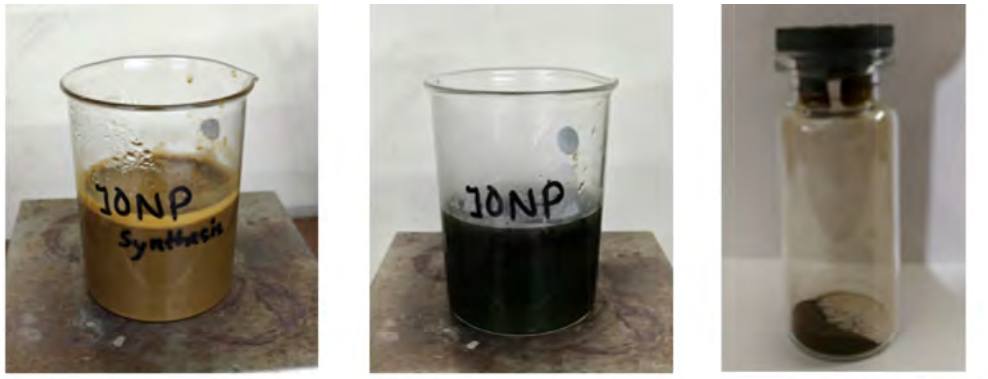

Fig. 01: Visual observation of IONPs biosynthesis

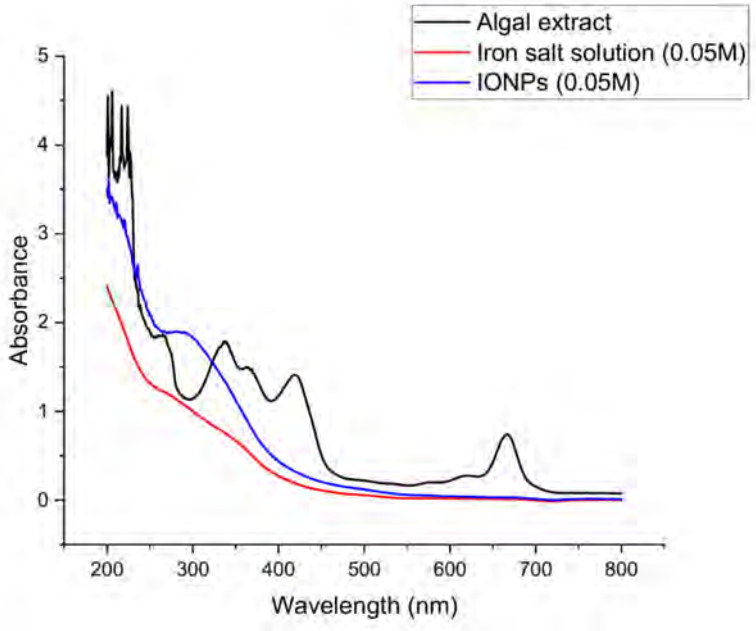

Fig. 02: UV-Visible Spectrum of algal extract, Iron salt- $FeCl_3$ + $FeSO_4$ (0.05M) solution and IONPs (at 0.05M)

3.3. Optimization of synthesis

Effect of molar concentrations

The synthesis of IONPs was studied with four distinct molar concentrations (0.01M, 0.02M, 0.05M, and 0.1M) and their UV-Vis spectrum as depicted in Fig. 03. At the lowest concentration (0.01M), weak absorbance (≤ 0.5 A) and broad peaks in the 300–400 nm range were observed, suggesting that nanoparticle formation was minimal due to lack of sufficient precursor. At a concentration of 0.02 M, absorbance showed declining trends with increase in time, accompanied by diverse peak around 300-340 nm, indicating that nanoparticle development is partial but incomplete.

At 0.05 M concentration, a more distinct, sharp and stable absorbance peak was noted with absorbance values surpassing 2.2A in the wavelength 320 nm. In contrast, a broad and sudden spiked absorbance peak was observed with 0.1M, which indicates aggregated IONPs synthesis with paramount impurities. The findings thus suggest that a precursor concentration of 0.05 M is ideal for generating high-quality IONPs (Mubaraki *et al.*,2023).

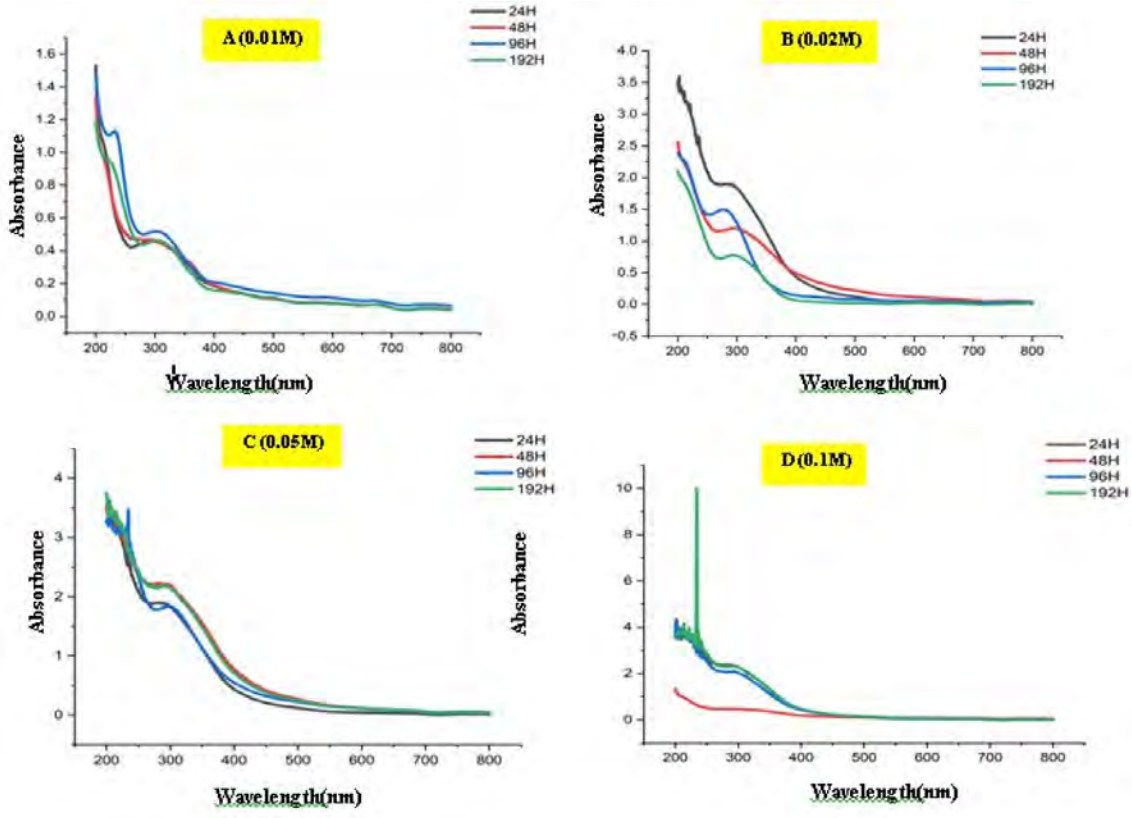

Fig. 03: Effect of different molar concentrations on IONPs green synthesis.

3.4. Effect of pH

The ideal acidic or alkaline conditions for nanoparticle formation were determined by evaluating the IONPs synthesis at several pH values (4, 6, 8, and 10) and the recorded UV-vis absorbance spectrum depicted in Fig. 04. At pH level of 4, there was minimal formation of IONPs, as indicated by low absorbance values (≤ 0.4 A) and the lack of distinct peaks. The acidic environment probably caused the dissolution of iron ions (Fe^{2+} /Fe^{3+}), thus hindering nucleation. At pH of 6, a slight increase of absorbance was observed (0.75–1.25A) with broad peaks beyond 380 nm that suggested partial aggregation due to incomplete precipitation (Mahdavi *et al.*, 2013; Yew *et al.*, 2016). At pH 8, the results were seen to be improved with moderate peaks occurring around 350 nm and absorbance from 1.0–1.4 A. Such peaks suggested polydispersity indicating uneven growth kinetics. Conversely, the highest absorbance (>1.6 A) at pH 10 featured a sharp and narrow peak at 330–350 nm which is a indicative of monodisperse IONPs (Parveen *et al.*, 2018). All these results corroborate the optimal growth range and also showed the inability to synthesize IONPs in acidic environment by the experimental cyanobacterial extract.

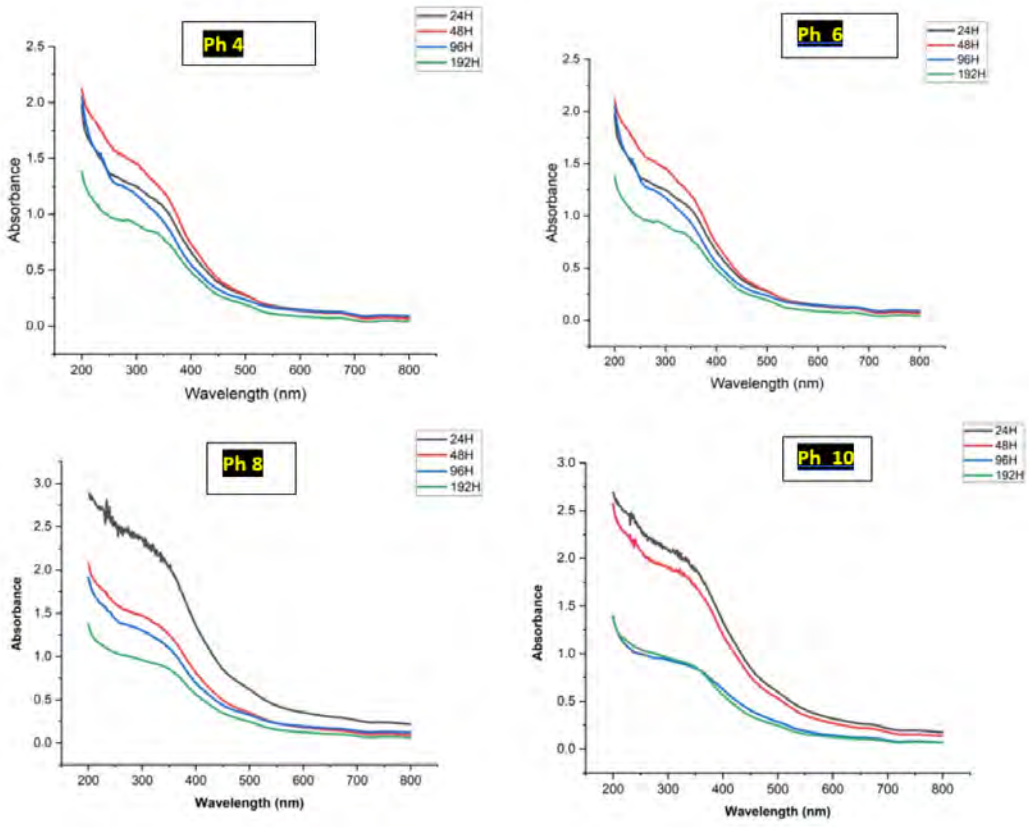

Fig. 04: Effect of different pH on IONPs green synthesis

3.5. Effect of Temperature

The effect of temperature reaction on formation of IONPs was studied at room temperatures, 40^{0}C, 50^{0}C, and 60^{0}C, while keeping the other optimal conditions constant (precursor concentration of 0.05M and pH level of 10,) and the UV-vis absorbance spectrum of the experiments were depicted in Fig.05.

The absorbance spectra at 40°C exhibited relatively high values (≥2.0 A) suggesting impure synthesis of IONPs. Raising the temperature to 50°C led to an absorbance of 1.0A and the sharpest and narrowest peak at 300-320 nm, indicating a better yield and crystallinity of the IONPs, whereas, the absorbance spectra at room temperature as well as at 60^{0}C showed relatively lower values (≤1.0A) with

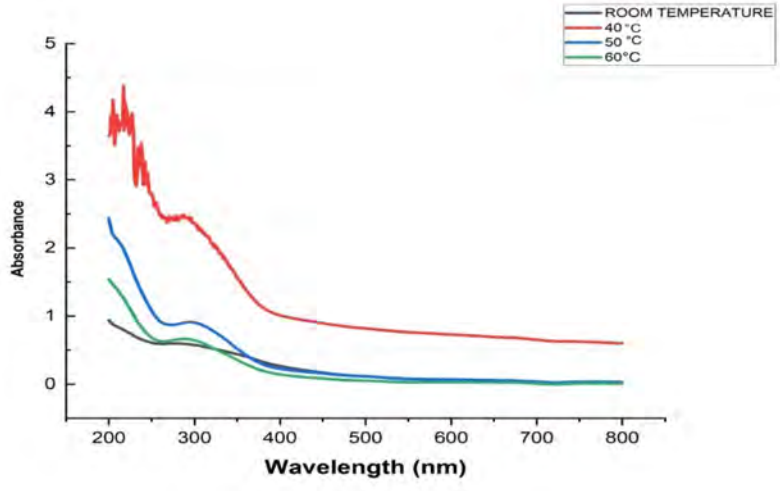

Fig.05: Temperatures effect on green synthesis of IONPs.

extensively broader peak which suggests lesser synthesis of IONPs.

The optimal synthesis was thus achieved at 50^{0}C with the precursor concentration of 0.05 M, at pH 10 which could be attributed to the alkaline pH and the moderate temperature that favour the formation of stable and uniform iron oxide nanoparticles due to enhanced reduction and nucleation processes as reported by Parveen *et al.* (2018).

3.6. Effect of IONPs on early seedling growth (In Vitro)

The impact of IONPs and ionic iron-$FeCl_3$ + $FeSO_4$ (0.05M) on seed germination and seedling growths were evaluated for two selected crop species, mustard (*Brassica rapa*) and rice (*Oryza sativa*). The percentage of in-vitro seed germination with control, ionic iron- $FeCl_3$ + $FeSO_4$(0.05M) and different doses of green synthesized IONPs are depicted in Fig. 06. In case of *B. rapa*, IONPs showed a hormetic response on seedling growth by stimulating in low to moderate concentrations and inhibiting in higher doses (Table 02). At a concentration of 5 mg/ml IONPs, the lengths of shoots and roots reached their maximums of 2.28 cm and 1.98 cm, respectively, indicating a 15% rise in shoot length and a 36% rise in root length.

The experimental results indicate that IONPs at optimal doses function as nano-fertilizers, improving iron bioavailability and stimulating metabolic activity. However, at a concentration of 10 mg/ml, there was a marked 29% reduction in shoot growth as compared to control, while root length increased 58% likely due to stress-induced prioritization of root development for nutrient scavenging or ROS mitigation (Mittler *et al.*, 2004).

In contrast, seeds treated with ionic iron- $FeCl_3$ + $FeSO_4$(0.05M) showed complete growth inhibition, with no shoot or root emergence, highlighting the severe phytotoxicity of free iron ionson *B. rapa* seeds compared to green synthesized nano-formulations.

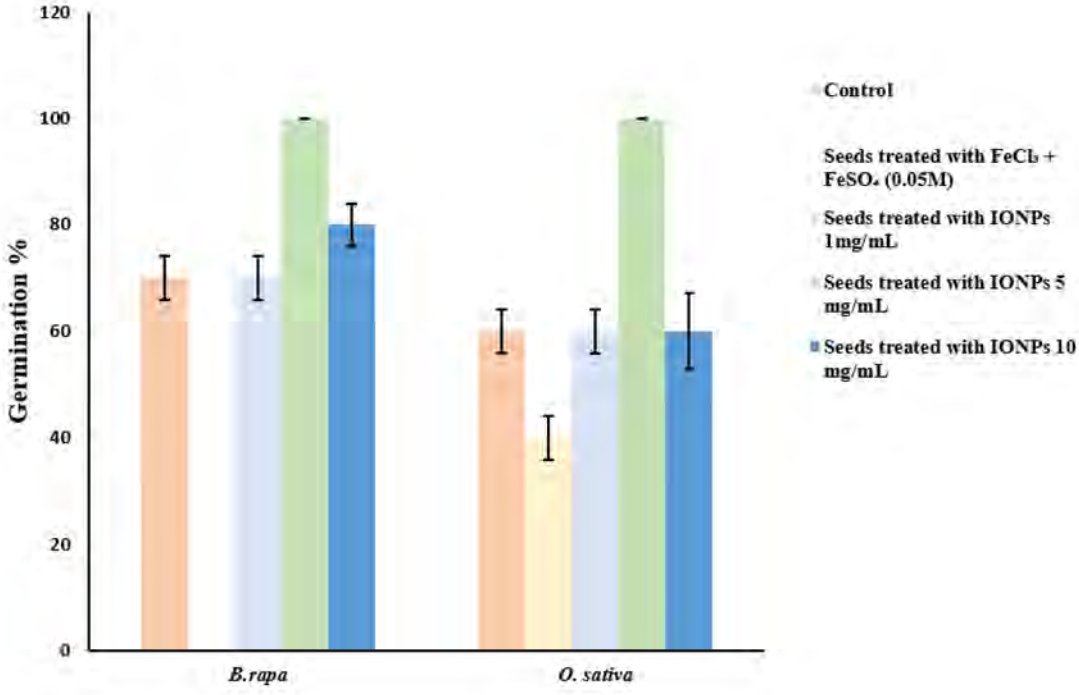

Fig. 06: In vitro seedling growth in percentage of *B. rapa* and *O. sativa* with IONPs and ionic iron conditions.

Table 2

Effect of IONPs and ionic iron- $FeCl_3$ + $FeSO_4$(0.05M) on seedling growth of *Oryza sativa* and *Brassica rapa*.

Treatment	*B. rapa* shoot (cm)	*B. rapa* root (cm)	*O. sativa* shoot (cm)	*O. sativa* root (cm)
Control	1.98	1.46	2.86	4.86
$FeCl_3$ + $FeSO_4$	0.00	0.00	2.6	1.9
IONPs 1mg/ml	1.5	1.58	3.7	1.7
IONPs 5mg/ml	2.28	1.98	3.84	4.2
IONPs 10mg/ml	1.4	1.5	2.24	2.86

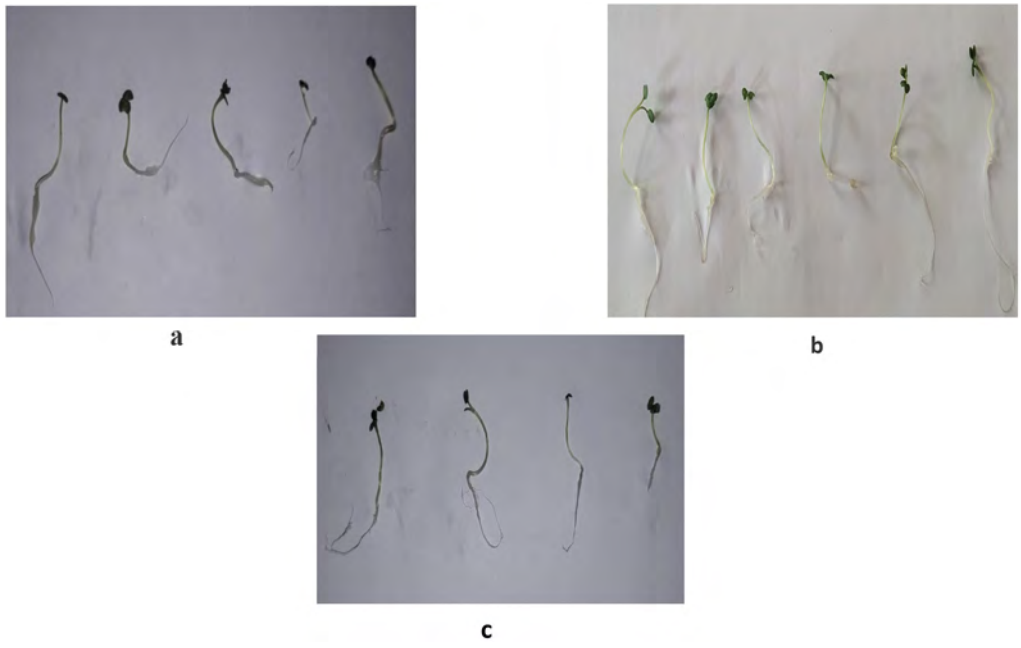

Fig. 07: *B. rapa* seedlings (a) control, (b) treated with 5mg/ml, (c) treated with 10mg/ml IONPs IONP, (Treated with IONP 10mg/ml (seedling growth)

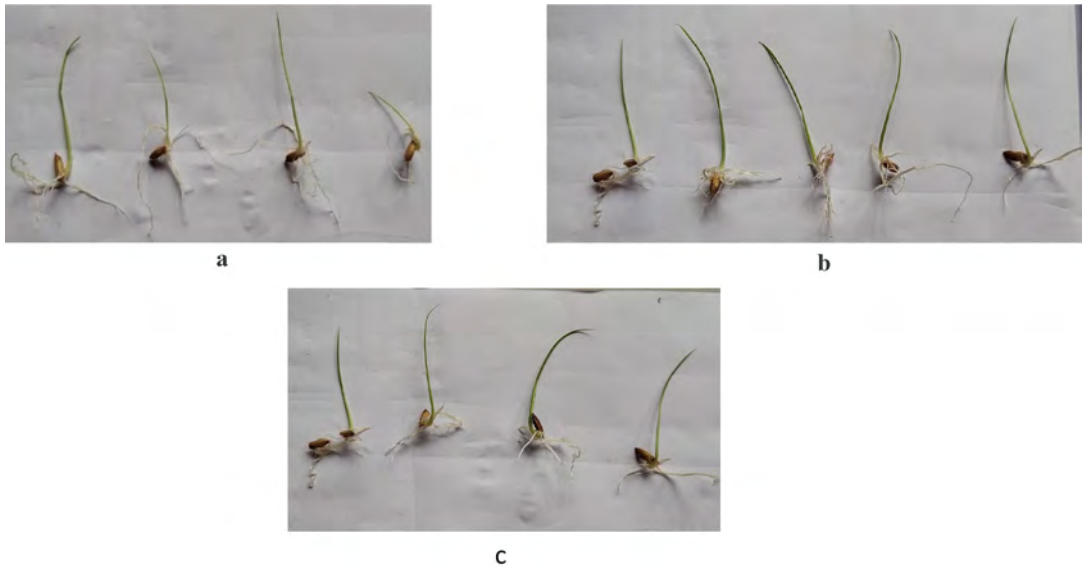

Fig. 08: *O. sativa* seedlings (a) control, (b) treated with 5mg/ml IONPs (c) treated with 10mg/ml IONPs

The effect of IONPs and ionic iron- $FeCl_3$ + $FeSO_4$(0.05M) showed similar pattern in the seedling growth of *O. sativa*. In case of ionic iron- $FeCl_3$ + $FeSO_4$(0.05M), around 9% reduction of shoot growth along with severely stunted (61% reduced) roots were resulted which could be attributed to the phytotoxicity of free iron ions.

On the other hand, the application of IONPs at a concentration of 1 mg/ml resulted in an increase by 29% in shoot length and 65% reduction in the *O. sativa* species. It indicates that at this particular dose, there is a trade-off between stimulation of the shoot and inhibition of the root. At a concentration of 5 mg/ml IONPs, shoot growth showed 34% increase, while root length was reduced by 14% suggesting enhancement of iron bioavailability without causing considerable stress to the roots. At a concentration of 10 mg/ml IONPs, however, shoot and root lengths decreased significantly to 22% and 41% respectively, indicating toxicity induced by nanoparticles at high doses.

3.7. Vigor Index (VIGOR) of B. rapa and O. sativa

In the VIGOR analysis of *B. rapa* seedlings, 5 mg/ml IONPs resulted in the highest Vigor Index of 425.36 which

was 43.5% greater than the control value of 240.66. This indicated that growth could be boosted by better iron bioavailability or stress priming. In contrast to the control, 1 mg/ml IONPs caused a slight decrease in VIGOR of 10.56%, indicating in adequate iron consumption; while 10 mg/ml IONPs caused VIGOR to drop by 32.99%, showing nanoparticle toxicity brought on by oxidative stress or excess iron release.

Remarkably, ionic iron- $FeCl_3$ + $FeSO_4$(0.05M) completely inhibited the growth resulting no VIGOR at all, which highlights the advantages of nano-iron for controlled delivery. The findings emphasize the potential of 5 mg/ml IONPs as a nano-fertilizer for mustard, as it effectively promotes growth and mitigates stress (Servin & White, 2015). However, higher concentrations necessitate careful handling to prevent phytotoxicity.

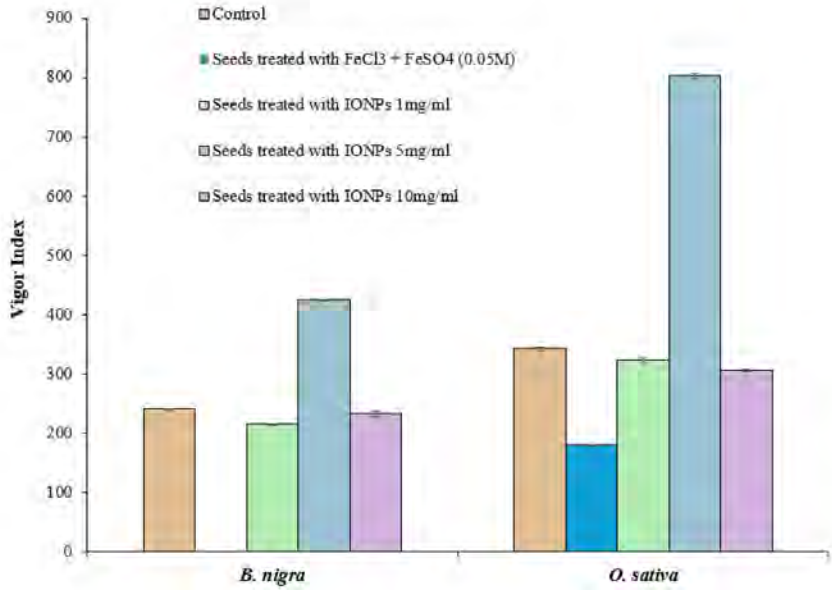

Fig. 09: Effect of ionic iron- $FeCl_3$+ $FeSO_4$ (0.05M) and different doses of IONPs on VIGOR of the tested seeds.

The VIGOR analysis conducted on *O. sativa* showed the peak of 802.66 at 5 mg/ml IONPs treatment surpassing the control value of 342.8 by 134%. This indicated that seedling VIGOR was increased as a result of enhanced iron bioavailability and metabolic stimulation (Liu and Lal, 2015). IONPs at 1 mg/ml slightly lowered VIGOR about 5.48% which may be due to root inhibition (Table. 02), indicating homeostatic control between shoot stimulation and root stress at lower doses. Atthe 10 mg/ml treatment, VIGOR decreased to 10.73% indicating the toxicity of the nanoparticles. It is worth mentioning that ionic iron- FeCl3 + FeSO4(0.05M) resulted in the lowest VIGOR of 47. 5%. These findings suggest that the controlled release of iron from IONPs reduces toxicity due to its gradual dissolution and uptake (Liu and Lal, 2015).

4. Conclusion

The ethanolic extract of the rice field soil cyanobacterium, *Oscillatoria princeps* Vaucher ex Gomont, efficiently acts as a reducing agent for the conversion of ionic iron into iron nanoparticles. The optimization process for IONPs green synthesis using major independent variables was achieved at a precursor concentration of 0.05 M, at pH 10, and a temperature of 50°C. When tested with the synthesized IONPs for growth and germination, showed varied responses in both the crops, B. rapa and O. sativa. VIGOR drastically increased for both the crops when seeds treated with 5 mg/ml IONPs which indicates that the application of the saidgreen synthesized INOPs could reduce the toxicity in in-vitro conditions. The study also paves a way for future investigation in field level for sustainable agriculture.

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Comparative volatile profiling of *Curcuma caesia* leaf and rhizome essential oil using two-dimensionalg as chromatography and time of flight mass spectrometry

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ABSTRACT

The comparative metabolomic analysis of *Curcuma caesia* (black turmeric or kali haldi) provide a thorough profiling of its volatile metabolites by using two-dimensional Gas chromatography Time-of-Flight Mass Spectrometry (GC×GC TOF MS). This study uncovered a total of 157 and 121 compounds from leaf and rhizome essential oil of black turmeric, respectively. Sesqui terpenoid compounds with an area percentage of 61.5% and 45.39% was found to be the dominating class of secondary metabolites in rhizome and leaf essential oil respectively followed by monoterpenoid. The predominant constituents found from the essential oil of this species are epicurzerenone, curzerene, camphor and eucalyptol. This technique also resolved many coeluted constituents and helps to detect many novel components from this species. This study will be helpful for further research into its therapeutic application.

1. Introduction

These days, the global resurgence of interest in natural products for medicinal and health care purposes has led to an increase in demand for aromatic plants (Mohanty *et al*., 2023). With more than 80 species, the genus *Curcuma* (Zingiberaceae) offers potential medicinal uses. *Curcuma* species are attracting a lot of interest worldwide for use in the creation of possible new medications to treat a variety of illnesses because they are abundant in bioactive compounds and are recognized for their anti-inflammatory, anti-microbial, anti-oxidant, anti-aging and anti-cancer qualities (Sasikumar, 2007). *Curcuma caesia* referred to as black turmeric or kali haldi, is a perennial, underappreciated medicinal herb that is endangered in South East Asia (Liu *et al*., 2013). This species is native to India's central and northeast regions and is rare in the North Hill Forest of Sikkim, the slopes of the Himalayas, and the Pappi Hills of East Godavari (Paliwal *et al*., 2011). Beneficial elements in *C. caesia* can be used as natural supplements and substitutes for medications to treat illnesses. Because of its current status as an endangered species, researchers are taking the initiative to develop novel techniques, such as tissue culture and plant cells, in order to keep this species from going extinct (Ibrahim *et al*., 2023). The essential oil from *C. caesia* rhizome as long been used to treat spleen enlargement, bronchitis, asthma and tuberculosis (Donipati and Sreeramulu, 2015). It is also used to treat anthelmintic disorders, cancer, hemorrhoids, leprosy, asthma, menstrual disorders, fever, inflammation, epilepsy wounds, vomiting, aphrodisiacs, gonorrhea discharges, and more are among the conditions (Das *et al*., 2013). Essential oil of *Curcuma* species shows the presence of different types of volatile constituents like sesquiterpenes, monoterpenes, and other aromatic chemicals include metabolites such curcumol, â-elemene, germacrone and curdione, are used by Chinese for medicinal uses to treat a variety of diseases, including cancerous tumours (Dosoky and Setzer, 2018; Soumya *et al*., 2019).

Previously, GC-MS analysis have identified many bioactive constituents such as eucalyptol, camphor, ar-tumerone, alloaromadendrene, retinal, α-santalol, (*Z*)-β-ocimeme, borneol, *ar*-curcumene, β-caryophyllene, 1,8-cineole, γ-curcumene etc. from *Curcuma caesia* essential oil (Mukunthan *et al*., 2014; Pakkirisamy *et al*., 2017; Pandey

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and Chowdhury, 2003). As, the gas chromatography mass spectrometry (GC-MS) is convenient for separating mixtures of substances, but the low sensitivity and weak resolving power of single capillary column makes it ineffective for in-depth metabolomics investigations (Ray *et al*., 2017). Compounds with similar boiling point causes overlapping of the peak in GC-MS because of a single column. However, two-dimensional gas chromatography GC×GC TOF MS is more efficient than GC-MS simply because it separates mixtures more quickly, sensitively and with greater precision. Particularly, because GC×GC is setup with double columns that separate elements in two dimensions, it aids in the separation of constituents that coelute at the same retention time (Ray *et al*., 2017; Singh *et al*., 2021). First dimension (1D) of the GC×GC instrument is designated as the first column, and the second dimension (2D) is designated as the second column. A modulator connects the first and second columns, transferring the fraction of each peak from one to another (Amaral *et al*., 2019; Stefanuto *et al*., 2021). Compound elution in the first column is comparatively slow as in GC, while the received fractions elute within 1-4 seconds in the second column (Amaral *et al*., 2019). The eluted chemicals are quickly recognised with a sophisticated detector like time-of-flight mass spectrometry(TOF-MS). Comparing TOF-MS to an MS detector, the former offers faster acquisition and produces more data (Stefanuto *et al*., 2021). The separation efficiency of 2 DGC×GC is greatly improved when compared to 1D GC since, the components that give botanical isolates their aroma is frequently trace or minor metabolites. Previously, the essential oils extracted from other species such as, *Curcuma zedoaria*, *Curcuma angustifolia*, *Nicotiana tabacum*, *Pandanus fascicularis*, and *Alpinia galanga*, GC×GC TOF MS analysis has detected more components than GC-MS analysis (Nasim *et al*., 2017; Zhu *et al*., 2005; Parveen *et al*., 2018; Singh *et al*., 2021; Jena *et al*., 2020). In the current study, the volatile constituent analysis of the essential oil of *C. caesia* leaves and rhizomes from Odisha was conducted using GC×GC TOF MS technique. This provides a greater understanding of effect of extraction technique of volatile constituents. This analysis could contribute to an improved awareness of the pharmacological actions essential oil of *C. caesia* against a range of diseases.

2. Materials and methods

2.1. Collection of plant sample and extraction of essential oil

The fresh and healthy leaves and mature rhizomes of *Curcuma caesia* were collected from Kandhamal, Odisha. The species was identified and authenticated by a taxonomist and the voucher specimens was deposited at Herbarium of Department of Biotechnology, Rama Devi Women's University, Bhubaneswar, Odisha. The healthy mature rhizomes and fresh leaves having 200g and 100g fresh weight respectively were cleaned and finely chopped and were separately subjected to hydro-distillation in a Clevenger apparatus for the extraction of the volatile oil over a period of 4 and 5 hours, respectively. Anhydrous sodium sulfate (Na_2SO_4) was used for the removal of moisture traces from the collected essential oils. The pure volatile oils were kepting lassvials at 4°C until further analysis. The yield percentage was computed by dividing the essential oil volume by the sample's fresh weight (% v/w).

2.2. Identification of volatile constituents by GC×GC TOF-MS analysis

The comparative metabolomics profiling of Leaf and rhizome essential oil of *C. caesia* was examined using GC×GC TOFMS system. The pure essential oil was processes and analysed in a Leco, Pegasus 4 DTOFMS system with Agilent 7890 BGC, a cold jet modulator, MPS2 auto sampler and a secondary oven. The system was equipped with Rxi 5-MS having 0.25mm I.D. × 30m length × 0.25mm film thickness as primary (1D) column and Rxi 17Sil MS with 0.25mm I.D. × 2m length × 0.25mm film thickness as secondary (2D) column. The carrier as Helium was introduced to the system with a flow rate of 1.5mL/min. The essential oil sample was inserted to the GC×GC inlet using with a split ratio of 40:1. The temperatures of the source and transfer lines were kept at 250°C in MS. The primary column's temperature programs were 50°C for one minute, 5°C per min ramping up to 230°C and holding it for five minutes, and 10°C per min ramping upto 260°C and holding it for one minute. There was a 10°C offset in the heating of the secondary column compared to the primary. A hot pulse of 0.75 seconds was maintained, the modulator temperature was kept at 15°C relative to the first column, and the modulation time was set at 5.5 seconds. At a TOFMS acquisition rate of 100 per s, the spectrum was acquired. The Chromato TOF program, version 3.34, was used to capture chromatogram spots and characterise peaks. Contour plots were used to manually identify peaks. By matching their spectra with reference components found in the NIST (2020) and Adams (2007) libraries, the constituents were identified.

3. Result and discussion

3.1. Essential oil yield

Using hydro-distillation method, the oil yield percentage in *C. caesia* rhizome and leaf samples on a fresh weight basis were 0.51% and 0.2% (v/w), respectively. This result concurs with the earlier studies which reported the 0.7% leaf oil yield based on fresh weight (Borah *et al*., 2019)

and 1.5% essential oil from fresh rhizome by using hydro-distillation method (Pandey and Choudhury, 2003).

3.2. Analysis of volatile constituents through GC × GC TOF MS

In the current study, GC×GC TOF MS is used extensively to evaluate the volatile constituents in the essential oil of fresh leaves and rhizomes, a prevalent technique that uses double columns with different stationary phases. By addressing co-elution concerns, this dual-column method improved the separation of co-eluted chemicals according to their polarity. Chroma TOF software's automatic peak processing capability made it easier to identify peaks in the GC × GC chromatogram, which led to the creation of several peaks in the GC × GC contour plot. GC × GC TOF MS provided notable benefits over conventional GC-MS, principally because of its improved resolving capability. Using this progressive technique, formerly unresolved peaks from the 1D column were effectively resolved, and co-eluting compounds were detected quickly and precisely by TOFMS.

The essential oils extracted from the *C. caesia* rhizome and leaves had 278 components in total, according to the results of a sophisticated analytical method. By this thorough investigation, compounds in the rhizome oil were 121 and compounds in the leaf oil were founded as 157. The total area percentage covered by rhizome and leaf essential oil were 90.54% and 87.50% respectively.

Alkanes, alkenes, alkyl halide, alcohols, aldehyde, alkyne, esters, ketones, ether, diterpenes, sesquiterpenes, monoterpenes, prenylated coumarins, furans, azulene, and fatty acids were among the wide range of potential chemicals found in the isolated volatile oil from *C. caesia* rhizomes. With relatively lower percentages for the primary components, GC×GC TOFMS analysis detected a wider range of biologically active compounds and produced a more detailed profile. Significant components found in rhizome GC × GC TOFMS included epicurzerenone (31.76%), curzerene (14.90%), camphor (6.36%), isoborneol (4.49%), eucalyptol (4.68%), isobornyl acetate (3.13%), elemene (1.93%), curcumenol (1.84%) (Table-1). The main compounds identified in rhizome EO using GC×GC TOFMS in the study by (Lenka *et al*., 2025) were linalool (0.98%), isoborneol (0.99%), germacrene B (1.56%), (Z) β-farnesene (0.99%), eucalyptol (7.56%), epicurzerenone (4.23%), ar-turmerone (2.59%), curzerenone (11.45%), β-eudesmol acetate (1.26%), zingiberene (0.99%), curzerene (4.56%), camphor (3.59%) and camphene hydrate (0.98%).

While GC × GC TOFMS examination of leaf shown major compounds as camphor (7.76%),curzerene (7.09%), eucalyptol (6.75%), (E)-α-famesene (6.65%), isoborneol (6.16%), epicurzerenone (4.30%), isofuranodienone (4.29%), endo-borneol (3.49%), α-elemenone (2.46%). Further investigation revealed other important phytoconstituents present in leaf oil, such as epicurzerenone (3.7%), curzerene (3.46%), α-Farnesene (2.67%), eucalyptol (2.69%), camphor (6.59%), germacrone (1.57%), curzerenone (3.85%), heptacosane (4.26%), camphene (1.85%), borneol (1.58%), α-pinene (0.99%) and isobornylformate (1.02%) according to (Lenka *et al*., 2025).

When it comes to separating volatile compounds, GC×GC TOFMS is more sophisticated than traditional GC-MS because it has a better resolving power. Because of this benefit, GC×GC TOFMS is able to quickly and accurately detect the coeluting chemicals. The 2D column was found to have more accurately resolved many of the 1D column's unresolved peaks. Compounds such as camphor ($RT_{1=}$740.5, $RT_{2=}$3.27), and N-tetrahydrofurfury l-3-chloro-propanamide (RT_1=740.5, RT_2=2.98), α- Myrcene (RT_1=1450, RT_2=4.95), 5-methylpyrimidine (RT_1=1450, RT_2=4.14) & 3-methylene-tridecane (RT_1=1450, RT_2=2.05) were the co-eluting compounds. These substances eluted out of the 1D column simultaneously, but the 2D column did so at various times.

4. Conclusion

The present study the chemical composition of *Curcuma caesia* leaf and rhizome essential oil has been identified through the GC×GC TOF MS analysis.Using GC×GC TOF MS system variety of minor compounds were detected from this species. From rhizome essential oil total 121 compounds comprising 90.54% of the total oil composition were revealed with Epicurzerenone (31.76%), Curzerene (14.90%) and Camphor (6.36%) as major compounds. In GC×GC TOF MS of leaf EO, total compounds detected were 157 comprising area percentage of 87.50%, where the major compounds found were camphor (7.76%), curzerene (7.09%), and eucalyptol (6.75%). Further research is necessary to comprehend the variability in chemical composition and its implications for biological activity, as the relative abundance of some compounds may differ based on factors like geographical origin, plant part used, and extraction method. The GC-MS and GC×GC TOF MS analysis, considered together, offer important information about the volatile makeup of *Curcuma caesia* essential oil, setting the stage for further research into its potential uses in flavouring, cosmetics, and medicine.

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Feral Flora of Koraput District in Odisha, India: Diversity, Ecology and Utilization

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ABSTRACT

Feral plants are the species that have escaped cultivation, and established in natural or semi-natural habitats, are an important yet often overlooked component of regional biodiversity. The present study investigates the diversity, distribution, utilization, and ecological significance of feral plant species in Koraput district of Odisha, India, a region recognized for its complex terrain, rich cultural heritage, and high floristic diversity. Extensive field surveys were conducted across agricultural margins, wastelands, roadsides, and forest fringes from 2020 to 2024 documenting feral species through standard floristic methods, and ethnobotanical observations. A total of 41 feral plant species belonging to 37 genera, and 29 families were recorded, of which Acanthaceae emerged as the most represented family. Dominance of herbs, and shrubs reflects the adaptability of these growth forms to disturbed landscapes. Many of the species identified were naturalized exotics that have become integral components of the local flora. The findings highlight the dynamic interaction between human activity, and vegetation composition, emphasizing the adaptive strategies of feral species.

1. Introduction

Feral plants, represent an important ecological link between cultivated, and wild flora (Scossa, and Fernie, 2021). These plants originate from domesticated species that have adapted to survive, reproduce, and establish self-sustaining populations in natural or semi-natural habitats without direct human assistance (Bagavathiannan *et al.,* 2008). Their presence provides valuable insights into the dynamics of plant domestication, ecological adaptation, and the processes of natural selection acting on formerly cultivated taxa (Gering *et al.,* 2019). In many regions, feral plants not only become invasive, and replace local flora, but also play important ecological roles by contributing to vegetation diversity, soil stabilization, and the maintenance of local pollinator networks (Brooker *et al.,* 2023). These plants occupy an intermediate position between cultivated, and wild floras, often retaining characteristics of domestication while adapting to spontaneous growth in disturbed or marginal environments. Globally, studies on naturalized, and feral plants have emphasized their dual role as both contributors to biodiversity, and potential ecological disruptors (Pyšek *et al.,* 2020).

In India, several comprehensive accounts of alien, and naturalized plants have been published, documenting their origin, spread, and ecological effects (Reddy *et al.,* 2008; Inderjit *et al.,* 2018). The Indian Alien Flora Database (ILORA) provides updated records of naturalized, and invasive taxa, revealing that a significant proportion of them are former cultivars that have naturalized over time (Pant *et al.,* 2021). In rural, and tribal regions of India, communities often exploit both wild, and feral plants for food, medicine, fodder, and household uses (Mishra and Chaudhury, 2012).

Koraput district of southern Odisha is globally recognised as an important agriculturally rich site due to its traditional rice-based agro-ecosystems, and associated crop genetic diversity. Floristic studies have reported over 2,500 species of flowering plants from the region, many of which

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are used for food, medicine, or ritual purposes (Rout *et al.*, 2016). However, the feral component of this flora remains largely undocumented. The present study aims to document the diversity, and distribution of feral plant species in the district, and record their ethnobotanical uses, and cultural significance among local communities.

2. Methodology:

2.1. Study Area

The present investigation was conducted in Koraput district, located in the southern part of Odisha, India, between latitudes 18°14'-19°13' N, and longitudes 82°5'-83°25' E (Adhikary *et al.*, 2019).

2.2. Field Survey and Data Collection

Koraput is inhabited by several tribal communities, including the Bhatra, Paroja, Kondh and Gadaba, who depends heavily on surrounding vegetation for food, medicine, and other subsistence needs (Mishra and Chaudhury, 2012). Extensive field surveys were conducted between 2020 and 2024 during different seasons (pre-monsoon, monsoon and post-monsoon) to collect plant specimens, and record observations on feral plant species. The surveys covered various habitats, fallow lands, roadsides, field margins, abandoned croplands, and village peripheries where feral plant growth was prominent. The study followed standard ethnobotanical and floristic survey methods (Martin, 1995). Plant specimens were collected, photographed with high resolution digital camera, and tagged with relevant ecological data, including habitat type, and associated species. GPS coordinates were recorded for spatial mapping of occurrences.

To document utilization patterns, semi-structured interviews, and group discussions were conducted with local tribal informants, and traditional healers following the Participatory Rural Appraisal (PRA) approach (Chambers, 1994). A total of 85 informants (45 male, 40 female; aged 25-75 years) representing different villages were interviewed in the Odia, and local tribal dialects with prior informed consent. Existing literature on the flora of Koraput district was reviewed to supplement the field data. Relevant information was extracted from published articles, books, and reports.

2.3. Identification and Classification

Collected specimens were processed, dried and mounted using herbarium techniques (Bridson and Forman, 1998). Identification was done through comparison with authentic herbarium specimens housed at the Central National Herbarium, Howrah, and by consulting relevant floras (Haines, 1921-1925; Saxena and Brahmam, 1994-1996).

2.4. Data Analysis

Plants were categorized based on their habit, habitat, family composition, association with other plants, and use types. The collected data were analyzed using descriptive statistical methods such as frequency, and percentage. Microsoft Excel was employed to generate graphs, and charts for clear visualization, and rapid interpretation of the findings. The results were subsequently interpreted in relation to the research objectives, and examined alongside existing literature on feral flora to provide a comprehensive understanding of the study area.

3. Results and Discussion

A total of 41 feral plant species belonging to 29 families were documented from the Koraput district (Fig. 1). Family Acanthaceae was well represented with 6 species, indicating its role in the spontaneous vegetation of the district. Seven families were represented by 2 species each, while the rest 21 were represented by only one species each. This indicates that feral plants are widely distributed in families. The recorded species comprise diverse life forms: shrubs (14 species), herbs (11 species), climbers (8species), and trees (8 species) (Fig. 2). Shrubs were dominant, followed by herbs, indicating that persistent, hardy species with quick vegetative propagation have a higher tendency to become feral. Ornamental use was the most prevalent (Fig. 3), accounting for 30 species (73.17%). These include attractive flowering plants such as *Agave americana, Ipomoea cairica, Clitoriaternatea, Mirabilis jalapa* and *Canna indica,* which have escaped garden cultivation, and naturalized in nearby habitats. Food plants comprised 6 species (14.63%), including *Annona squamosa, Panicum sumatrense, Sesamum indicum* and *Eleusine coracana,* which indicate agricultural escape, and traditional utility. The "Others" category of 5 species (12.17%), such as *Santalum album, Morus alba* and *Simarouba glauca,* represent species valued for timber, oil, cultural, religious, or ecological purposes.

Species such as *Lantana camara* and *Ipomoea cairica* exhibit invasive traits, potentially threatening native biodiversity. Conversely, species like *Annona reticulata, Coffea arabica* and *Sesbania sesban* hold immense local socioeconomic importance, supporting supplementary food, fodder, and agroforestry applications. The presence of the above mentioned species indicates strong ethnobotanical associations. Moreover, culturally valued plants, such as *Nyctanthes arbor-tristis, Lawsonia inermis* and *Santalum album* reflects the potential of feral plants for commercial, pharmaceutical and conservation-based utilization.

Table 1

Details of Feral Plants in Koraput district, Odisha

Sl. No	Scientific Name of the Species	Flowering & Fruiting	Habitat & Ecology	Uses	Distribution in India
1	*Agave americana* L. (Asparagaceae)	June-October	Common in hill slopes in association with *Lantana camara, Cymbopogon citratus* etc.	Popular for its fibre, and the flower stalks, and the base leaves of *Agave americana* can be roasted, and consumed.	Almost throughout.
2	*Alocasia macrorrhizos* (L.) G. Don (Araceae)	May-September	In the margin of wetland in associations with *Amorophophallus pae oniifolius*	Rhizome, petiole of leaves are edible	Almost throughout; cultivated as well as naturalized.
3	*Annona reticulata* L. (Annonaceae)	April-November	Ruderal habitat in association with *Ipomoea carnea, Chromolaena odorata* etc.	Fruits are edible	A.P, A., B., D., Ka., Ke., M.P, M., O., T.N, U.P, W.B.
4	*Annona squamosa* L. (Annonaceae)	April-December	Ruderal habitat in association with *Samanea saman, Ipomoea carnea, Chromolaena odorata* etc.	Popular for its edible fruits	A.P, A., B., D., G., Ka., Ke., M.P, M., O., R., T.N, U.P, W.B. (Cultivated, and almost naturalised).
5	*Antigonon leptopus* Hook. & Arn. (Polygonaceae)	September-January	Common in waste land along with *Justicia adhatoda, Mangifera indica, Ficus racemosa* etc.	Used as an ornamental plant.	Almost throughout.
6	*Barleria cristata* L. (Acanthaceae)	October-February	Common in roadside as well as waste lands with the association of *Nyctanthes arbor-tristis, Lantana camara, Senna occidentalis, Justicia betonica* etc.	Used as ornamental plants as well as medicinal plants	Throughout
7	*Barleria prionitis* L. (Acanthaceae)	September-February	Common in roadside as well as waste lands with the association of *Clerodendrum infortunatum, Aeschynomene aspera* etc.	Used as an ornamental plant as well as medicinal plants; used in decorating butterfly gardens.	Throughout.
8	*Brugmansia suaveolens* (Humb. & Bonpl. ex Willd.) Bercht. & J. Presl (Solanaceae)	October-March	Common in wasteland in association with *Lantana camara, Ipomoea carnea, Sida acuta, Cascabela thevetia*	Ornamental plant.	Ar.P, A., G., H.P, J & K, Ka., Ke., M., Man, Meg, Miz, N., O., S., T.N, U.K, U.P.

9	*Canna indica* L. (Cannaceae)	April-December	Common in marshy land with *Typha domingensis*, and some species of *Cyperus*.	Used as ornamental plants	Almost throughout.
10	*Caryota urens* L. (Arecaceae)	February-December	In Hill slopes associated with *Diospyros melanoxylon, Mallotus philippensis, Grewia asiatica* etc.	Sometimes used as an ornamental plant.	Almost throughout.
11	*Cascabela thevetia* (L.) Lippold (Apocynaceae)	Throughout the year	Available in Roadside in association with *Lantana camara, Ipomoea carnea, Sida acuta, Brugmansia suaveolens* etc.	Used as an ornamental plant, and is also mythologically significant	A & N, A.P, A., B., D., D & D, Ka., Ke., M.P, Meg, O., R., S., T.N, U.P, W.B.
12	*Clerodendrum chinense* (Osbeck) Mabb. (Verbenaceae)	August-January	Roadside hedges in association with *Ricinus communis, Vitex negundo, Leonotis nepetifolia* etc.	Used as an ornamental plant because of its fragrant flowers.	Ar.P, A., Man, Meg, N., O., W.B.
13	*Clitoria ternatea* L. (Fabaceae)	August-February	Common climber in association with *Dicliptera paniculata, Chromolaena odorata, Achyranthes aspera, Ocimum americanum* etc.	Used as an ornamental plant as well as a medicinal plant. It is a mythologically significant plant	Almost throughout.
14	*Coffea arabica* L. (Rubiaceae)	February-December	Found in Wasteland in association with *Colebrookea oppositifolia, Chromolaena odorata, Clerodendrum indicum* etc.	It is well known for its famous liquor.	Cultivated as well as naturalized throughout the country especially southern part of the country, and A & N.
15	*Cosmos sulphureus* Cav. (Asteraceae)	August-January	Wasteland in association with *Aeschynomene indica, Sesbania sesban, Themeda triandra* etc.	Often used as an ornamental plant.	Almost throughout.
16	*Crinum asiaticum* L. (Amaryllidaceae)	August-December	Fringes of forest, margin of wetlands in association with *Alocasia macrorrhizos, Shorea robusta* etc.	Used as an ornamental plant as well as a medicinal plant	Almost throughout.
17	*Ecbolium ligustrinum* (Vahl) Vollesen (Acanthaceae)	July-October	Fringe of forest wasteland in association with *Sida acuta, Calotropis procera* etc.	Often used as an ornamental plant.	A.P, A., B., Ke., M., O., T.N, W.B.

18	*Eleusine coracana* Gaertn. (Poaceae)	September-February	Available in road side in association with *Sida acuta, Celosia argentea* and other grasses.	Food yielding plant.	Almost throughout.
19	*Euphorbia tithymaloides* L. (Euphorbiaceae)	September-January	Common wasteland plant in association with *Chromolaena odorata, Lantana camara* etc.	Used as an ornamental plant as well as a medicinal plant.	Almost throughout.
20	*Hedychium coronarium* J. Koenig (Zingiberaceae)	August-December	Margin of wetlands, canal in association with *Coccinia grandis, Alocasia indica, Hellenia speciosa, Ludwigia perennis* etc.	Used as an ornamental plant for its fragrant flower.	A & N, A.P, Ar.P, A., B.,J., Ka., Ke., M.P, Man, Meg, Miz, N., T.N, U.P, U.K, W.B.
21	*Hellenia speciosa* (J. Koenig) S.R. Dutta (Costaceae)	June-December	Margin of Waste water bodies as well as in the margin of the forest in association with *Sida cordata, Alocasia indica, Hedychium coronarium, Senna tora;* in some areas *Hellenia speciosus* is introduced, and has become an invasive species.	Crepe Ginger has many historical uses in Ayurveda, where the rhizome has been used to treat fever, rash, asthma, bronchitis, and intestinal worms.	Almost throughout.
22	*Ipomoea cairica* (L.) Sweet (Convolvulaceae)	July-December	Found in forest, wet grassland, at edges of rivers, and lakes, also along roadsides, waste places, and cultivated ground in association with *Ficus racemosa, Crotalaria retusa* etc.	Sometimes used as an ornamental plant.	Almost throughout.
23	*Ipomoea nil* (L.) Roth (Convolvulaceae)	August-January	Common in waste lands in association with *Clerodendrum infortunatum* and *Ricinus communis*	Used as an ornamental plant.	Almost throughout.
24	*Justicia gendarussa* Burm.f. (Acanthaceae)	September-January	Common wasteland plant in association with *Plumeria rubra, Kalanchoe pinnata* etc.	Used as a medicinal plant	Almost throughout.
25	*Kalanchoe pinnata* (Lam.) Pers. (Crassulaceae)	October-March	Wasteland plant in association with *Justicia gendarussa,*	Used as an ornamental plant as well as a medicinal plant	Almost throughout.

			Jatropha curcas, Combretum album etc.		
26	*Lantana camara* L. (Verbenaceae)	Throughout the year	Invasive in all the waste areas, besides the railway tract in association with *Chromolaena odorata* etc.	Used as an ornamental plant as well as in a butterfly garden. Now, it is becoming popular in making handicrafts, and toys, and fire woods hedge plants	Almost throughout.
27	*Lawsonia inermis* L. (Lythraceae)	April-September	Road side in association with *Vitex negundo, Duranta erecta, Chromolaena odorata* etc.	Used as ornamental plant.	A.P, A., B., Goa, G.,J., Ka., Ke., M.P, M., Man, Meg, O., R., S., T., U.K, U.P, W.B.
28	*Michelia champaca* L. (Magnoliaceae)	February-October	Common in Hill slopes in association with *Agave americana, Diospyros melanoxylon* etc.	Used as ornamental plant.	Almost throughout.
29	*Mirabilis jalapa* L. (Nyctaginaceae)	August-December	Roadside plant in association with *Sida acuta, Ipomoea hederifolia, Croton bonplandianus,* and other grasses.	Used as ornamental plant.	A.P, A., B., Ch., D., G., H., H.P,J., Ka., Ke., M.P, M., Meg, O., R., T.N, T., U.K, U.P, W.B.
30	*Morus alba* L. (Moraceae)	February-June	Road side plant in association with *Ficus benghalensis, Jatropha curcas* etc.	It is widely cultivated to feed the silkworms employed in the commercial production of silk. Fruits are edible, and have anticancer properties.	Almost throughout. Cultivated throughout tropical, and subtropical regions.
31	*Nyctanthes arbor-tristis* L. (Oleaceae)	September-December	In wastelands, Forest along with other trees	Ornamental as well as medicinal plant, and mythologically significant.	Almost throughout; naturalized.
32	*Paederia foetida* L. (Rubiaceae)	July-November	Common climber in association with *Plumeria obtusa, Pithecellobium dulce,* and *Plumbago zeylanica*	Leaves are edible, and have medicinal uses.	Ar.P, A., Man, Meg, Miz, N., O., S., W.B.
33	*Passiflora incarnata* L. (Passifloraceae)	June-October	Roadside plant in association with *Bridelia stipularis, Aeschynomene indica, Cordia dichotoma* etc.	Used as an ornamental plant for its showy flowers.	Ke., O., and other parts of country
34	*Panicum sumatrense* Roth ex Roem. & Schult. (Poaceae)	October-February	Roadside in association with *Alternanthera sessilis, Chloris barbata, Senna tora, Cenchrus polystachios* etc.	Food plant.	A.P, A., B., Ch., D., Goa, H., H.P,J., Ke., M.P, M., Meg, O., P., R., T.N, Tel, T., U.K, W.B.

35	*Santalum album* L. (Santalaceae)	July-November	Margin of forest, road side in association with *Shorea robusta, Mallotus philippensis, Woodfordia fruticosa, Cleistanthus collinus, Millettia pinnata* etc.	Wood yielding plant.	A.P, Ka., Ke., M.P, M., O., R., T.N, U.P.
36	*Sesamum indicum* L. (Pedaliaceae)	July-October	Road side plant in association with *Physalis minima, Alternanthera sessilis, Eleusine indica, Triumfetta rhomboidea, Leucas cephalotes* etc.	Oil yielding plant.	Almost throughout.
37	*Sesbania sesban* (L.) Merr. (Fabaceae)	September-January	Road side, and margin of cultivated lands in association with *Aeschynomene aspera, Abutilon indicum, Corchorus capsularis* etc.	Often cultivated for green manure.	Almost throughout.
38	*Simarouba glauca* DC. (Simaroubaceae)	February-August	Waste land as well as road sides in association with *Acacia auriculiformis, Solanum sisymbrifolium, Trema orientalis, Cryptolepis dubia* etc.	Edible oil yielding plant.	Almost throughout.
39	*Thunbergia alata* Bojer ex Sims (Acanthaceae)	September-January	Forest margin in association with *Laportea interrupta, Sida cordifolia, Lantana camara* etc.	Ornamental plant.	Naturalised throughout the country.
40	*Thunbergia fragrans* Roxb. (Acanthaceae)	August-January	Forest margin in association with *Trema orientalis, Chromolaena odorata, Alternanthera sessilis, Ardisia humilis* etc.	Ornamental plant.	Widely distributed throughout the country.
41	*Zephyranthes citrina* Baker (Amaryllidaceae)	July-October	Wasteland plant in association with *Andrographis paniculata, Ruellia prostrata, Phyllanthus simplex, Euphorbia hirta,* and other grasses.	Ornamental plant	Almost throughout.

Abbreviations : A.P:, Andhra Pradesh, A & N:, Andaman & Nicobar, Ar P: Arunachal Pradesh, A.: Assam, B.: Bihar, Ch: Chhattisgarh, D & D: Daman & Diu, D.: Delhi, G.: Gujarat, H: Haryana, H.P: Himachal Pradesh, J & K: Jammu & Kashmir, J.: Jharkhand, Ka: Karnataka, Ker : Kerala, M.P: Madhya Pradesh, M.: Maharashtra, Man: Manipur, Meg: Meghalaya, Miz: Mizoram, N.: Nagaland, O.: Odisha, R.: Rajasthan, S: Sikkim, T.N: Tamil Nadu, Tel: Telangana, T.: Tripura, U.K: Uttarakhand, U.P: Uttar Pradesh, W.B: West Bengal

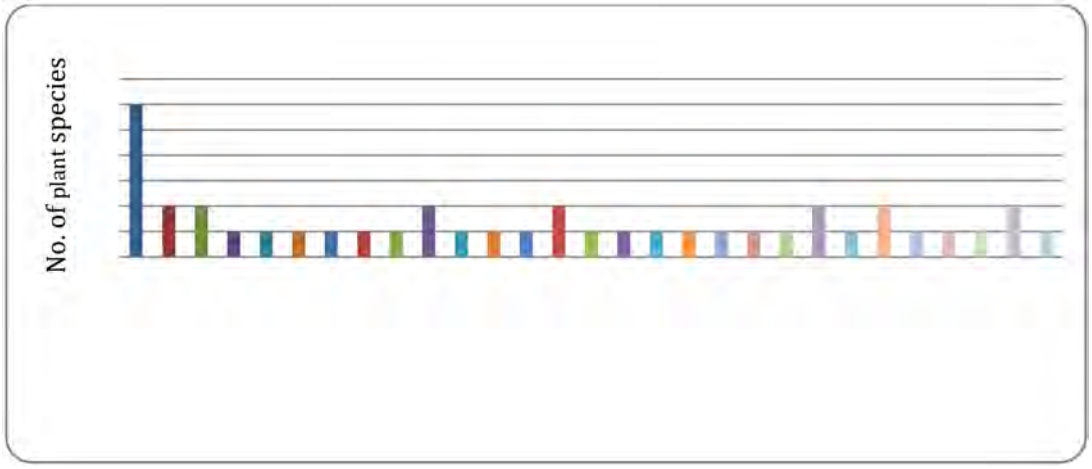

Fig. 1. Family-wise distribution of feral plants in Koraput district of Odisha

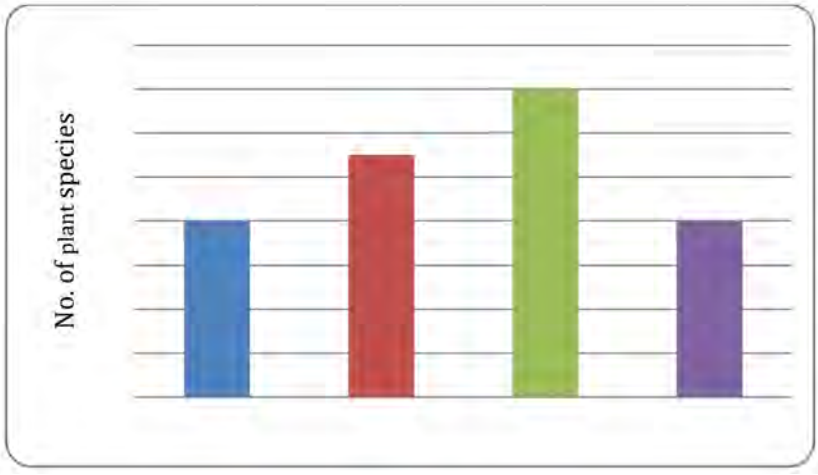

Fig. 2. Habit-wise distribution of feral plants in Koraput district of Odisha

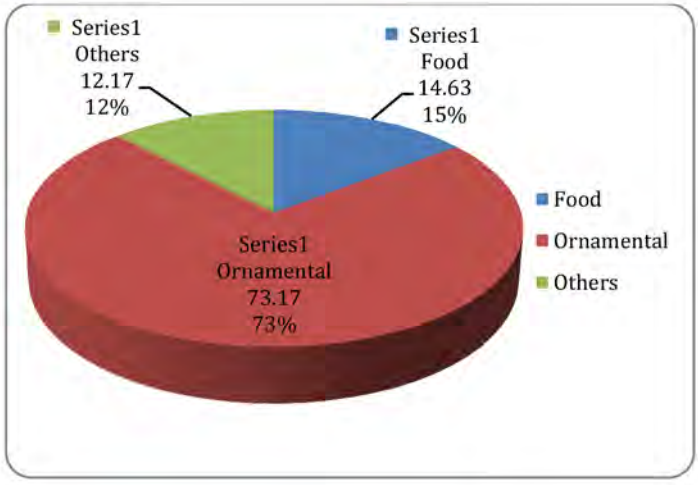

Fig. 3. Usage of feral plants in Koraput district in percentage

4. Conclusion

The present study provides a comprehensive account of feral plant species occurring in the Koraput district of Odisha. The naturalization of these species in Koraput reflects ecological adaptability, anthropogenic influence, and dispersal dynamics. These feral species, play important ecological, aesthetic, nutritional, and socio-economic roles. While many feral plants enhance biodiversity, and offer useful ecosystem services, some species may also pose ecological challenges due to their invasive potential, emphasizing the need for monitoring, and sustainable management. This inventory provides a valuable baseline for further studies on feral flora diversity, ecological behaviour, economic potential, and conservation strategies in the region. Promoting awareness about the potential benefits, and risks associated with feral plants can support biodiversity conservation, sustainable resource utilization, and environmental planning in Koraput district.

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Quorom Sensing: The Microbial diversity and their Communication in the diverse applications in medicine and plant science

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ABSTRACT

Quorum sensing (QS) has emerged as a pivotal area of research, shedding light on how bacteria and microorganisms communicate through chemical signals known as autoinducers. This process coordinates collective behaviours by regulating diverse genes and signalling pathways. This review explores innovative strategies to manipulate QS, focusing on quorum quenching and QS inhibitors as promising tools to reduce bacterial virulence. These approaches have demonstrated effectiveness in aquaculture and agriculture by disrupting QS to prevent infections and enhance plant resistance. QS also holds potential for developing biosensors that enable early disease detection and for preventing biofilm formation—an essential step in combating antimicrobial resistance. Beyond microbial control, QS mechanisms are being harnessed in cancer therapy, particularly in targeted drug delivery systems. Emerging technologies such as nanomaterials, hydrogels, and microplastics offer novel methods to fine-tune QS activity. Overall, this review highlights the expanding role of QS in regulating bacterial behaviour and its far-reaching implications for human health, diagnostics, and therapeutic innovation.

1. Introduction:

Quorum sensing (QS) is a sophisticated communication mechanism employed by bacteria to coordinate group behaviors in response to changes in population density and species composition within their environment. This system relies on the synthesis, detection, and response to extracellular signalling molecules known as autoinducers (Waters and Bassler, 2005). As bacterial populations grow, the concentration of autoinducers increases. Upon reaching a threshold level, these molecules trigger a collective response among the bacterial community.

QS governs a wide array of complex behaviours, including virulence (Winson *et al.*, 1995), biofilm formation antibiotic resistance, bioluminescence, and adaptation to environmental stimuli. The emergence of QS has transformed our understanding of bacteria from solitary, simple organisms to highly interactive communities capable of coordinated actions. Many QS-regulated processes are ineffective when performed by individual cells, underscoring the importance of collective behaviour.

Ecological Adaptation & Specificity

Quorum Sensing systems are highly specialized, reflecting the ecological pressures and lifestyles of different bacteria. Autoinducers are chemical signals used in QS and are reoften species-specific, ensuring precise communication and minimizing cross-talk in mixed microbial communities.

Historical Milestones

1960s-1970s: Discovery of extracellular signaling in *Streptococcus pneumoniae* and bioluminescent marine bacteria like *Vibrio fischeri* laid the groundwork for QS research.

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1980s: Identification of luxI and luxR genes in *V. fischeri* revealed the genetic basis of QS-regulated bioluminescence. These genes encode the enzyme for autoinducer synthesis and its receptor, respectively.

1990s: Genome sequencing uncovered homologs of QS genes in diverse bacteria, confirming QS as a widespread regulatory mechanism.

Molecular Mechanisms

Quorum Sensing involves the production, release, and detection of autoinducers. Once a threshold concentration is reached typically at high cell density these molecules bind to receptors that activate transcriptional regulators, altering gene expression to coordinate group behaviors like biofilm formation, virulence, and sporulation.

QS research has expanded to encompass broader aspects of microbial communication.

Subsequent discoveries, such as autoinducer-2 (AI-2) in *Vibrio harveyi,* revealed that QS can facilitate interspecies and even interkingdom communication . This opens avenues for manipulating bacterial interactions within the human body. Studies have shown that QS-deficient mutants in many plant and animal pathogens exhibit reduced virulence . Chemical strategies to disrupt QS-either between bacteria or between bacteria and viruses-offer promising therapeutic potential for disease treatment .

2. Gram - Negative Bacteria:

2.1. Vibrio fischeri: The Model Organism for QS

- Pioneering Studies: Engebrecht *et al.* (1983-1987) (3) identified the LuxI/LuxR system and AHL signals.
- QS Mechanism
  - LuxI produces 3-oxo-C6-HSL ? activates LuxR ? induces luxCDABE ? bioluminescence.
  - Low Cell Density (LCD): C8-HSL delays autoinduction.
  - High Cell Density (HCD): Positive feedback maintains light production.
- Ecological Role: Mutualistic relationship with squid; light helps squid avoid predation.

2.2. Pseudomonas aeruginosa: A Complex QS System

- Hosts: Opportunistic pathogen in immuno compromised individuals (AIDS, CF, UTIs).
- QS Circuits:
  - LasI/LasR ? 3-oxo-C12-HSL (initiates QS cascade).
  - RhlI/RhlR ? C4-HSL (activated downstream of Las system).
  - PQS system ? 2-heptyl-3-hydroxy-4-quinolone (PqsABCDE operon).
- Functions:
  - Regulates virulence factors like LasB elastase and pyocins.
  - Las and Rhl positively/negatively regulate PQS.
  - All three systems integrate for coordinated pathogenesis and biofilm formation.
- Clinical Relevance: Drives drug resistance, persistence in hospitals.

2.3. Vibrio harveyi: Multi-Signal QS

- Three QS Systems:
  - HAI-1: Intraspecies.
  - CAI-1: Genus-level (Vibrio).
  - AI-2: Inter-species communication.
- Signal Integration:
  - Phosphorylation cascade leads to regulation of LuxR and AphA.
  - At LCD: sRNAs inhibit LuxR; at HCD: LuxR is activated ? bioluminescence.
- Broader Implication: Enables cross-species communication in microbial communities.

2.4. Other Gram-Negative Bacteria with LuxIR-Type Systems

- *Agrobacterium tumefaciens:*
  - Uses 3-oxo-C8-HSL to regulate Ti plasmid transfer.
- *Pseudomonas aureofaciens:*
  - Produces phenazine antibiotics; PhzI/PhzR QS system.
- *Pseudomonas fluorescens 2P24:*
  - Uses PcoIR to aid rhizosphere colonization.
- *Sinorhizobium meliloti:*

- SinI/ExpR system regulates EPS for plant symbiosis.

- *Serratia:*
  - QS includes two-component regulatory systems.

2.5. Research Implications

- High specificity of QS: Each AHL typically only activates its corresponding receptor.
- QS as a Target:
  - Disrupting QS offers a potential strategy for anti-virulence therapy.
  - Could reduce resistance and pathogenicity without selective pressure from antibiotic

3. Gram Positive Bacteria

Streptococcus pneumoniae: Multiple QS Systems

ComABCDE System:

- Analogous to Agr; central to competence, biofilm formation, and bacteriocin production.
- Enables horizontal gene transfer, contributing to:
  - Antibiotic resistance
  - Genetic diversity
  - Virulence adaptation

BlpABCSRH System:

- Regulates bacteriocins and immunity proteins.
- Promotes competitive exclusion in microbial communities.

LuxS/AI-2 System:

- AI-2 acts as a universal signal molecule (interspecies).
- Regulates:
  - Biofilm formation on abiotic surfaces
  - Fratricide (killing sibling cells)
  - Virulence traits

All Three Systems:

- Involved in cooperative and competitive behaviours.
- Offer multiple entry points for therapeutic disruption.

Broader Role of AI-2 in Gram-Positive Bacteria

- AI-2 is not unique to *S. pneumoniae:*
  - Also found in: *S. aureus, B. anthracis, B. burgdorferi, C. difficile, E. faecalis.*
- Implicated in:
  - Biofilm architecture
  - Antibiotic resistance
  - Cell adhesion

4. Quorum Quenching (QQ) and Quorum Sensing Inhibitors

Since the 1940s, the widespread use of antibiotics has driven the rise of microbial resistance. The intense selection pressure resulting from excessive antibiotic application has continually fostered the emergence of drug-resistant strains. Because this selection pressure is inevitable, there is a constant need to develop new antibiotics. Quorum quenching (QQ) presents a promising alternative by targeting quorum sensing (QS) systems to mitigate bacterial pathogenicity without promoting resistance (Paluch *et a*l., 2020).

QS inhibitors (QSIs) typically induce QQ through three primary mechanisms:

1. Targeting signalling molecules: This includes inhibiting their production or degrading them using QQ enzymes. Among these, degradation is the most recognized approach. Common QQ enzymes-such as lactonases, acyltransferases, oxidases, and reductases-break down signalling molecules, preventing them from reaching threshold concentrations and thereby halting QS activation.
2. Blocking signal receptors: Antagonists are used to competitively inhibit the binding of signalling molecules to their receptors.
3. Disrupting downstream signalling: This is achieved by inhibiting kinases and interfering with transcriptional regulators of operon genes.

The potential applications of QQ are vast and encouraging. For instance, *Vibrio harveyi*, a significant marine pathogen, poses a threat to shrimp farming and causes substantial economic losses. The growing problem of antibiotic resistance highlights the value of QS-targeted strategies. Studies have shown that AI-2 signalling molecules in *V. harveyi* play a key role in regulating virulence in brine shrimp. Disrupting genes like luxS or luxP can reduce shrimp

mortality, while compounds such as (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone can inhibit LuxR, effectively interfering with the QS circuit without increasing resistance pressure.

Additionally, QQ can suppress *V. harveyi* luminescence and toxin T1 expression, lowering its infectivity and impact on aquaculture (Manefield *et al.*, 2000). In plant pathogens like *Erwinia carotovora*, reducing AHL signals via the AiiA enzyme enhances resistance to soft rot in vegetables such as cabbage and potatoes. The discovery of farnesol in *Candida albicans* has expanded QS research to fungi where increased farnesol levels inhibit biofilm formation and reduce resistance (Zibafar *et al.*, 2009).

Given the challenges posed by antibiotic misuse, QQ offers an innovative and potentially resistance-free strategy, making it a compelling area for further research. However, clinical application of QQ requires careful validation due to possible toxicity and resistance concerns. Comprehensive safety evaluations and solutions to ecological and practical hurdles are essential. Moreover, some studies suggest that QQ-based treatments might eventually face resistance issues similar to antibiotics (Defoirdt 2018). Therefore, future research must strive for a deeper understanding of QQ mechanisms and address these emerging challenges.

5. Quorum sensing quenching and human disease treatment

Since the quorum sensing (QS) system plays a key role in enabling communication among pathogenic bacteria that pose risks to human health, targeting and inhibiting QS has emerged as a critical area of research for disease prevention. Notable applications of QS include deploying biosensors for detecting bacterial presence, interfering with biofilm formation to combat antibiotic resistance, and exploring its potential in developing antitumor therapies.

6. Biosensor and human disease prevention

Quorum sensing (QS)-based biosensors offer rapid and accurate methods for assessing bacterial populations across diverse settings, including clinical diagnostics, food safety, and environmental monitoring. By detecting QS signalling molecules, these biosensors enable early identification of bacterial contamination, allowing for timely interventions to prevent infections and spoilage (Paluch *et al.*, 2020).

A key requirement for these biosensors is high sensitivity to homoserine lactone (HSL) signals, which are central to the QS circuits of many pathogenic bacteria. Detection is typically achieved through homologous binding proteins, such as LuxR, which selectively recognize specific signalling molecules.

In water quality monitoring-a field critical to public health-QS-based biosensors have shown great promise. Researchers have developed sensor modules based on QscR that enable efficient detection of bacterial contamination in water systems (Cheng *et al.*, 2021).

In the domain of food safety, biosensors are being used to monitor food spoilage via luminescence responses triggered by volatile organic compounds. However, further development is needed to enhance their sensitivity and specificity to QS signals in complex food matrices. One notable advancement is the *Vibrio harveyi*-based whole-cell biosensor, which detects the AI-2 signalling molecule produced by *Campylobacter jejuni*. This system lowers the detection limit by a factor of 100, enabling both quantitative analysis and enhanced food production safety.

A key research question moving forward is whether QS circuits or homologous binding proteins can be engineered or adapted to improve biosensor universality-allowing a single system to detect a wider range of pathogens.

Ultimately, the broader application of QS-based biosensors in clinical and food industry settings holds significant promise for improving public health through more effective detection, prevention, and management of bacterial infections.

7. Biofilm and resistance

Traditional antimicrobial drugs directly target bacterial cells, which often leads to the development of resistance, especially in environments with high selection pressure .In contrast, quorum sensing (QS)-based strategies work by disrupting bacterial communication rather than killing bacteria outright. This approach reduces virulence and may enhance the effectiveness of existing antibiotics, offering a promising avenue for next-generation antimicrobial treatments. Notably, modulating QS pathways has been shown to potentiate antibiotic efficacy against resistant strains. As such, integrating QS-targeting mechanisms into biosensors and therapeutic interventions presents a multifaceted strategy for addressing bacterial resistance and improving public health outcomes.

8. Cancer

Cancer is a critical noncommunicable disease and treatment resistance necessitates the development of innovative therapies. Bacterial-mediated cancer therapy, particularly using quorum sensing (QS) systems, offers a

promising approach for targeted drug delivery with minimal side effects. Research has shown that the unique microenvironment of necrotic tumor tissue attracts the preferential accumulation of *Salmonella* (Forbes *et al.* 2003). Leveraging this characteristic, therapeutic proteins can be selectively expressed through QS once *Salmonella* accumulates in the tumor, reducing damage to healthy tissues. This strategy bypasses the need for tumor surface markers, a common limitation in conventional therapies.

Despite these advancements, several challenges remain. These include preventing bacterial spread after therapeutic action maximizing tumor-targeting specificity, and managing the virulence factors or immune responses triggered by bacterial QS. To mitigate risks, most studies employ attenuated or detoxified bacterial strains vetted for safety.

In parallel, the global crisis of rising antibiotic resistance has sparked interest in alternative strategies. Quorum quenching (QQ) enzymes and quorum sensing inhibitors (QSIs) offer a promising solution by disrupting biofilm formation and virulence factor expression without exerting selective pressure-thus reducing the emergence of resistant strains. This non-selective pressure strategy may serve as both an antibiotic alternative and a novel approach for cancer therapy. While QS-based technologies have shown promise in clinical and industrial application, real-world bacterial ecosystems are far more complex than those in controlled laboratory settings. Therefore, further research is crucial to improve the specificity and targeting accuracy of QS systems, paving the way for safer and more effective therapies.

9. Novel QS Regulation Mode

As the threat posed by harmful bacteria to human health increases, quorum sensing (QS) has become increasingly important in disease prevention and control. In recent years, many novel and unexpected QS regulatory mechanisms have been discovered. For instance, emerging materials such as nanomaterials and hydrogels have shown potential when integrated with QS-based strategies. Additionally, QS is being explored for its role in the bacterial degradation of microplastics (MPs), offering promising environmental and health benefits. Another innovative approach involves the use of bacteriophages to disrupt QS pathways and protect human health.

These new strategies significantly expand the application scope and value of QS in both medical and environmental contexts. In the current post-antibiotic era, QS technologies offer a promising alternative for mitigating bacterial virulence without promoting resistance. As a result, research into quorum sensing inhibitors (QSIs) and quorum quenching (QQ) enzymes has become highly active. However, challenges remain i.e particularly the low bioavailability and potential toxicity of many QSIs, as noted by (Gómez *et al.*, 2019).

10. Nanomaterial Regulation

Nanomaterials, owing to their superior physicochemical properties, not only have the potential to directly modulate quorum sensing (QS) but also significantly enhance the efficacy and stability of quorum sensing inhibitors (QSIs). Consequently, nanocomposites make quorum quenching (QQ) a more feasible strategy for bacterial control.

Nanomaterials have found extensive applications across biomedical and environmental domains due to their distinctive properties. They serve as optical sensors ,electrochemical sensors and bacterial biosensors facilitating early disease detection when bacterial populations reach pathogenic levels. Beyond diagnostics, nanomaterials play a crucial role in preventing bacterial colonization and spread as well as suppressing the release of virulence factors. Their ability to disrupt and inhibit biofilm formation further diminishes bacterial virulence and resistance, streamlining both prevention and treatment approaches. However, despite their promise, clinical use of nanomaterials in quorum sensing (QS) regulation is largely limited to gastrointestinal and respiratory diseases. This constraint stems from difficulties in processing and delivering nanomaterials with precision (Mogosanu *et al.*, 2015). Advancing modification techniques could greatly expand their therapeutic reach. For instance, injectable nanomaterials might be engineered to combat systemic or bloodstream infections while topical formulations could enable transdermal therapies for bone and joint infections.

To realize these applications, focused research is required to address challenges related to dosage optimization, biosafety, stability, storage, and transportation. Most critically, the human microbiome presents a far more complex environment than in vitro laboratory conditions, necessitating extensive in vivo studies to validate the safety and effectiveness of nanomaterial-based QS modulation strategies.

11. Hydrogel Regulation

Hydrogels are a promising class of materials that have attracted growing research interest in recent years. Advances in hydrogel design now enable these materials to respond to a variety of environmental stimuli, such as temperature, pH, or light. This responsiveness allows for the controlled

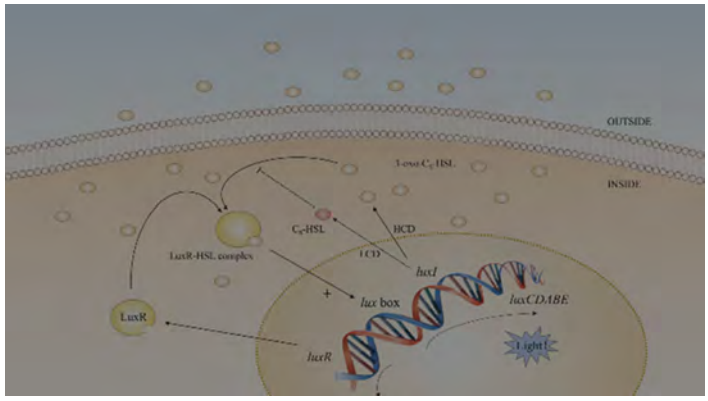

FIGURE 1 : Regulation of bioluminescence in *V. fischeri.* At LCD, luxI synthesis of C8 HSL would compete to inhibit the binding of 3 oxo C6 HSL to LuxR. When the cell density reaches a threshold, it is able to produce enough 3 oxo C6 HSL to combine with LuxR to form a complex, thereby initiating the transcription of luxCDABE and forming bioluminescence. Small ellipses represent signaling molecules and large ellipses represent proteins. (13)

release of specific substances, offering a novel strategy to regulate or inhibit bacterial quorum sensing (QS), which is central to the development and persistence of many infectious diseases. Recent studies have demonstrated the potential of hydrogels in modulating QS-related processes, including the inhibition of biofilm formation, suppression of virulence factor production and anti-inflammatory effects, antimicrobial efficacy,detection capabilities and applications in food packaging and seafood preservation .

For instance, elevated levels of reactive oxygen species (ROS), commonly produced during bacterial infections, can exacerbate inflammation and hinder the treatment of posttraumatic osteomyelitis .The accelerated release of HBPL under such conditions enables effective ROS scavenging, disruption of biofilm formation, and interference with QS gene expression. Additionally, Seto *et al.,* 2019. designed a hydrogel capable of detecting *Pseudomonas aeruginosa* infections in a cell-free environment, demonstrating high sensitivity and practical applicability.

12. QS and MP degradation

Microplastics (MPs), defined as plastic particles or fragments smaller than 5 mm in diameter and are now globally distributed and pose significant risks to environmental organisms. As apex predators, humans are susceptible to the accumulation of MPs through biomagnification, which may lead to adverse health effects .

One promising strategy for addressing this issue involves leveraging bacterial quorum sensing (QS). Certain bacteria utilize MPs as transport vectors, adhering to their surfaces to form biofilms and create a "plastisphere"-a unique, miniature ecosystem that facilitates the long-distance transport and diffusion of microorganisms. Members of the Rhodobacteraceae family are particularly abundant in the plastisphere, likely due to the critical regulatory role of QS in biofilm formation. Targeting QS mechanisms to enhance MP degradation is emerging as a key research direction. However, research on QS-mediated MP degradation remains limited and is not yet fully understood. Since QS can enhance bacterial recognition and breakdown of MPs, introducing QS signalling molecules or their analogs to activate degradation pathways represents a promising strategy. In addition, genetic engineering of QS systems to promote the expression of MP-degrading enzymes offers another innovative approach. In conclusion, harnessing bacterial QS systems holds great potential to mitigate the environmental impact of MPs and, more critically, to reduce the associated risks to human health.

Given the growing threat of antimicrobial resistance and increasing interest in interkingdom quorum sensing (QS), bacteriophages have garnered significant attention as potential alternatives to traditional antibiotics .Some bacteriophages remain dormant within their bacterial hosts until activated by bacterial communication signals. Virulent phages follow a strictly lytic cycle, replicating rapidly and lysing their host, while temperate phages can either destroy the host cell or persist through lysogeny, integrating their DNA into the host genome. In cases of polylysogeny, where multiple prophages coexist in a single bacterium, external stressors such as DNA damage can disrupt this balance and trigger the lytic cycle. However, DNA damage does not always initiate phage activation; instead, host responses to infection and QS signals can also drive the shift from lysogeny to lysis.

13. Conclusion

Quorum sensing (QS) plays a crucial role in bacterial communication, with significant advancements in understanding its influence on gene expression and

regulatory pathways. It enables complex microbial interactions and ecological adaptation, sparking growing interest in its effects across biological kingdoms. QS is increasingly recognized as a strategic approach to address major global challenges such as microbial pathogenicity, antibiotic resistance, and even cancer. By modulating pathogen virulence and leveraging viral agents to control harmful bacteria, QS offers a promising alternative to excessive antibiotic use. Its integration with nanomaterials and hydrogels further amplifies its potential as a powerful tool for microbial management. Nonetheless, the complexity of QS networks and the unpredictability of natural environments pose considerable hurdles that demand deeper investigation and validation. Ultimately, QS has evolved beyond a mere microbial signalling system to become a vital mechanism for human-directed control of microbial behaviour.

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Iron fortification induced photochemical changes in Photosystem II activity in mungbean [*Vigna radiata* (L.) R. Wilczek)] as evident by Chlorophyll *a* fluorescence transient analysis

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ABSTRACT

Mung bean (*Vigna radiata*) is an important nutrient-rich leguminous crop with an increasing significance as food in the world having excellent source of protein, dietary fiber, minerals, vitamins to promote good health. The consumption of iron is one of the major problems of human nutrition worldwide and lack of iron causes iron deficiency nutrient disorder commonly called anemia.To enrich iron as a micronutrient dependent phytic acid and phenol compounds, the present study was carried out in order to figure out the effect of iron at 20µM, 100µM, 200µM dose of iron beside control in hydroponic condition for 7 days and 14 days. The growth, biochemical and performance of photosystem–II activity were analysed. The low dose Fe enhanced the growth and photosynthetic activity of ~0.95 fold at 20µM till 14 days that significantly reduce growth and PS-II activity at 100 µM and 200µM treatments. Enhance growth and chlorophyll content as well as chlorophyll *a* fluorescence activity at ÄJ and ÄI in double normalization OJIP transient graph confirm its role in higher photosynthetic activity and its possible utilization in iron fortification in mung bean sprouts rather than its deleterious activity in high dose (100 to 200 µM) with reduced growth, chlorophyll, carbohydrate, protein content and photosynthetic activity having potential health benefiting food in human healthcare.

1. Introduction

Mung beans are most widely used pulse crop in India and are cheap source of food that contain carbohydrate (55%-65%), protein, fat, vitamins, micronutrients (Nair *et. al.*, 2015). Micronutrient malnutrition affects more than one-half of the world`s population, especially women, teenage girls and preschool children (McMillen *et al.*, 2022). Iron enrichment in mung bean sprouting have additional proteolytic cleavage activity for proteins & are even higher during sprouting. Mung bean carbohydrates are easily digestible in humans as compared to other forms of legumes. Both seeds and sprouts of mung bean produce lower calories compared to other cereals, which makes it more attractive to obese and diabetic individuals.

Iron, cobalt and zinc are the essential nutrients that relatively harmless but can be toxic in large amounts or certain forms. Metal accumulate in ecological food chain through uptake at primary producer level and then through consumption at consumer levels as plant roots are the primary contact site for metal ions. Iron is an important constituent of several plant proteins and enzymes like leghemoglobin, cytochromes, ferrodoxin, catalase, peroxidase, aconitase and superoxide dismutase (Marschner, 1995). As a critical component of proteins and enzymes, iron plays a significant role in basic biological processes such as photosynthesis, chlorophyll synthesis, and for the maintenance of chloroplast structure and function, respiration, nitrogen fixation, uptake mechanisms (Kim and Rees, 1992) and DNA synthesis through the action of the

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ribonucleotide reductase (Reichard, 2002). It is also an active cofactor of many enzymes that are necessary for plant hormone synthesis, such as ethylene, lipoxygenase, 1-aminocyclopropane acid-1-carboxylic oxidase (Houben and de Poel, 2019), or abscisic acid. Because of iron's redox properties and its ability to form complexes with diverse ligands, this element is a constituent of many electron carriers and enzymes; therefore, it plays an important role in plant metabolism. On the other hand, low solubility of inorganic iron at physiological pH levels and its high reactivity in the presence of oxygen, generates toxic hydroxyl radicals, & represents a severe difficulty (Hell and Stephan, 2003).

In aerobic soils, iron is predominantly found in the ferric form (Fe+3) with extremely low solubility whereas soluble ferrous (Fe+2) form available to plant because of low redox potential (Schmidt, 1993). Plants have developed sophisticated mechanisms to take up small amounts of soluble iron. Non-graminaceous plants release protons, secrete phenolics, reduce Fe+3, and take up iron (Ishimaru *et al.,* 2011). Once iron is solubilized, Fe+3 is reduced to Fe+2 by a membrane-bound Fe+3 reductase oxidase. Fe is then transported into the root by an iron-regulated transporter (Ishimaru *et al.,* 2011) reported that graminaceous plants rely on an Fe+3 chelation system during the secretion of mugineic acid (MA) family phytosiderophores. MAs are secreted to the rhizosphere through TOM1, and they then chelate Fe+3. In rice, the resulting Fe-MA complex is transported by the yellow stripe family transporters (OsYSL15) (Nozoye *et al.,* 2011). Approximately 80% of iron is found in photosynthetic cells where it is essential for the biosynthesis of cytochromes and other heme molecules, including chlorophyll, the electron transport system, and the construction of Fe-S clusters (Hansch and Mendel *et al.,* 2009). In the photosynthetic apparatus, two or three iron atoms are found in molecules directly related to photosystem II (PS-II), 12 atoms in photosystem I (PS-I), five in the cytochrome complex, and two in the ferredoxin molecule (Varotto *et al.,* 2002). Such distributions show that iron is directly involved in the photosynthetic activity of plants, and consequently their productivity (Briat *et al.,* 2007).

Iron availability is assumed to affect the natural distribution of species, and it may limit the growth of fast-growing economically important plants (Tagliavini and Rombolà, 2001). Iron can also be potentially toxic at high concentrations. The ability of iron to donate and accept electrons means, that if iron is free within the cell, it can catalyze the conversion of hydrogen peroxide into free radicals. Free radicals can cause damage to a wide variety of cellular structures, and they can ultimately kill the cell (Crichton *et al.,* 2002). To prevent that kind of damage, life forms have evolved a biochemical protection mechanism by binding iron atoms to proteins. Biofortification of mung bean with boron, zinc and iron alters its grain yield and nutrition.Increasing Fe uptake in plant can remodel metabolism, increasing efficiency of Fe utilization and store Fe (Kroh and Pilon, 2020). Higher Fe uptake by plant is reported to reduce the protein synthesis in the leaf (Saikia and Baruah, 2012) and may exert toxic effects at higher doses (Rani *et al*, 2022). Food fortification is an important approach for the increase in iron intake and reductions in iron deficiency (Kujur, 2019). Chlorophyll molecules capture light energy during photochemical reactions of photosynthesis and the excess energy is dissipated as heat (Non-Photochemical Quenching) or emitted as chlorophyll a fluorescence (Maxwell and Johnson 2000). Increasing photosynthesis leads to a decrease in extra energy dissipation. Thus, the efficiency of the photochemical reactions and the degree of heat dissipation is estimated by measuring the yield of chlorophyll a fluorescence which induces OJIP transient (Stirbet and Govindjee, 2011; Tsai *et al.,* 2019). The Photosystem II (PSII) activity in form of chlorophyll a fluorescence could be used as a screening technique to distinguish effect of iron in mung bean which has not studied in depth in *Vigna radiata*. This experiments aim in analysis of low dose iron fortification in *V. radiata* to have its effect on growth, secondary metabolites and photosynthetic signature through OJIP transient analysis.

2. Materials and methods

2.1 Hydroponic culture

Seeds of mung bean (*Vigna radiata*) were aseptically surface sterilized with 5 min each of 0.01% teepol and sodium hypochloride and then washed in distilled water for two to three times. Seeds were placed on a plastic net fitted on a glass culture jar (250 ml) filled with distilled water up to the net. Germinated 10 days old seedlings were treated with 20 µM, 100 µM, 200 µM FeSO4 solution with a control for 7days and 14 days. 3rd and 4th leaf leaves from apex were subjected to different biochemical parameters and chlorophyll fluorescence analysis with three replications.

2.2 Morphological growth analysis

Root, shoot and leaf lengths were measured in different concentration and time intervals with a measurement scale and the mean data was obtained from replicated data. Observations of stomata were made from the peal of leaves under Olympous BX53 microscope attached with digital camera

2.3 Chlorophyll analysis

The extraction and estimation of chlorophyll and carotenoid was undertaken using Arnon`s method (Arnon, 1949). 0.5g of plant leaf materials of the respective concentration were taken in a mortar and pestle and grinded with 80% ethanol. Extracted samples were centrifuged at 10,000 rpm for 10 min at 4^0C. The supernatants were collected and absorbance measured at 663nm, 645nm and 470nm using UV-VIS spectrophotometer. The calculation of the concentration of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were calculated using the following equations:-

Chlorophyll a: [12.25*A663nm]-[2.79*A645nm]

Chlorophyll b: [21.5*A645nm]-[5.1*A663nm]

Total chlorophyll: [7.15*A663nm] + [18.73*A645nm]

Carotenoid: (1000*A470nm)-(1.82*Chlorophyll a)-(85.2*Chlorophyll b)/198

2.4 Estimation of total carbohydrate

Total carbohydrate content was estimated following anthrone-sulphuric acid method of McCready *et al.* (1950) with modifications (Aarrouf *et al.*, 1999). Anthrone (0.2%) in concentrated H_2SO_4 was used as reagent. Spectrophotometric optical density was recorded at 630nm. Quantization was done with standard graph using glucose.

2.5 Estimation of total phenol

0.5g of the plant leaf was weighed and homogenized in 10mL of 80% ethanol. The homogenate were centrifuged at 10,000 rpm for 20 mins. The supernatants was heated in water bath at 90^0C for 1h and evaporated to dryness. The residue was dissolved with 5mL of distilled water. 2mL of aliquots from each test tubes were taken for analysis. The volume was made up to 3mL by distilled water including the test tubes containing the samples, test tubes containing standard solution. A test tube with 3mL of distilled water serves as blank. 0.5mL Phenol (Folin-Ciocalteu) reagent was added to all the test tubes and kept in dark for 3 min. After 3 min, 2mL of 20% Na2CO3 solution was added to all the test tubes, mixed thoroughly and placed in boiling water bath for exactly 1 min. The mixture was then cooled and absorbance was recorded at 650nm using the UV-VIS spectrophotometer. The amount of phenol in the test samples was determined from the standard curve.

2.6 Estimation of proline content

Extraction and determination of proline content was done by Ninhydrin method. 0.5g of the plant leaf material of the respective concentration (Control, 20 µM, 100 µM, and 200 µM) was homogenized in 1mL of 70% pure ethanol and were taken in test tubes. 2mL of reaction mix (1% Ninhydrin + 60% acetic acid + 20% ethanol + 2% water)) was added to all the test tubes including standard solution (1 ml of proline solution; 11.5 mg of L-proline dissolve in 70% ethanol for stock). A test tube with 1mL ethanol and 2mL of reaction mix serves as blank. All the test tubes were then kept in boiling water bath at 95^0C for 30 min and were cooled down to room temperature. The contents were centrifuged for 1 min at 10,000rpm. The supernatant was collected and the absorbance was recorded at 520nm using UV-VIS spectrophotometer. The amount of proline in the test samples was determined from the standard curve.

2.7 Estimation of protein content

The protein content was determined by using Bradford method (Bradford, 1976) with BSA as the standard. 2g of plant leaf material of respective concentration was homogenized in 2mL of chilled protein extraction buffer (Protein extraction buffer: 0.0625M TrisHCl buffer pH 6.8 + 20mM PFSM + 200µL, 2-Marcaptoethanol + 3% SDS) with some sterilized sea sand. Then the extract was boiled in hot water bath for 2 min at 95^0C. The extracts were centrifuged at 10,000rpm for 15 min at 4^0C. The supernatants were collected and stored in refrigerator. 100µL of the supernatant of each concentration was pipetted out in a series of test tubes. 5 mL of the Bradford reagent (100mg Coomassie Brilliant blue G-250 + 50mL 95% ethanol + 100mL of 85% (w/v) Phosphoric acid +850 ml distilled water and filtration through Whatman 1 paper after complete dissolvation. Solution was prepared before use for better solubility of colour with 1N NaOH. To obtain a brown colour, Bradford reagent and complete removal of blue colour of solution, repeated filtration can be done). The test tube containing 100µL distilled water and 5mL of Bradford reagent serves as blank. The absorbance was measured at 595nm using UV-VIS spectrophotometer.

2.8 Measurement of OJIP parameters

The chlorophyll fluorescence measurement data can be analysed by the OJIP test, which is based on the theory of energy flow in thylakoid membranes, thereby facilitating a better understanding of the relationships between the biophysical side of photosynthesis and fluorescence signals. The OJIP phase of the Chl a fluorescence induction curve is referred to as "fast transient" lasting for less than a second; it is obtained after a dark-adapted sample is exposed to saturating light. In the OJIP curve, 'O' stands for "origin" (minimal fluorescence), 'P' for 'peak' (maximum fluorescence), and 'J' and 'I' for inflection points between the 'O' and 'P'

levels.Hansatech Instruments Ltd (Kings Lynn, UK) developed a new tool- mPEA (multifunctional Plant Efficiency Analyzer) allowing for fast and very informative sub millisecond time resolution analysis of the functional status of the photosynthetic apparatus in plants.

2.9 Chlorophyll a fluorescence analysis

Parameters related to Chlorophyll a fluorescence were measured by a Multifunction Plant Efficiency Analyzer (M-PEA) (Hansatech Instruments Ltd, UK) following the method of Strasser *et al.,* (2010) standard method. Dark adaptation leaves (20 min in dark adaptation) were illuminated with red light (at 650 nm of 3,000 µmol (photon) m^{-2} s^{-1} provided by an array of light-emitting diodes with a light pulse exposure for 1 sec) from the middle part of a leaf blade using leaf clips. The Chl a fluorescence transient were measured that contained, initial (F0) to the maximal (Fm) fluorescence value, which has been recorded at 20 µs (O-step), 2 ms(J-step), 30 ms (I-step), and 0.5-1 s (P-step) (Šimiæ *et al.*, 2014). Photosynthetic data were collected for 118 points in 1 sec and the PSII behavior was determined on the basis of parameters recorded and derived from the OJIP transientsbased on Energy Fluxes in chloroplast membranes. The chlorophyll a fluorescence parameters and equations of the OJIP-test have followed (Tsimilli-Michael and Strasser, 2008). The detail table is available in our earlier paper (Das, *et al.*, 2022) which was followed from Stirbet *et al.*, (2018).

2.10 Statistical analysis

Mean data are calculated from the replicated data samples for morphology, biochemical, and OJIP transients data and the standard deviations were analyzed in MS Excel. Analysis of variance as well as Duncan's Multiple Range Test were made for all mean data for different treatments (Sokal and Rohlf, 1995).

3. Results and Discussions

3.1. Morphological and biochemical analysis

Comparative analysis of shoot and root growth found significant difference in 20 µM Fe treatment as compared to control irrespective of the duration of treatment. The 200 µM Fe have shown decrease of shoot and root growth with a little prompted shoot growth in 20 µM Fe treatment for 7days. Leaf area found enhanced in lower doses which was gradually decreased in higher doses (Fig. 1, Table 1). Decreased shoot length, root length and leaf area with increase in Fe concentration was also reported earlier (Rasafi, *et al.,* 2016). Toxic concentration of iron caused growth inhibition and significant decline in fresh and dry matter yield in green gram seedlings (Jafar *et al.,* 2015).

The carbohydrate content of seedlings showed a decreasing trend with increase in the concentration of iron on subsequent days of exposure. The maximum decrease in carbohydrate content was found in 200 µM iron concentration and increased content was observed in 20 µM solution compared to control. Phenol content found increasing trend in all the treatments with increasing concentration. The significant increase was noted in 200 µM after 14 days of treatment (Table 2). Proline content was decreased in 20 µM Fe treatment compared to control which was increased significantly ~2.4 fold than control and ~3.1 fold increased than 20 µM Fe treated plants after 14 days of treatment (Table 2). Accumulation of proline in plants is increased which is either due to its enhanced synthesis or reduced degradation (Verbruggen and Hermans, 2008).

Protein content was increased in 20 µM iron concentration. Then it decreased with increase in the concentration of Fe in *V. radiata*. The protein content varied from 12.91 mg g^{-1}f.w. 20 µM to 10.15 mg g^{-1}f.w. in 200 µM

Fig. 1. Effect of Fe on growth of *V. radiata* after 14 days of treatment. (a) Control, (b) 20µM, (c) 100 µM, (d) 200 µM

Table 1

Effect of different concentration of iron on growth of *V. radiata* treated for 7 and 14 days.

Treatments	7 days			14 days		
	Shoot length (cm± SD)	Root length (cm± SD)	Leaf area (cm^2± SD)	Shoot length (cm± SD)	Root length (cm± SD)	Leaf area (cm^2± SD)
Control	18.11±0.14a	7.51±0.14a	2.32±0.12a	22.51±0.22a	9.81±0.14a	4.69±0.12b
20 µM	18.51±0.28a	6.52±0.12b	2.13±0.14n	21.32±0.21b	8.32±0.22b	4.89±0.11a
100 µM	16.80±0.14b	5.81±0.11c	2.03±0.11c	18.11±0.11c	6.75±0.23c	3.43±0.11c
200 µM	16.12±0.28c	5.63±0.13c	1.89±0.12d	17.30±0.13d	6.21±0.22d	2.29±0.14d

Table 2

Effect of iron on the carbohydrate (mg g^{-1}f.w. ± SD), phenol, proline (µg gf.w.± SD) on *V. radiata* treated for 7 and 14 days.

Treatments	7 days			14 days		
	Carbohydrate	Phenol	Proline	Carbohydrate	Phenol	Proline
Control	17.85±0.73	1.02±0.05	1.13±0.13	16.12±0.5	1.13±0.13	2.86±0.10
20 µM	18.78±0.93	1.11±0.09	1.56±0.17	17.82±0.61	1.20±0.08	2.13±0.32
100 µM	12.35±0.87	1.31±0.03	1.71±0.15	13.94±0.6	1.38±0.02	6.80±0.10
200 µM	11.81±0.73	1.34±0.06	2.18±0.15	12.41±0.9	1.41±0.03	6.81±0.10

in 7 day plants which was further decreased significantly to 8.64 mg g^{-1}f.w. in 14 days old 200 µM Fe treated plant. Protein content decreases with increase in the concentration of iron in the growing medium. Protein content was found to be more in the plants grown in 20µM concentration and minimum in the plants grown in 200 µM Fe concentration. These ions damage membranes, DNA and proteins due to the free radical productions. Toxic concentration of iron decreases carbohydrate content in green gram. Carbohydrate content increased in 20 µM of Fe concentration and significant reduction was observed in 200 µM Fe concentration due to Fe toxicity.

3.2. Photosynthetic pigments

Total Chl content increased significantly in 20 µM to 7.45 µg g^{-1} at 7 days and 10.14 µg g-1 at 14 days of treatment compared to control which were subsequently decreased in 100 and 200 µM upto ~4 µg g^{-1} (Table 3). Out of the Chl a and Chl b, the amount of Chl b was increased or decreased as per the dose. Carotenoid content decreased significantly at 20 µM in 7 days as well as 14 days. Chl a :b ratio altered significantly in all treatments irrespective of duration of treatment. The concentration of chlorophyll and carotenoid decreased with the increase in the Fe concentration.

3.3. OJIP analysis

The OJIP graph of 7 days and 14 days treated plant showed significant differences in variable fluorescence of different steps of OJIPcurves (Figs. 2a-d). Chla fluorescence transient shows normal OJIP curves in all the treatments. Comparatively high PS-II fluorescence activity in 20 µM Fe treatment in 'P' transient point followed by transient point 'I' and 'J' at 7 days of treatment (Fig. 2b) which was significantly decreased in 'I' and 'J' point at 14 days of treatment. ΔVt graph after double normalization showed significant reduction of fluorescent transient point ΔJ and ΔI at 200 µM Fe treatment followed by 100 µM Fe treatment (Figs 2b,d). That clearly showed a clear inhibition of Fe induced decrease in photosynthetic activity. In contrary, low dose of Fe treatment enhanced photosynthetic activity and growth promotion. Chl a fluorescence analysis was found to be a powerful tool to study the light reactions of photosynthesis (Strasser *et al.,* 2004; Goltsev *et al.,* 2016) and also to quantify the abiotic stress (Goussi *et al.,* 2018; Dhokne *et al.,* 2022)

3.4. Chlorophyll a fluorescence analysis

Quantum yield (at t=0) of energy dissipation i.e. Fo/Fm could not show much significant variation in different concentration of Fe and duration of treatment (Fig. 2e,f). Relative variable fluorescence at 'J' step (Vj) was highest in 20µM Fe and lowest in 200 µM Fe which was pronounced in14 days. Relative fluorescence in I step (Vi) also significantly varied among the treatment varieties. Significantly low ABS/RC (Absorption per Reaction Centre) was found in 20 µM concentration for 14 day treated plant, Dissipation per Active

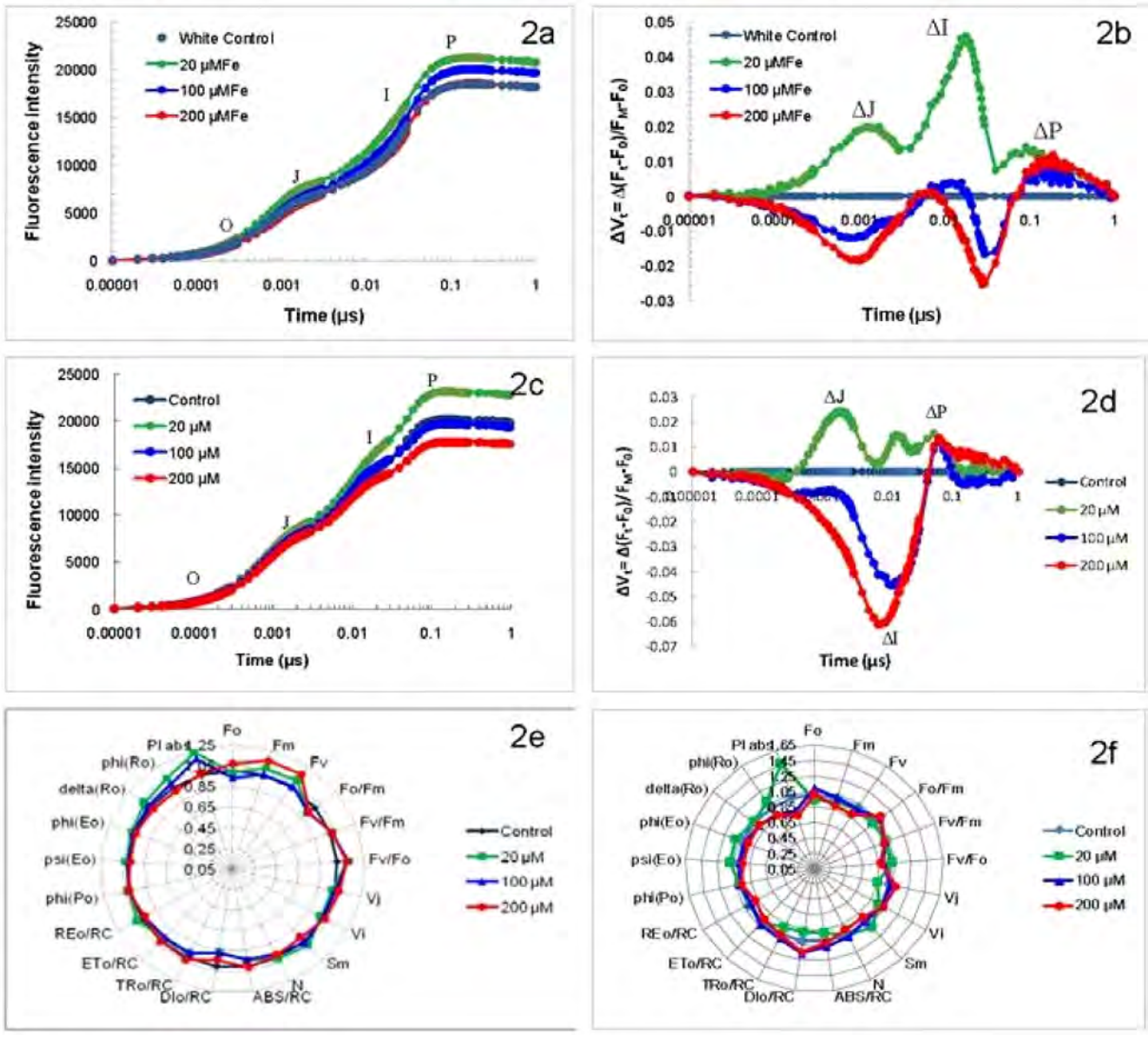

Fig. 2. Effect of Fe on PSII activity on OJIP transient graphs (2a) and delta Vt graph (2b) at 7 days and 14 days treatments respectively ((2c-2d). Radar plot of different photosynthetic parameters at 7days (2d) and 14 days of Fe treatment (2f).

Table 3

Effect of different dose of iron on the photosynthetic pigments (µg/g f.w. ± SD) of *V. radiata* treated for 7 and 14 days.

Treatments	7 days				14 days			
	Chl a	Chl b	Total Chl	Carotenoids	Chl a	Chl b	Total Chl	Carotinoids
Control	2.71±0.13b	4.40±0.07ab	7.12±0.04b	21.05±0.07a	3.27±0.06ab	5.30±0.06b	8.57±0.08b	19.06±0.06a
20 µM	2.90±0.11a	4.55±0.06a	7.45±0.02a	11.18±0.05c	3.66±0.13a	5.48±0.06a	9.14±0.05a	12.12±0.15d
100 µM	2.23±0.13bc	3.56±0.08b	5.79±0.05c	17.03±0.05b	2.88±0.02c	4.08±0.2c	6.96±0.06c	13.04±0.14c
200 µM	2.08±0.07c	2.73±0.17c	4.02±0.08d	20.08±0.09a	1.99±0.14d	2.54±0.2dc	4.53±0.03d	18.11±0.12b

Reaction Centre (DIo/RC), Trappingper Active Centre (TRo/RC) also found significantly increased in 200 µM Fe treatment as contrary to 20 µM Electron Transport per Active Reaction Centre (ETo/RC) found lowest in 200 µM 14 day plant which was as good as control in 20 µM Fe treatment. The flux of electrons transferred from QA to final PSI acceptors per active PSII (REo/RC) was maximum 20 µM followed by 100 µM and 200 µM in 14 days treatment. No variation was noticed in the ϕ(Po) whereas probability of trapped excitation, which was used in electron transport beyond QA i.e.Ψ(Eo) and the maximum quantum yield for primary photochemistry (at t=0) i.e. Quantum yield of the electron transport beyond QA Φ(Eo) recorded highest in 20 µM Fe treatment. Quantum yield for reduction of end electron acceptors at the PSI acceptor side (ϕRo) showed a maximum in 20 µM Fe treatment. The electron transfer efficiencies to PSI acceptor (ϕRo) was found with similartr end (Fig. 2f). The performance indices, Absorption Basis (PIabs) found significantly decreased in 200 µM Fe treatment and significantly increased in 20 µM Fe treatment as compared to control.

Fv/Fm ratio found no significant variation among the studied varieties which is the maximum quantum yield of primary PSII photochemical reactions, that is mainly used to state the primary health as reported in various plant (Kalaji *et al.*, 2017; Stirbet *et al.*, 2018). The presence of stress signature resulting due to either inactivation of PSII or inhibition of quenching might be the causes of low Fv/Fm (Long *et al.*, 1994). The values of Fo/Fm, Kn, ABS/RC and DIo/RC decreased in Fe treatment in 200 µM. Little changes change in Fo, DI0/CSM, TRo/RC, Kp in treatment. The energy fluxs ΦE0, Ψ0, ΦP0 of PSII were not affected. Radar Plot of different OJIP parameters showed significant variation in RE0/RC, ϕR0 which was reflected in PIabs.

5. Conclusion

The shoot, root growth and leaf area significantly increased in 20 µM Fe treated plant which reduced significantly in 100 and 200 µM treatment in both 7 and 14 days. Chlorophyll content also increased in low doses that reduced significantly in 200 µM Fe treatment for 14 days. PSII activity-related parameters like photosynthetic efficiencies, electron transfer from PSII to PSI acceptor which was better in 20 µM Fe rather than 200 µM Fe, in accordance to other biochemical parameters. Carotenoids, proline, polyphenol were increased with high Fe treatment with protein content in leaf. This suggests the use of low dose Fe fortification in green mung for future nutritional use.

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Nanomaterials in sustainable agriculture: Green synthesized nanoparticles for crop protection and plant growth promotion

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ABSTRACT

The urgent need to increase global food production while maintaining the ecological balance has accelerated the adoption of sustainable agricultural practices. Green-synthesized nanoparticles (GNPs), derived from plants, algae, fungi, bacteria, and agricultural by-products, have emerged as promising alternatives to conventional agrochemicals. Unlike chemically synthesized nanoparticles, GNPs are produced using eco-friendly reducing and stabilizing agents such as polyphenols, flavonoids, and microbial metabolites, ensuring biocompatibility and reduced toxicity. Their unique physicochemical properties including high surface-to-volume ratio, controlled release, and functional versatility enable diverse applications in agriculture, including nutrient delivery, growth stimulation, pathogen control, and stress tolerance. Metallic nanoparticles (Ag, Au, Cu, Fe), carbon-based nanomaterials, and polymeric systems have demonstrated efficacy as nano-fertilizers, biostimulants, and nano-pesticides, enhancing nutrient-use efficiency, improving photosynthesis, modulating phytohormones, and suppressing pathogens such as *Fusarium, Alternaria*, and *Xanthomonas.* Moreover, GNPs support soil fertility and microbial interactions, promoting circular and climate-smart agriculture. This review highlights recent advances in biosynthetic approaches, mechanisms of action, and agricultural applications of GNPs, emphasizing their potential to reduce chemical inputs, mitigate environmental risks, and achieve sustainable crop production.

Introduction

Feeding the rapidly growing global population which is projected to reach 9.7 billion by 2050, will require a 60-70% increase in food production while still maintaining ecological balance and sustainability. Conventional agricultural practices, largely dependent on chemical fertilizers and pesticides, have played a key role in enhancing crop yields but have also led to soil degradation, groundwater contamination, and ecological imbalance (Zulfiqar *et al.*, 2019).

Nanotechnology has emerged as a revolutionary tool in this context, offering innovative solutions for crop improvement and sustainable farming. Among its approaches, the green synthesis of nanoparticles (GNPs) which employs biological agents such as plant extracts, microorganisms, and biopolymers has gained considerable attention as a safer and more sustainable alternative to conventional chemical or physical synthesis methods (Hosseingholian *et al.,* 2023).

The applications of GNPs in agriculture are diverse, ranging from nano fertilizers and nano pesticides to nano-enabled delivery systems. Nano fertilizers release nutrients in a controlled manner, reducing leaching losses and improving nutrient-use efficiency compared to traditional fertilizers (Zulfiqar *et al.,* 2019). A novel concept, nano priming, uses nano-agrochemicals to enhance seed germination and activate stress-responsive genes, thereby improving early plant establishment (An *et al.,* 2020). In

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addition, nanoparticles such as iron, carbon nanotubes, and copper-based nanomaterials contribute to soil fertility by increasing nutrient retention and reducing contamination from heavy metals and agrochemical residues (Elsayed *et al.,* 2025).

Overall, the development and application of green-synthesized nanoparticles hold immense promise for sustainable agriculture by improving nutrient efficiency, enhancing stress tolerance, and supporting soil and ecosystem health. This review provides an overview of recent advances in the green synthesis of nanoparticles, their role in crop protection and plant growth promotion, and their potential as eco-friendly alternatives for sustainable agricultural practices.

Green Synthesis of Nanoparticle

Synthesis by microbes

Microorganisms facilitate nanoparticle biosynthesis through multiple biological mechanisms. Extracellular enzyme activity plays a key role, as many microbes secrete enzymes particularly reductases into their environment, which act as natural electron donors to reduce dissolved metal ions and initiate nanoparticle nucleation. Microbial metabolic pathways further contribute, with certain bacteria and fungi utilizing respiratory or photosynthetic electron transport systems to transfer electrons to metal ions, enabling sustainable biologically driven redox reactions for nanoparticle formation. Intracellular synthesis also occurs, where microorganisms internalize metal ions and, through interactions with enzymes, proteins and other biomolecules, catalyze reduction and guide-controlled nanoparticle assembly within the cytoplasm or specialized organelles. Additionally, microorganisms release diverse biomolecules including proteins, peptides, polysaccharides, and secondary metabolites that bind metal ions, promote reduction, and act as natural capping and stabilizing agents, effectively controlling particle size, morphology, and surface properties (Duran *et al.,* 2013). Together, these extracellular, intracellular, and biomolecule-mediated processes form an integrated, eco-friendly system for the biosynthesis of stable and well-defined nanoparticles.

Synthesis by plant

Plant-mediated green synthesis of nanoparticles relies on the reducing and stabilizing properties of bioactive compounds present in plant extracts, including polyphenols, flavonoids, and terpenoids, which donate electrons to metal ions, facilitating their reduction and subsequent nanoparticle formation (Hosseingholian *et al.,* 2023). The reaction medium's pH and temperature are important variables as ideal pH levels encourage metal ion reduction, regulate nucleation and development, while temperature affects reaction kinetics and shapes the size and shape of the nanoparticles (Hussain *et al.,* 2016). Furthermore, biomolecules that bind to the surface of nanoparticles, inhibits aggregation, and improves stability such as proteins, polysaccharides, and organic acids act as natural capping and stabilizing agents. These capping agents also play a key role in determining the size, shape, and surface properties of the synthesized nanoparticles, ensuring uniformity and functional performance. Collectively, the interplay of reducing compounds, reaction conditions, and stabilizing biomolecules enables an eco-friendly, controlled, and efficient approach to nanoparticle synthesis using plant extracts.

Green synthesized nanoparticles for management of plant diseases

Green-synthesized nanoparticles (GNPs) represents a sustainable alternative for crop protection, providing effective control of plant pathogens while reducing reliance on chemical pesticides. These nanoparticles, which are made from biological agents including bacteria, fungi, algae, and plant extracts, reduce expenses, eliminate hazardous chemicals during synthesis, and support the ideas of circular agriculture (Sharma *et al.,* 2025). By penetrating pathogen cells, biofilms, and plant tissues, their high surface-to-volume ratios and adjustable characteristics allow potent antibacterial action at low dosages [Fig 1] (Al-Khattaf *et al.,* 2021).

Silver nanoparticles (AgNPs) are widely studied for their ability to suppress *Fusarium, Alternaria* and *Xanthomonas* through membrane disruption, ROS generation, and inhibition of spore germination. Gold nanoparticles (AuNPs), though less intrinsically antibacterial, are valued for their stability, low toxicity, and multifunctional roles, including biofilm inhibition, systemic resistance induction, and fungicide delivery (Sharma *et al.,* 2025; Geoprincy *et al.,* 2013). Copper nanoparticles (CuNPs, CuO-NPs) retain copper's classic antimicrobial effects while enhancing nutrient uptake and controlling pathogens such as *Phytophthora infestans* and *Agrobacterium tumefaciens* [Table 1] (Geoprincy *et al.,* 2013).

Carbon-based nanomaterials (CNTs, graphene oxide, quantum dots) and iron nanoparticles (FeNPs, Fe_3O_4) further expand applications by enabling agrochemical delivery, nutrient absorption, and immune stimulation. GNPs function as nano-fungicides, seed treatments, and elicitors, activating defence enzymes such as peroxidase and phenylalanine ammonia-lyase, thereby enhancing systemic acquired resistance with minimal residues (Sharma *et al.,* 2024).

Table 1

Green synthesized nanoparticles with their function in disease management

Nanoparticle type	Green synthesis source	Pathogens controlled	Mechanism of action	Reference
Silver	Extracts of neem, Tulsi, green tea, curry leaves	*Fusarium oxysporum*, *Alternaria alternata*, *Xanthomonas* spp., *Pseudomonas syringae*	Membrane disruption, ROS generation, inhibition of enzymes activity, suppression of spore germination	[Geoprincy *et al.*, 2013]
Gold	Hibiscus, lemon peel, mango leaves	*Sclerotinia sclerotiorum*, *Rhizoctonia solani*	Induction of systemic resistance, inhibition of biofilms	[Al-Khattaf *et al.*, 2021]
Copper	Garlic extracts, citrus peel, fungal biomass, algae	*Phytophthora infestans*, *Alternaria solani*, *Agrobacterium tumefaciens*	Copper ion release, enzyme inhibition, disruption of cell wall and metabolic pathways	[Gour et al., 2019]
Carbon	Bamboo extract, green tea, lignin waste	*Colletotrichum* spp., *Botrytis cinerea*	High adsorption for nano encapsulation, oxidative stress, induction, immune stimulation	[Sharma et al., 2024]
Iron	Plant polyphenol, algae	*Penicillium* spp., *Fusarium* spp.	Growth promotion, improved nutrient assimilation	[Herlekar *et al.*, 2014]

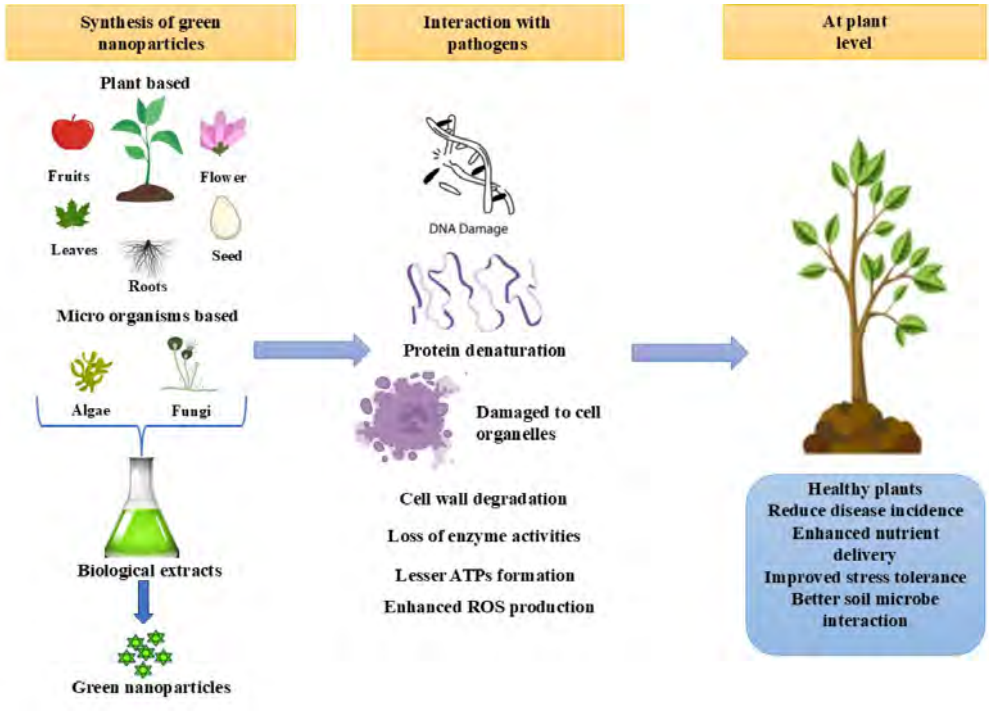

Fig 1: Green nanoparticles in plant disease management

Green synthesized nanoparticles in plant growth promotion

The application of nanotechnology in agriculture has expanded beyond crop protection to include plant growth promotion, with green synthesized nanoparticles emerging as sustainable tools for improving crop productivity (Singh *et al.*, 2019). GNPs are made using plant extracts, algae, fungi, bacteria, or agricultural wastes as stabilizing and reducing agents, in contrast to traditionally created nanoparticles. By using natural capping compounds that enhance plant uptake and activity, this environmentally friendly method reduces environmental effect while introducing biocompatibility. GNPs can serve as nano-fertilizers, biostimulants, micronutrient supplements, elicitors of plant defence, and carriers of bioactive compounds due to their large surface area, regulated reactivity, and adjustable release (Begum *et al.*, 2023; Bahrulolum *et al.*, 2021).

Mechanisms of Plant Growth Promotion

GNPs enhance growth through several mechanisms. Better nutrient delivery plays a significant part as metallic nanoparticles like iron, copper, and zinc offer vital micronutrients in bioavailable forms that overcome soil immobilization and boost nutrient-use efficiency. Similarly, carbon-based nanomaterials like graphene oxide (GO) and carbon quantum dots (CQDs) stimulate root development and improve nutrient absorption by influencing rhizosphere microbes (Mathur *et al.*, 2024; Gour *et al.*, 2019).

The control of phytohormones is another important contribution. It has been demonstrated that GNPs alter auxins, gibberellins, and cytokinins, which enhances root elongation, shoot development, and seed germination. For example, gold nanoparticles increase photosynthetic efficiency by upregulating associated genes, while silver nanoparticles made with neem or aloe extracts speed up seedling establishment (Singh *et al.*, 2019).

In addition, GNPs can induce systemic resistance and stress tolerance. When they interact with plant cells, they cause mild oxidative stress, which triggers antioxidant and defence signalling pathways. By strengthening resistance to temperature stress, salt, and drought, this priming effect enables plants to continue growing in challenging environments (Das *et al.*, 2018).

GNPs also contribute to soil and microbial health. Iron and zinc nanoparticles enrich soil fertility while promoting beneficial plant growth promoting rhizobacteria (PGPR) and mycorrhizal fungi. In hydroponics, carbon nanostructures help adsorb toxins, regulate pH, and provide a favourable environment for root growth (Mathur *et al.*, 2024).

Applications of Specific Nanoparticles

Silver Nanoparticles (AgNPs): Beyond antimicrobial roles, green AgNPs enhance germination, chlorophyll content, and photosynthetic efficiency by improving electron transport (Khan *et al.*, 2023).

Gold Nanoparticles (AuNPs): Biogenic AuNPs act as metabolic modulators, improving enzyme activity, carbohydrate transport, and supporting overall plant health (Das *et al.*, 2018).

Copper Nanoparticles (CuNPs): Essential for lignin synthesis and enzyme function, green CuNPs can serve as slow-release micronutrient fertilizers (Gour *et al.*, 2019).

Iron Oxide Nanoparticles (Fe_3O_4): Fe_3O_4 nanoparticles help reduce iron chlorosis and stimulate root hair formation, thereby enhancing nutrient uptake and chlorophyll synthesis (Herlekar *et al.*, 2014).

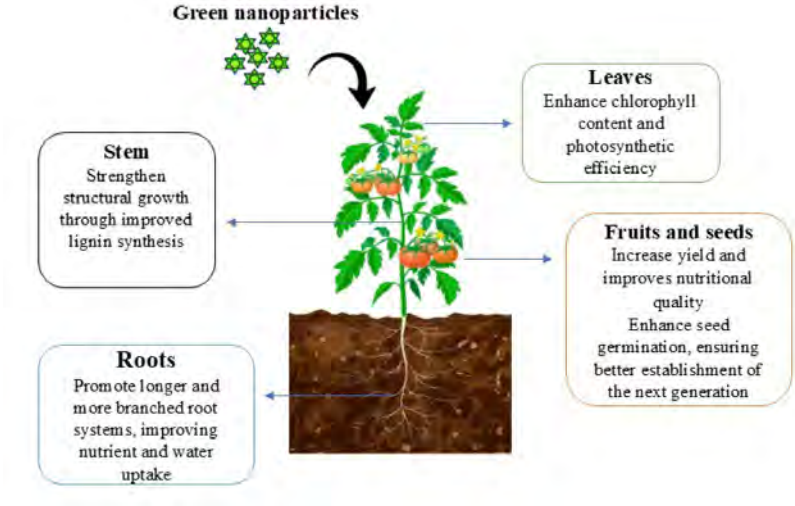

Fig 2: Effect of green nanoparticles on plant growth and development

Carbon-Based Nanoparticles (GO, CQDs): Improves soil water retention, increases microbial activity, and facilitates hormone transport, while also serving as carriers for biofertilizers (Sharma *et al.,* 2024).

Future prospects

Although green-synthesized nanoparticles (GNPs) offer promising applications in sustainable agriculture, their large-scale adoption requires further refinement. Future research should focus on standardizing eco-friendly synthesis methods, ensuring reproducibility, and lowering production costs for field-scale applications. Comprehensive safety assessments are essential to evaluate nanoparticle interactions with plants, soil microbes, and ecosystems, thereby minimizing potential risks. Their effectiveness will be further increased by the creation of intelligent nano formulations that integrate several capabilities, such as pathogen resistance, stress tolerance, and nutrient delivery. In addition, combining GNPs with precision agriculture technologies like sensors, AI-powered monitoring, and managed delivery systems may maximize resource efficiency while minimizing environmental effects. Bridging the gap between lab research and actual agricultural operations would require strong policy frameworks, farmer training, and awareness campaigns. GNPs have the ability to completely transform farming systems with these developments, ensuring ecological balance and promoting global food security.

Conclusion

The integration of green synthesized nanoparticles into modern agriculture represents a pivotal step towards achieving sustainable food production. By utilizing biological agents for synthesis, these nanoparticles minimize environmental risk while offering multifaceted benefits in crop protection, nutrient delivery, and stress management. Silver and copper nanoparticles have demonstrated potent antimicrobial activity, gold nanoparticles act as metabolic modulators, while iron and carbon-based nanomaterials improve nutrient assimilation and soil health. Such multifunctional roles underscore their potential as eco-friendly alternatives to synthetic agrochemicals.The cumulative evidence highlights that GNPs not only enhance crop productivity but also contribute to long-term soil fertility and ecological resilience. Their ability to reduce chemical dependence, activate plant defence mechanisms, and improve growth parameters makes them vital tools for future farming systems. Thus, the adoption of GNPs can play a crucial role in addressing the dual challenge of increasing food demand and maintaining environmental sustainability.

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Effect of arbuscular mycorrhizal fungus and plant growth promoting *rhizobacteria inoculation* on morpho-physiology of *Cicer arietinum* L. grown under phosphorus and iron deficiency in hydroponics

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ABSTRACT

Hydroponics is soilless cultivation technique to grow plants in a nutrient solution. It has advantages over conventional soil-based cultivation, which has challenges of soil-borne pathogens and pests. However, it has incidence of water borne pathogens and plant lack association of beneficial microbes. In the current study the beneficial microbes the arbuscular mycorrhizal fungus (AMF) *Claroideoglomus claroideum* and plant growth promoting rhizobacteria *Stenotrophomonas hibiscicola* were inoculated to the experimental plant *Cicer arietinum* grown in hydroponic with phosphorus (P) and iron (Fe) deficient Hoagland solution. The morphology, biochemical content photosynthetic pigment, total carbohydrate, reducing sugar, protein, free amino acid, phenol and proline, antioxidative enzyme activity APX, GPX and POD in *C. arietinum* was assessed after 30 days of growth. The combined inoculation of AMF and PGPR resulted highest improvement root dry biomass both under P-deficiency (63%) and Fe deficiency (67%). The shoot dry biomass was enhanced by 40% and 58% under P-deficiency and Fe-deficiency respectively with combined AMF and PGPR inoculation. The maximum increase in carbohydrate (55%), protein (58%) and free amino acid (85%) content was recorded with AMF + PGPR inoculation under Fe-deficiency. The microbial inoculations caused a decline in the antioxidative enzyme and maximum decrease of 20-39% under P deficiency and 18-29% under Fe deficiency with AMF + PGPR inoculation. Compared to the single microbe, the combined microbial inoculation showed optimal morpho-physiology of *C. arietinum* under P and Fe-deficiency.

1. Introduction

Nutrients are vital elements essential for plant growth, metabolism and structural development. Each element has a specific function and when those elements are lacking, plants exhibit recognisable deficiency symptoms. Phosphorus (P) is one of the crucial macronutrients that is an integral part of the cell membrane, the backbone of DNA and RNA, structural component of energy-rich molecules like ATP/ ADP etc. It plays an important role in the assimilation of carbon and nitrogen, in energy and lipid metabolism, and in the development of root and reproductive organs in plants. Deficiency of P causes inhibition of primary root elongation, stunted growth and decline photosynthetic efficiency (Ha and Trans, 2013). Iron (Fe) is another essential element, required in small quantities but indispensable for plants. It is involved in chlorophyll synthesis and critical for the maintenance of chloroplast structure and function (Rout, 2015). The Fe deficiency disrupts multiple metabolic pathways in plants, including chloroplast structure deformation, a decline in chlorophyll synthesis, reduced photosynthetic efficiency and impaired respiration, ultimately leading to reduced crop productivity (Wang *et al.,* 2022).

In natural conditions, plants live with numerous microbes. Some of the plant-associated microbes are beneficial, which promote plant growth and increase resistance to stress, including nutrient deficiency (Morcillo and Manzanera, 2021). Beneficial bacteria in the rhizosphere are known as plant growth-promoting rhizobacteria (PGPR),

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which may be free-living or symbiotic. PGPR, through their metabolic activities such as organic acid production, nutrient solubilization, nitrogen fixation, siderophore production, etc., enhance nutrient uptake in plants (Singh *et al.,* 2022). The mycorrhiza fungi form a symbiotic association with plant roots, and their mycelia extend beyond the rhizosphere to support water and nutrient acquisition. The root colonisation of arbuscular vesicular mycorrhizal fungi (AMF) increases P uptake in host plant (Jiang *et al.,* 2021). AMF is also known to mobilise micronutrients (Zn, Fe) through secretion of chelators (Haselwandter *et al.,* 2020).

Hydroponics is a new generation soilless cultivation technique that involves growing plants in a nutrient solution. It is gaining popularity in urban areas where cultivable agricultural land is limited. Though the hydroponic culture has several advantages, it requires high nutrient input and lacks beneficial microbes. Recent studies have introduced PGPR in hydroponic culture and obtained better yields than non-inoculated plants (Azizoglu *et al.,* 2021; Ikiz *et al.,* 2024). However, studies integrating beneficial microbes, PGPR and AMF in hydroponics with low nutrient input are limited. Thus, the current study is designed to assess the 'effect of AMF and PGPR inoculation on morpho-physiology of *Cicer arietinum* L. grown under P and Fe deficiency in hydroponics'. Objectives of the current study are to analyse the morphology, biochemical content and antioxidative enzyme activities of *C. arietinum* with AMF and PGPR inoculation under P and Fe deficiency. As the study is novel approach in hydroponics, the findings will provide a new dimension to integrate useful microbe to minimize nutrient input and optimal plant growth.

2. Materials and methods

2.1 Experimental plant

Seeds of the leguminous plant *Cicer arietinum* L. belonging to the family Fabaceae were collected from Odisha University of Agriculture and Technology (OUAT), Bhubaneswar. The viability of the seeds wasassessed by the germination test.

2.2 AMF inoculum

The AMF *Claroideoglomus claroideum* (CMCC/AM 2705) was procured from The Energy and Resources Institute (TERI), New Delhi. The starter culture was mass propagated in the host plant *Zea mays* (maize) by pot culture. The AMF inoculated maize seedlings were grown in a sterilised potting mix (soil: sand: organic manure, 3:2:1 v/v) maintained at 60±2% RH, 25±2°C temperature and 12 h dark/light (700-900 µmol m-2s-2) for 90 days in a greenhouse. The host plant root was assessed for AMF root colonization (Phillips and Hayman, 1970). The potting soil was examined for AMF spores by the wet sieving and decanting method (Gerdemann *et al.,* 1963). The AMF root colonization was 100% and the spore density was quantified as 114/100g dry soil.

2.3 Plant growth promoting rhizobacteria inoculum

The bacterial strain *Stenotrophomonas hibiscicola* strain ATCC 19867 (NCBI accession no. PV342550) was obtained from the Microbiology Laboratory, Botany Department, Utkal University. The *S. hibiscicola* was a Gram-negative bacillus, isolated from the rhizosphere of a leguminous weed, *Tephrosia purpurea* (L.) Pers. growing on the bauxite mine soil of Panchpatmali, Koraput, Odisha. Plant growth-promoting attributes of the bacteria were assessed by testing indole-3-acetic acid (IAA) production (Khiangte *et al.,* 2021), siderophore production (Dave *et al.,* 2006), solubilization of P (Joshi *et al.,* 2021), and Zn (Kamran *et al.,* 2017).

2.4 Experimental design

The experimental design was a randomised 3×4 factorial design with three nutrient conditions: control (C), phosphorus deficiency (P DEF), and iron deficiency (Fe DEF), along with four microbial inoculations: non-inoculated (NI), arbuscular mycorrhizal fungus (AMF), plant growth promoting rhizobacteria (PGPR) and combined inoculation (AMF + PGPR) in the experimental plant *C. arietinum.* The plant growth experiment was carried out in hydroponics with half-strength Hoagland solution (Hoagland, 1920).

For the hydroponic culture, the concentrations of phosphorus (KH_2PO_4) and iron (Fe-EDTA) were reduced to 1/10 of the control (half-strength Hogland solution) to make the nutrient solution P and Fe-deficient, respectively. For AMF inoculation, the overnight-soaked seeds placed over a Petri plate were sprinkled with the AMF spores (100 spores / 10 seed). For PGPR inoculation, the overnight-soaked seeds were immersed in 24 h cultured bacterial suspension (109 CFU/ ml) for 5-6 hours, followed by washing with sterile distilled water to remove traces of culture media. For AMF + PGPR inoculation, PGPR-inoculated seeds were sprinkled with AMF spores. The microbe-inoculated seeds were allowed to germinate on the Perti plate for 7 days and subsequently placed over a net covering the cup containing P and Fe-deficient Hoagland nutrient solution, where the plants were grown on the hydroponic system in plant growth chamber for 30 days at 25°C temperature, 60% RH, 12h of light/dark cycle with 500-1000 µmol m-2s-2 light intensity and pH5.5 of nutrient solution. Different growth and biochemical parameters were analysed in P and Fe-deficiency with microbial inoculations.

2.4.1 Morphological parameter

The morphological parameters such as root and shoot length, fresh weight (FW) and dry weight (DW) of *C. arietinum* with different treatments were analysed. A measuring scale was used to determine the length of the excised shoot and root sample. The fresh weight (FW) of shoot and root samples was measured in an electronic weighing balance. The fresh biomass was dried at 80°C in a hot air oven for 3 h to determine the dry weight (DW).

2.4.2 Biochemical parameters

Biochemical parameters such as photosynthetic pigment i.e. chlorophyll a, chlorophyll b, total chlorophyll and carotenoid (Arnon, 1949), total carbohydrate (Hedge and Hofreiter, 1962), reducing sugar (Nelson, 1994), protein (Lowry *et al.*, 1951), free amino acid (Moore and Stein, 1963), and proline (Bates *et al.*, 1973) content were estimated following standard methods.

2.4.3 Antioxidative enzyme activities

The antioxidative enzyme activities, like ascorbate peroxidase (APX) as per Wang *et al.* (1999), Glutathione peroxidase (GPX) following Hemeda and Klein (1990) and Peroxidase (POD) according to Van-Assche *et al.* (1988) in *C. arietinum* root and shoot samples were assessed.

2.4.4 AMF colonisation

For estimation of AMF root colonization (%) the root samples were cleaned, cut into 1 cm size, treated with KOH (10%) for 20-25 min, bleached with H2O2 for 5 minutes, followed by acidification with HCl (2%) for 5 min and dyed in 0.05% trypan blue stain (Phillips and Hayman, 1970). The roots were squashed gently and viewed under a microscope for studying AMF colonisation (Giovannetti and Mosse, 1980).

Mycorrhizal colonisation (%)=(No.of root segment colonized with with AMF)/(Total no.root segment examined)×100

2.5 Statistical analysis

For each treatment, 3 replicate cups each with 10 plants were maintained. Data represented were mean ± standard error of mean (SEM) obtained from 3 replicates. The statistical difference of the parameters among the treatments was analysed through Duncan's multiple range test at p=0.05 (Duncan, 1955).

3. Results and Discussion

3.1 Characterisation of PGPR

The bacterium *S. hibiscicola* was positive for IAA and siderophore production, along with phosphate solubilization (Figure 1 a-e). IAA production is a crucial parameter in evaluating the plant growth-promoting potential of PGPR, as it is a plant growth-promoting hormone. IAA regulates numerous physiological activities in plants, like stimulation of seed germination, cell division, apical dominance, phototropic responses, and overall plant growth

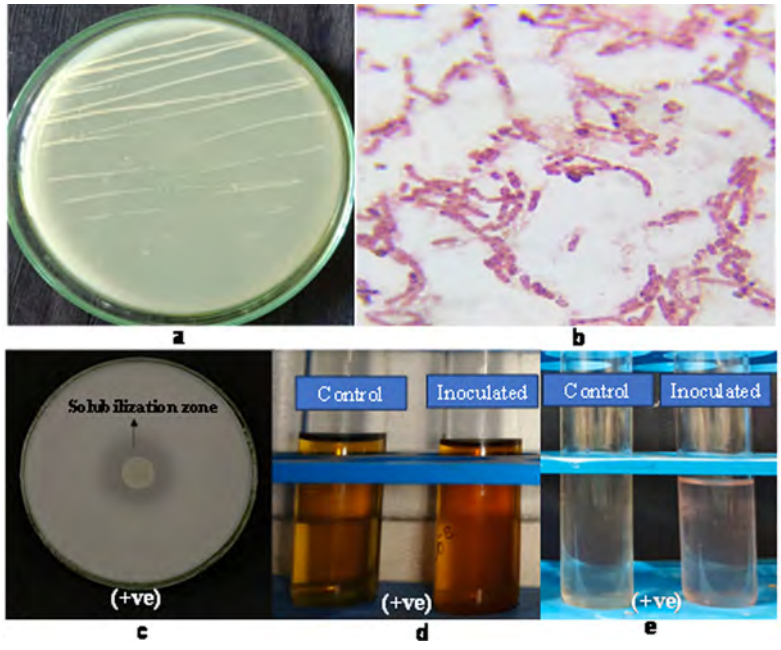

Fig 1: Biochemical characterisation of plant groth promoting rhizobacteria *Stenotrophomonas hibiscicola.* a. Pure culture on a petriplate, b. Gram staining, c. Phosphate solubilization, d. Siderophore production, e. Indole acetic acid (IAA) production.

rate (Mohite, 2013). Siderophore is a low molecular weight iron chelator secreted by PGPR, which helps to overcome the Fe-limiting condition in plants by increasing the bioavailability of iron (Radzki *et al.*, 2013). As the bacteria *S. hibiscicola* has been previously reported for PGP attributes namely IAA synthesis, siderophore production and phosphate solubilization (Upadhaya *et al.*, 2023) supports our findings.

3.2 AMF root colonization

The AMF *C. claroideum* root colonization in *C. arietinum* under P and Fe deficiency with AMF and PGPR inoculation was analysed (Figure 2). The AMF inoculated *C. arietinum* revealed the rate of colonisation as 90% in control, 78% in P deficient medium and 78% in Fe-deficient medium. However, combined inoculation of AMF and PGPR enhanced to a greater extent, i.e., 95% in the control, 82% in P-deficient medium, and 63% in Fe-deficient medium.The symbiosis of plant and AMF is highly dependent upon the optimum nutrient level. Any alteration in the nutrient medium reduced the mycorrhizal colonisation efficiency (Wang *et al.*, 2022) which might be due to the reason behind reduced colonization under P and Fe deficient condition.

3.3 Growth and morphological parameters

The seed germination rate of *C. arietinum* was recorded as 99%. After 30 days of plant growth in hydroponics growth and morphological parameters of *C. arietinum* were analysed (Figure 3). The growth analysis of *C. arietinum* showed that P and Fe deficiency declined the root and shoot length, fresh biomass, and dry biomass compared to the control (Table 1). However, with microbial inoculations,

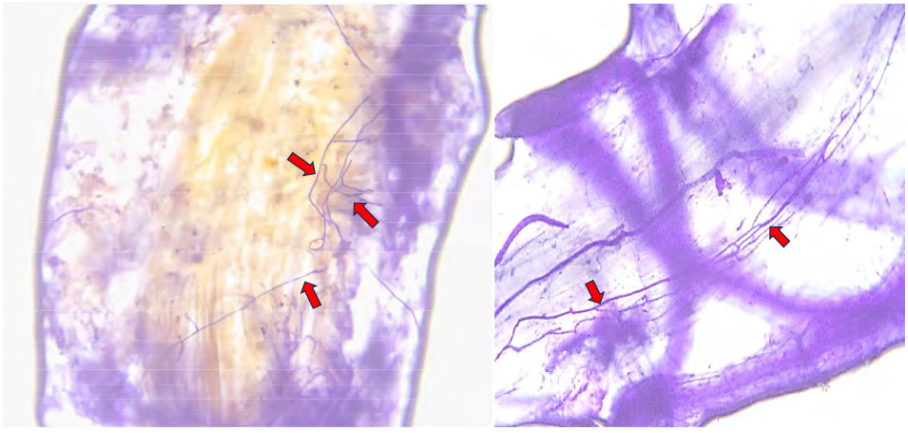

Fig 2: Arbuscualr mycorrhizal fungus (AMF) *Claroideoglomus claroideum* root colonization in *C. arietinum* root. The arrow marks showed AMF hyphae.

Table 1

Impact of arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR) inoculation morphological parameters of *C. arietinum* under P and Fe deficiency.

Treatments		Root length (cm)	Shoot length (cm)	Root FW (g)	Shoot FW (g)	Root DW (g)	Shoot DW (g)
Control	NI	11.9 ± 0.45b	29 ± 0.07c	0.18 ± 0.01c	0.58 ± 0.003b	0.017 ± 0.01b	0.08 ± 0.004b
	AMF	14.5 ± 1.13a	36.4 ± 0.18a	0.24 ± 0.003ab	0.78 ± 0.009ab	0.020 ± 0.001a	0.09 ± 0.002a
	PGPR	12.8 ± 0.64b	32.2 ± 0.29b	0.23 ± 0.003ab	0.66 ± 0.007b	0.019 ± 0.001a	0.08 ± 0.003b
	AMF+ PGPR	15.3 ± 0.70a	38.1 ± 0.25a	0.26 ± 0.007a	0.85 ± 0.003a	0.023 ± 0.002a	0.10 ± 0.004a
P Deficiency	NI	7.7 ± 0.77d	25.4 ± 0.28d	0.11 ± 0.003d	0.50 ± 0.005b	0.011 ± 0.003c	0.06 ± 0.005c
	AMF	11.9 ± 0.34b	33.1 ± 0.07b	0.17± 0.003c	0.72 ± 0.003ab	0.017 ± 0.01b	0.07 ± 0.002b
	PGPR	9.9 ± 0.09c	28.2 ± 0.09c	0.13 ± 0.006d	0.56 ± 0.003b	0.013 ± 0.001c	0.06 ± 0.005c
	AMF+ PGPR	12.5 ± 0.50ab	34.6 ± 0.1b	0.18 ± 0.003c	0.79± 0.009a	0.018± 0.001b	0.08 ± 0.001b
Fe Deficiency	NI	9.2 ± 0.14c	22 ± 0.05d	0.14 ± 0.007d	0.38± 0.006c	0.012 ± 0.001c	0.05 ± 0.005c
	AMF	10.9 ± 0.19c	24.8 ± 0.13d	0.16 ± 0.003cd	0.46 ± 0.004c	0.015 ± 0.004c	0.05 ± 0.007c
	PGPR	13.6 ± 0.22ab	29.6 ± 0.1c	0.20 ± 0.007b	0.46 ± 0.05c	0.019 ± 0.001a	0.07 ± 0.001b
	AMF+ PGPR	14.3 ± 0.10a	30.4 ± 0.09b	0.22 ± 0.007b	0.65 ± 0.05b	0.020 ± 0.001a	0.07 ± 0.008b

Fig 3: Hydropnically grown *C. arietinum* inoculated with arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR) under P and Fe-deficiency.

there was improvement in the morphological parameters. Under P-deficiency, the highest enhancement in root length (62%), shoot length (40%), fresh biomass root (63%), fresh biomass shoot (58%), dry biomass root (63%) and dry biomass shoot (40%) was recorded with AMF + PGPR inoculation. Under Fe-deficiency, AMF + PGPR inoculation resulted the highest increase in root length (58%), shoot length (40%), fresh biomass root (57%), fresh biomass shoot (71%), dry biomass root (67%) and dry biomass shoot (58%) was observed.

Limited P availability results in constrained root growth, adversely affecting uptake of other nutrients and stress tolerance (Kavanová *et al.*, 2006). AMF inoculation is well known to overcome phosphorus deficiency (Das *et al.*, 2022), which aligns with the present finding. The synergistic interaction of AMF and PGPR enhances P uptake (Chen *et al.*, 2023), which justifies the current findings. F is one of the micronutrients required for chlorophyll synthesis, electron transport, DNA synthesis, etc. (Rout *et al.*, 2015). Fe-deficiency causes metabolic disruption, reduced chlorophyll content, impaired ATP production and root elongation (Wang *et al.*, 2022). The Fe-chelator siderophore produced by PGPR binds to Fe3+ and makes it available to the host plant (Patel *et al.*, 2018), which contributes to plant growth enhancement under Fe-deficiency. Further,

enhancement of Fe uptake by AMF inoculation has been emphasised under Fe-deficient conditions (Rahimi *et al.*, 2021), which supports the current observation of the highest growth promotion with AMF + PGPR inoculation.

3.4 Biochemical parameters

3.4.1 Photosynthetic pigment content

The photosynthetic pigment analysis of *C. arietinum* showed reduced chlorophyll a, b, total chlorophyll, and carotenoid pigment content under P and Fe deficiency compared to the control (Figure 4 a-d). However, microbial inoculation improved the photosynthetic pigment content under the nutrient deficiency. The co-inoculation of AMF + PGPR resulted in the highest enhancement of photosynthetic pigment content. Under P-deficient conditions, AMF + PGPR inoculation resulted in enhancement of the chlorophyll a, chlorophyll b, total chlorophyll and carotenoid by 65%, 57%, 62% and 52% respectively. Under Fe-deficiency, the chlorophyll a, b, total chlorophyll, and carotenoid contents howed an increase of 52%, 74%, 60% and 56% respectively, withAMF + PGPR inoculation.

Phosphorus, being the core element of ATP and ADP, provides energy for chlorophyll biosynthesis (Khan *et al.*, 2023) and Fe serves as a vital cofactor for numerous enzymes involved in chlorophyll biosynthesis (Rai *et al.*, 2021). Thus, P and/or Fe deficiency affects pigment synthesis, hinders the electron transport process and photosynthesis (Carstensen *et al.*, 2018). Inoculation of beneficial microbes like AMF and PGPR have reported to overcome the constraints of P and Fe deficiency by improving nutrient uptake through P mobilization and siderophore production (Molnar *et al.*, 2023). The integrated inoculation of AMF and PGPR positively affecting nutrient acquisition and utilisation efficiency of plants has been reported (Adesemoye *et al.*, 2008) which supports our findings.

3.4.2 Carbohydrate, reducing sugar, protein and free amino acid content

The carbohydrate, reducing sugar, free amino acid and protein content in *C. arietinum* under P and Fe deficiency are presented in Figure 5 (a-d). The nutrient-deficient condition resulted in reduced primary metabolite content compared to the control. However, the inoculation of

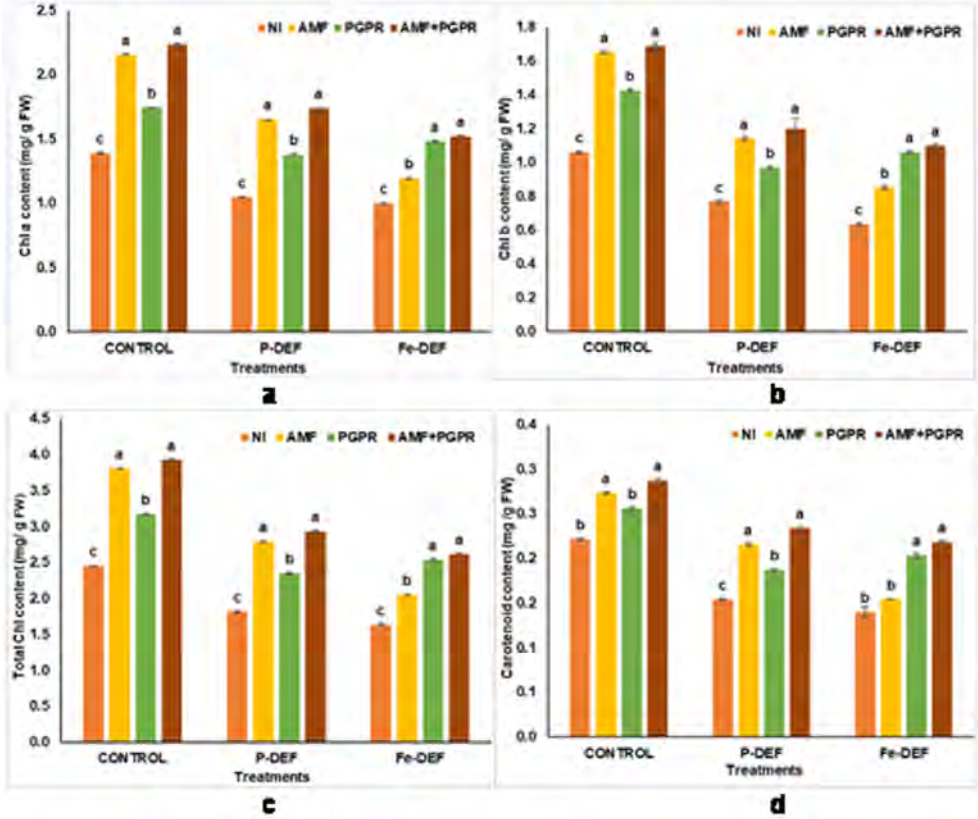

Fig 4: Impact of arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR)inoculation on photosynthetic pigment content of *C. arietinum* under P and Fe deficiency. **a**. Chlorophyll a, **b**. Chlorophyll b, **c**. Total chlorophyll and **d**. Carotenoid pigment content. Data represented is (mean ±SE) obtained from three replicates. Mean values in bars with different letters indicate significant difference at p=0.05, tested through DMRT.[NI: Non inoculated, AMF: Arbuscular mycorrhizal fungi, PGPR: Plant growth promoting rhizobacteria, AMF + PGPR: Combined inoculation of AMF and PGPR, P-DEF: Phosphorus deficiency, Fe-DEF: Iron deficiency.]

beneficial rhizospheric microbes enhanced the biochemical contents under the nutrient-deficient condition. Among microbial inoculation, integrated inoculation of AMF and PGPR resulted in the maximum enhancement in carbohydrate (47%), reducing sugar (42%), protein (57%) and free amino acid (62%) content under P deficiency. Under Fe deficiency, the AMF + PGPR inoculation caused highest increase in carbohydrate (55%), reducing sugar (38%), protein (58%) and free amino acid (85%) content in *C. arietinum.*

Limited P and Fe content affects chlorophyll biosynthesis, the electron transport rate, and disruption of photosynthetic machinery (Carstensen *et al.,* 2018), causing decline in reducing sugar synthesis. Fe deficiency leads to leaf chlorosis, damaging chloroplast structure, adversely affecting photosynthetic rate, which is directly linked with carbohydrate accumulation (Ivanov *et al.,* 2012). The nutrient also affects protein metabolism by impairing the enzyme cofactor (Huan *et al.,* 2022). However, inoculation of AMF and PGPR enhances the soluble sugar accumulation, supporting carbohydrate metabolism, and enhances the synthesis of amino acids under stress (Fan *et al.,* 2024). Integrated inoculation of AMF and PGPR leads to improved biochemical machinery through improved nutrient uptake (Fasusi *et al.,* 2023), corroborating the present finding.

3.4.3 Phenol and proline content

Under P and Fe deficiency the phenol and proline content of *C. arietinum* was higher, however the microbial inoculation caused decline in their level compared to the control (Figure 6 a-b). The combined microbial inoculation treatment (AMF + PGPR) showed the highest reduction in the phenol and proline content than the individual microbial treatments of AMF and PGPR. The phenol content was declined by 31% in P-deficient condition and 28% in Fe-deficient condition with AMF + PGPR treatment compared to NI. The proline content was reduced by 54% in P-deficient condition and 26% in Fe-deficient condition with AMF + PGPR inoculation compared to NI.

Nutrient deficiency is an abiotic stress that stimulates the production of secondary metabolites like phenolic

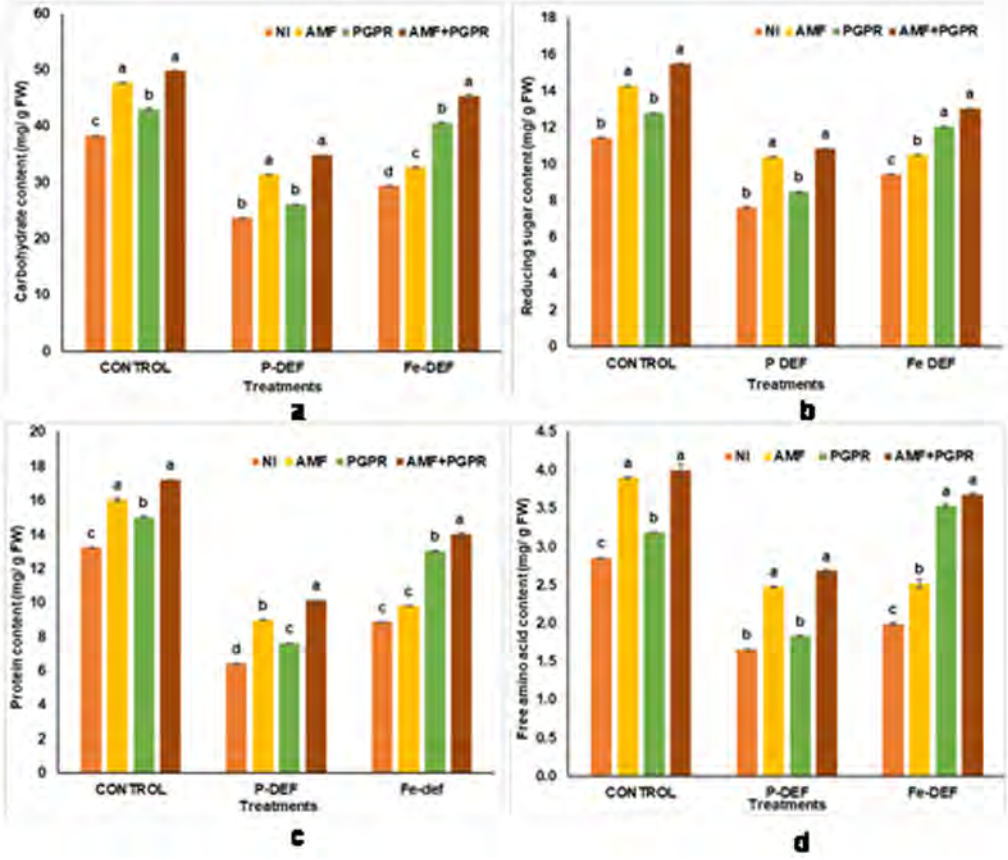

Fig 5: Impact of arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR) inoculation on biochemical parameters of *C. arietinum* under P and Fe deficiency. a. Carbohydrate, b. Reducing sugar, c. Protein and d. Free amino acid content. Data represented is (mean ±SE) obtained from three replicates. Mean values in bars with different letters indicate significant difference at p=0.05, tested through DMRT.[NI: Non inoculated, AMF: Arbuscular mycorrhizal fungi, PGPR: Plant growth promoting rhizobacteria, AMF + PGPR: Combined inoculation of AMF and PGPR, P-DEF: Phosphorus deficiency, Fe-DEF: Iron deficiency.]

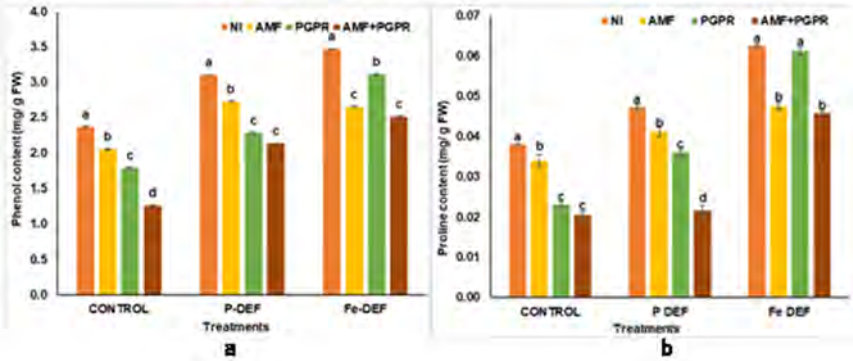

Fig 6: Impact of arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR) inoculation on phenol and proline content of *C. arietinum* under P and Fe deficiency. a. Phenol and b. Proline content. Data represented is (mean ±SE) obtained from three replicates. Mean values in bars with different letters indicate significant difference at p=0.05, tested through DMRT.[NI: Non inoculated, AMF: Arbuscular mycorrhizal fungi, PGPR: Plant growth promoting rhizobacteria, AMF + PGPR: Combined inoculation of AMF and PGPR, P-DEF: Phosphorus deficiency, Fe-DEF: Iron deficiency.]

compounds as a defence mechanism (Ahlawat *et al.*, 2024). Proline is a stress indicator, the production of which maintains water and nutrient balance, enhances antioxidant defence, stabilises membranes and enzymes (Göring and Thien, 1979). Thus, the abiotic stress due to nutrient limitations is the cause of higher phenol and proline content under P and Fe deficiency compared to the control. The synergistic effect of AMF + PGPR provides greater stress tolerance, improves nutrient acquisition, maintains the ROS level and enhances the antioxidant system. Similar reduction of phenol and proline content under stress was previously observed with microbial inoculation (Abdelaal *et al.*, 2024), which justifies the current finding.

3.4.4 Antioxidative enzyme activity

The antioxidative enzyme activity APX, GPX and POD under P and Fe limitation is presented in Figure 7 (a-f). The antioxidative enzyme activity was observed to increase under P and Fe deficiency compared to the control in both root and shoot. The microbial inoculations resulted in decline in the antioxidative enzyme activity analysed. Among the microbial inoculation, co-inoculation of AMF + PGPR provides improved results compared to individual treatment and control in ROS regulation. Under P deficiency, the co-inoculation of AMF + PGPR reduced the APX activity by 39% in the shoot and 29% in the root; GPX activity by 25% in the shoot and 33% in the root, and POD activity by 20% in the shoot and 37% in the root. Under Fe deficiency, the AMF+PGPR declined APX activity by 25% in shoot and 18% in root; GPX activity by 22% in the shoot and 26% in the root, and POD activity by 24% in the shoot and 29% in the root.

Under the nutrient deficiency stress, the production of ROS leads to oxidative damage. To counteract the stress, plants upregulate the antioxidative enzyme activity which scavenge the ROS and improves tolerance to oxidative stress (Rao *et al.*, 2025). The microbial inoculations reduce oxidative stress through nutrient acquisition, induce systemic tolerance, production of microbial antioxidants and metabolites under stress conditions (Singh *et al.*, 2020), which justify the current findings.

4. Conclusions

It was concluded that the combined inoculation of AMF and PGPR significantly improved the morpho-physiology of *C. arietinum* than individual microbial inoculations under P and Fe deficiency. The AMF root colonization by *C. claroideum* and mobilization of P through the fungal hypha attributed to compensate P deficiency. The siderophore production by PGPR *S. hibiscicola* enhanced the Fe uptake and counteract Fe deficiency. The morphological parameters showed enhancement due to compensation of nutrient deficiency. The improvement of photosynthetic pigment content and improvement of photosynthetic efficiency caused increased reducing sugar, total carbohydrate and protein content. The alleviation of nutrient stress resulted in decrease of phenol and proline level. The antioxidative enzyme activities declined by ROS management through microbial antioxidants and systemic stress tolerance. Thus, co-inoculation of AMF and PGPR in hydroponic culture is potential to reduce nutrient input.

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Conflict of interest: Authors declare that they do not have any conflict of interest.

Contribution: Conceptualization and experimental

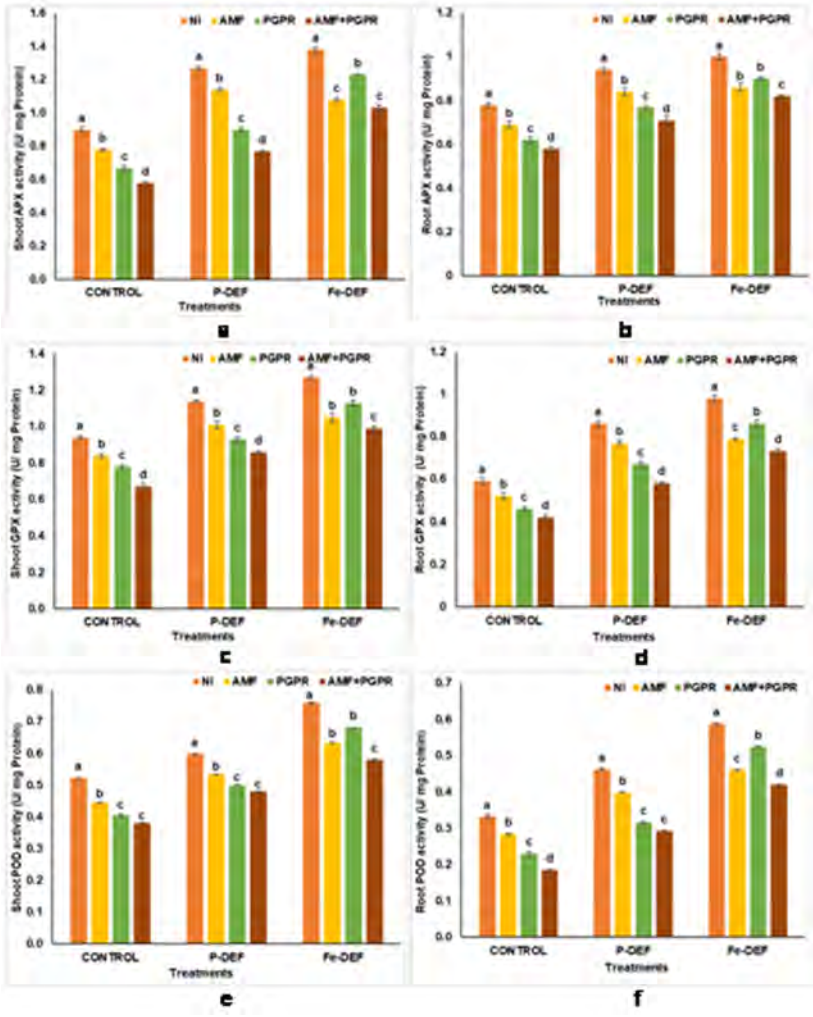

Fig 7: Impact of arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR) inoculation on antioxidative enzyme activities of *C. arietinum* under P and Fe deficiency. a. Shoot APX, and b. Root APX; c. Shoot GPX d. Root GPX; e. Shoot POD and f. Root POD. Data represented is (mean ±SE) obtained from three replicates. Mean values in bars with different letters indicate significant difference at p=0.05, tested through DMRT.[NI: Non inoculated, AMF: Arbuscular mycorrhizal fungi, PGPR: Plant growth promoting rhizobacteria, AMF + PGPR: Combined inoculation of AMF and PGPR, P-DEF: Phosphorus deficiency, Fe-DEF: Iron deficiency.]

design - Bandana Kullu, Ankita Tripathy; Experimental work and data analysis - Ankita Tripathy and Sushree Paramita; Supervision- Bandana Kullu; Draft Manuscript - Ankita Tripathyand Sushree Paramita; Editing and finalization of manuscript- Ankita Tripathy, Sushree Paramitaand Bandana Kullu. All authors have read and agreed to the final manuscript.

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Assessing the effects of arsenic toxicity on Mung (*Vigna radiata* L.) seedling development and metabolism and the counteraction of phosphorus

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ABSTRACT

The present study evaluated the effectiveness of phosphorus in reducing arsenic toxicity in Mung (*Vigna radiata* L.). Mung, a protein-rich plant, is sensitive to arsenic toxicity (0,100,200,300 µM), as evidenced by decreased germination of seeds in percentage, length of root and shoot, fresh biomass, total chlorophyll, soluble protein, amylase content, and increased lipid peroxidation, leading to oxidative stress. Phosphorus, which shared the same pathway from soil to plant tissues as arsenic, served as a fertilizer to relax plant morphology and physiological harm. Adding 20 ppm of phosphorus and 300 µM of high-grade arsenic considerably improved germination of seed percentage, root growth (40%), shoot growth(41%), fresh biomass (64.84%), and total chlorophyll (49.34%). Protein (40.24%) and amylase (48.27%) are more effective than arsenic alone in promoting plant development. At 300 µM + P, lipid peroxidation (MDA content) decreased by 84.90% compared to 300 µM arsenic alone. Exposure of 13-day-old seedlings to As increased superoxide dismutase (SOD), while catalase reduced at greater As concentration. Phosphorus modulates the generation of reactive oxygen species (ROS) by reducing superoxide dismutase (SOD) levels, whereas elevated catalase activity suggests a role in the detoxification of hydrogen peroxide (H_2O_2). In conclusion, phosphorus application in arsenic-affected soil served as a plant therapy, improving morphology, physiology, and antioxidant activity significantly.

Introduction

The carcinogenic dangerous metalloid arsenic is present in significant concentrations in the Earth's crust and is one of the most common 20th elements. Industrial discharges, agricultural practices and anthropogenic activities lead to the deposition of arsenic (As) in groundwater and soil, which can induce toxicity in crop plants (Samanta *et al.,* 2021). Soil acquires arsenic through natural processes, including volcanic eruptions and rock weathering, as well as anthropogenic activities such as pesticide application, mining, and wastewater discharge from textile, tannery, and electroplating businesses. Arsenic can migrate from soils to crops, particularly via irrigated groundwater and increased pesticide use, then accumulating in the edible portions of crops, so entering the human body through the food chain (Adhikary *et al.,* 2022).

The poisonous element arsenic exists in pentavalent form as arsenate (As V) and trivalent form as arsenite (As III). Plants absorb As V, but arsenate reductase transforms the less deadly arsenate As V into the more poisonous arsenite As III within root cells (Zhao *et al.,* 2009). As III enters through aquaporin channels, but As V, being comparable to phosphate (P) groups in biological systems, is delivered into cells by P co-transporters (Sharma *et al.,* 2021; Farooq *et al.,* 2016). Arsenic results in a significant reduction in germination %, shoot and root length, biomass, and chlorophyll content (Mahdieh *et al.,* 2013; Sadeghipour *et al.,* 2021). As produces oxidative stress, leading to cellular damage characterized by increased lipid peroxidation, H_2O_2 buildup. Arsenic-induced phytotoxicity elevates the stress metabolites malondialdehyde (MDA) and hydrogen peroxide (Gupta *et al.,* 2020). Plants alleviated metal stress through the synthesis of antioxidant enzymes, including Catalase

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(CAT), Superoxide dismutase (SOD), Glutathione reductase(GR), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX). Superoxide dismutase, a protective enzyme, eliminates superoxide anions by catalyzing their conversion into hydrogen peroxide, which is then catalyzed by catalase to produce oxygen and water. The regions in India susceptible to arsenic contamination include West Bengal, Uttar Pradesh, Assam, and Chhattisgarh. Besides chromium, cadmium, and lead, arsenic was detected beyond the permissible levels in majority of the hotspot blocks within the Hirakud Command Area, Odisha, India (Biswas *et al.*, 2025). Heavy metals impose significant limitations on the growth and productivity of pulse crops, even at low concentrations (Majhi *et al.*, 2023). Abiotic stressors impede seed germination and hinder seedling establishment. Arsenic negatively impacted water and nutrient status, cellular homeostasis, repair of membrane damage, synthesis of ATP, and photosynthesis (Das *et al.*, 2023). Pulses are cultivated plants within the legume family, mostly for their edible seeds. Seeds are an excellent source of protein, vital minerals, and fiber. The nodulation process, beneficial for nitrogen fixation, is also influenced by arsenic, a hazardous element (Pajuelo *et al.*, 2019).

Currently, researchers are increasingly focused on mitigating the impact of arsenic stress toxicity on plant growth. Phosphorus is crucial for root development, nutrient absorption, and the growth of leguminous crops (Mitran *et al.*, 2018). Researchers are intrigued since phosphorus and arsenic utilize the same mechanism to infiltrate plant tissues. Certain non-resistant plants can be rendered more resistant to arsenate by enhancing their phosphorus levels, which subsequently results in less arsenic buildup due to the inhibition of phosphate/arsenate uptake (Mehrag *et al.*, 2002). The interaction between phosphorus and arsenic varies among plant species and is contingent upon the susceptibility or resistance of the plant, as well as the concentration and duration of arsenic exposure. Arsenic availability in soil pore-water solution is reduced under aerobic water management with phosphorous supplement as compared to anaerobic water management in rice (Talukder *et al.*, 2012). Phosphorus can mitigate arsenic stress in various plant species, including chickpea (Gunes *et al.*, 2009), wheat (Pigna *et al.*, 2010), black gram (Srivastav *et al.*, 2013), *Brassica juncea* (Niazi *et al.*, 2017) and rice (Mishra *et al.*, 2022). The present experiment was conducted to assess the effect of arsenic toxicity and its combination with phosphate on *Vigna radiata* L. to enhance seedling development, morphology, photosynthesis, membrane protection and antioxidant metabolism. The current work may assist local farmers in formulating phosphate as a biofertilizer for usage in arsenic-contaminated soil.

2. Materials and methodology

2.1 Study site description and experimental design

The experiment was carried out at the Department of Botany, Model Degree College, Nayagarh, Odisha, during the January-May period of 2025 (Lat 20.112543º, long 85.154836º). The local farmers in Nayagarh, Odisha, provided the "Nayagarh local" kind of green gram seeds. The experiment's sandy loam soil, which had a pH of 7.1, was taken from the college field. Seeds were surface sterilized with Sodium Hypochlorite (4%, w/v) and washed thoroughly by distilled water. Sodium arsenate heptahydrate ($Na_2HAsO4,7H2O$) was chosen as arsenic source for experimental use. The 10 number of seeds were placed to germinate in each petri dish lined with Whatman No-1 filter paper and moistened with 3ml each of treatment solution as follows dH_20 only (Control) (T1), 100µm sodium arsenate (T2), 200µm (T3), 300µm (T4) and the combination of phosphate 20ppm K_2HPO_4 with arsenic treatment labeled as T5 (100µm+P), T6 (200µm+P), T7 (300µm+P). To guarantee accuracy, every experiment was carried out in triplicate and each independently. An assessment of seed germination percentage was calculated after 6 days as per the following formula.

Seed Germination percentage(%)= Number of seeds germinated in final day/ Total seeds sown in petri dish

A pot experiment was designed in which seeds sowed in 1kg plastic bags containing sandy loam soil and compost (3:1 ratio). Daily 50 ml of arsenic and phosphate solutions were given in each pot. Seedling growth analysis was conducted by measuring the length of roots and shoots, as well as the fresh weight of plants after 13 days. Leaf samples employed for the testing of biochemical parameters. Two set of plastic bags were used in which one set of bags were supplied with only arsenic and other set of bags was supplied with Arsenic and Phosphate as described in above treatments.

2.2 Analysis of growth and morphological parameters

The length of root, shoot and fresh biomass of arsenic treated and arsenic with phosphate treatments seedlings were measured and expressed in centimeter, gram respectively.

2.2.1 Photosynthetic pigment: Chlorophyll content

The pigment content was analyzed using Arnon's technique (Arnon, 1949). Leaf samples were utilized to measure the optical density (absorbance) at 645 nm and 663 nm utilizing a UV-spectrophotometer for chlorophyll, with 80% acetone serving as the blank. Total chlorophyll was quantified utilizing the methodology established by Singh

et al., 2017 and expressed in mg/g of fresh weight (FW).

2.2.2 Protein Concentration and Alpha-Amylase Activity

The protein concentration was quantified utilizing the process established by Lowry *et al.,* 1951. It was measured at a wavelength of 640 nm and expressed in metric unit mg protein/100 mg of fresh tissue weight. The amylase activity was assessed using the protocol established by Katsuni and Fukuhara (1969). Results were presented as mg of starch hydrolysed per 100 mg of fresh weight (FW).

2.2.3 Peroxidation of lipids

According to Heath & Packer's (1968) description, lipid peroxidation was quantified by measuring the amount of malondialdehyde (MDA) content as assessed by thio barbituric acid reactive material. The nonspecific absorbance at 600 nm was deducted from the absorbance of the supernatant, measured at 532 nm. The MDA was calculated using an attenuation coefficient of 155 $mM^{-1}cm^{-1}$ and expressed in metric unit n $molg^{-1}$ FW.

2.3 Extraction and assay of activities of antioxidant enzymes

An enzyme extract was procured by homogenizing leaves in a 0.1 M sodium phosphate buffer (pH 7.0) augmented with polyvinyl pyrrolidone. The homogenate was subjected to centrifugation at 15,000 rpm for 30 minutes using a chilled centrifuge. The supernatant was employed for the assessment of catalase and superoxide dismutase. The protocol was followed in accordance with Singh et al., 2017.

2.3.1 Catalase analysis

Catalase (CAT) was assessed following the approach established by Cakmak and Marschner (1992). The assay mixture consisted of enzyme extract, potassium phosphate buffer (pH 7.0), and 10 mM H_2O_2. The decomposition rate of H_2O_2 over one minute was evaluated at 240 nm, calculated using an extinction coefficient of 39.4 $mM^{-1}\cdot cm^{-1}$, and expressed in g/FW. One unit of catalase (CAT) is defined as the quantity of enzyme required to oxidize one mM of hydrogen peroxide (H_2O_2) per minute.

2.3.2 Superoxide dismutase analysis

Superoxide dismutase (SOD) was evaluated utilizing the NBT (nitroblue tetrazolium) photochemical assay as outlined by Beyer & Fridovich (1987). A mixture is prepared using enzyme extract, methionine, and sodium carbonate and made the final volume upto 4 ml. The photoreduction of NBT was spectrophotometrically assessed at 560 nm and contrasted with a blank sample lacking enzyme extract. One unit of SOD activity is described as the amount of enzyme required to provide 50% inhibition of NBT breakdown.

2.4 Statistical analysis

To guarantee accuracy, every experiment was carried out in triplicate and each independently. One-way ANOVA and Tukey tests were employed for statistical analysis in order to compare the treated and control groups. The results were represented as mean ± standard deviation and statistical significance was defined as $p \leq 0.05$. For statistical analysis Minitab version 21 was used for the analysis.

3. Result

3.1 Investigation of Germination and effect of arsenic and phosphate on morphological parameter

After six days, the germination percentages of Vigna radiata at arsenic doses of 100µM, 200µM, and 300µM were 92%, 75%, and 58%, respectively. The germination rate exhibited a progressive decline with increasing arsenic content. Seed germination was seen to be greater at a higher dosage of 300 µM As + P compared to the sole arsenic treatment, which yielded 68% (Fig. 2 A). A pot culture experiment was set with treatment of Arsenic and Phosphorous to determine the morphological and biochemical assays (Fig.1 A and B). The length of roots and shoots, as well as fresh biomass, decreased with rising arsenic levels. At an enhanced arsenic concentration of 300 µM, root length, shoot length, and fresh biomass were reduced by 56.82%, 52.37%, and 57.34%, respectively, in comparison to

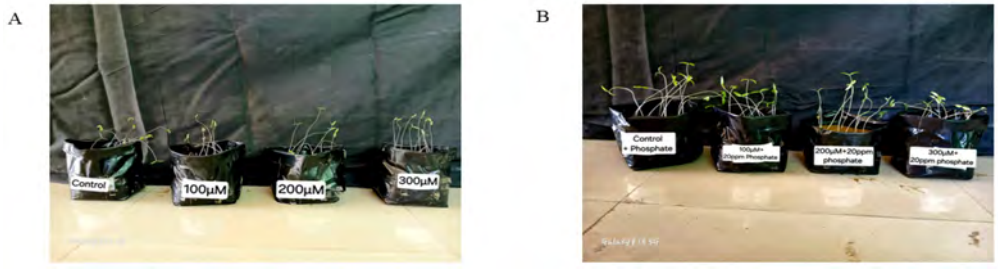

Fig.1 (A) Pot culture experiment for observation of Arsenic effect on *Vigna radiata* L. (B) Pot culture experiment for observation of phosphate and arsenic combination effect

the control group. Additionally, seedlings subjected to 300 µM arsenic and 20 ppm phosphorus shown enhancements of 40.08%, 41%, and 64.84% in root length, shoot length, and biomass, respectively, in comparison to those exposed solely to 300 µM arsenic (Fig. 2B; Fig. 2C; Fig. 2D). Arsenic coupled with phosphate produced optimal outcomes across all parameters.

3.1.1 Impact of arsenic and phosphate on total chlorophyll concentration, protein levels, and alpha-amylase activity

The total chlorophyll content of leaves was assessed using 13-day-old seedling leaves, revealing a substantial decrease in chlorophyll levels with rising arsenic exposure. The addition of 20 ppm phosphate, alongside 300 µM arsenic, increased chlorophyll content by 49.34% compared to the absence of phosphate (Fig. 3A). Likewise, the soluble protein content exhibited a decline with higher arsenic administration. The protein content decreased by 28%, 39.50%, and 55.32% at concentrations of 100µM, 200µM, and 300µM, respectively, in comparison to the control (Fig. 3B). The protein content rose from 0.668 mg at 100 µM arsenic to 0.887 mg at 100 µM plus 20 ppm phosphorus, and at a higher dosage of 300 µM, it climbed from 0.415 mg to 0.580 mg at 300 µM plus phosphorus. The activity of alpha amylase in *Vigna radiata* seedlings exhibited a comparable pattern. The administration of 20 ppm phosphate at 300 µM resulted in a substantial rise from 48.37% compared to 300 µM arsenic (Fig. 3C).

3.1.2 Impact on lipid peroxidation

Lipid peroxidation was reported to rise progressively with elevated MDA levels in arsenic conditions. The MDA levels increased by approximately 45.83%, 50%, and 54.71% at concentrations of 100µM, 200µM, and 300µM, respectively, in comparison to the control. The combination treatment of arsenic and phosphate decreased MDA levels, demonstrating its protective effect on seedlings. At 300µM + P, the reduction in MDA concentration was 84.90% compared to the result at 300 µM arsenic alone (Fig. 4A).

3.2 Impact on antioxidant enzyme activity

Our current investigation revealed a reduction in catalase activity at a greater concentration of arsenic, specifically 300µM (Supplementary Table 1; Fig. 4B). A contrary tendency was observed in the combined application of arsenic and phosphate. The results suggested that catalase played a dramatic role in mitigating the bad impact of arsenic following phosphate application. Conversely, SOD enzyme activity was elevated with the rise in arsenic content (Fig. 4 C). At 300µM + P, the reduction in SOD concentration was 78.94% compared to the result at 300 µM arsenic alone.

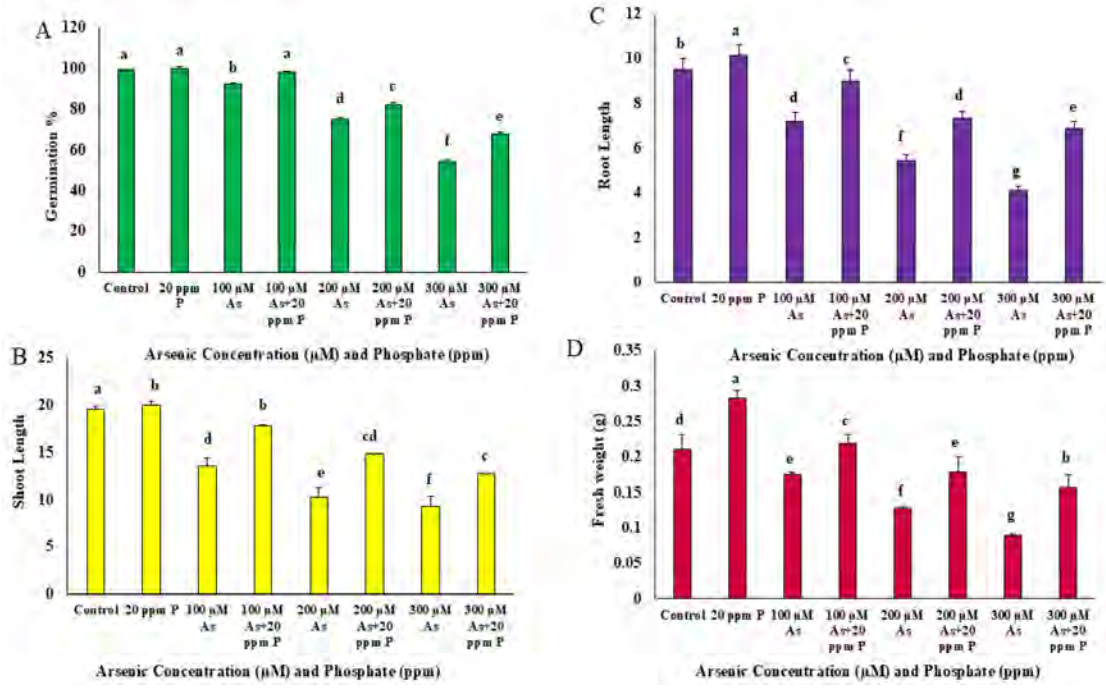

Fig. 2 (A) *Vigna radiata* L. germination rate after treatment with Arsenic and Phosphate. (B) *Vigna radiata* L. root length after treatment with Arsenic and Phosphate. (C) *Vigna radiata* L. shoot length after treatment with Arsenic and Phosphate. (D) *Vigna radiata* L. fresh biomass after treatment with Arsenic and Phosphate. The data is presented as the mean (average of triplicate value) ± Standard deviation. Statistical significance ($p \leq 0.05$) between treatment denoted by small letters, identical letters denoting non-significant differences.

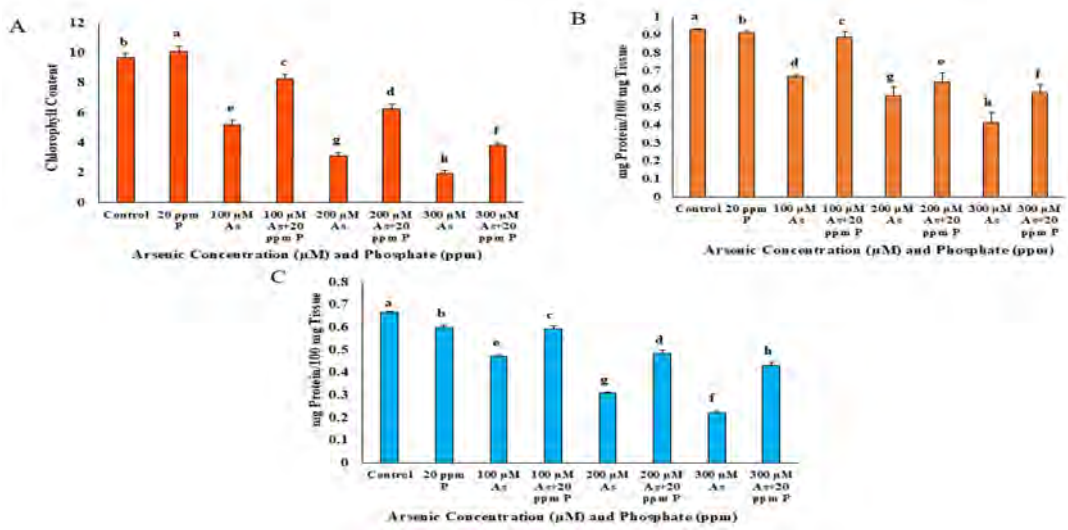

Fig. 3 (A) Chlorophyll content after treatment with Arsenic and Phosphate. (B) Protein content after treatment with Arsenic and Phosphate. (C) Amylase content after treatment with Arsenic and Phosphate.The data is presented as the mean (average of triplicate value) ± Standard deviation. Statistical significance ($p \leq 0.05$) between treatment denoted by small letters, identical letters denoting non-significant differences.

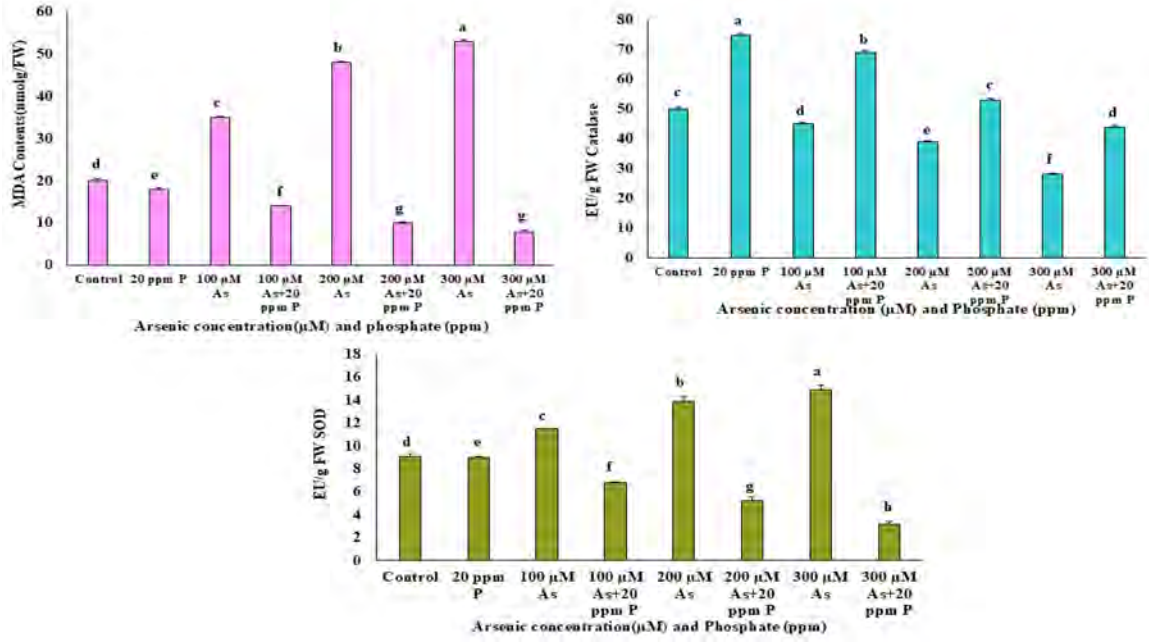

Fig. 4 (A) Peroxidation of Lipids after treatment with Arsenic and Phosphate. (B) Assessing Catalase activityafter treatment with Arsenic and Phosphate. (C) Assessing Super Oxide dismutase (SOD) activity after treatment with Arsenic and Phosphate.The data is presented as the mean (average of triplicate value) ± Standard deviation. Statistical significance ($p \leq 0.05$) between treatment denoted by small letters, identical letters denoting non-significant differences.

Discussion

Arsenic, a non-essential metalloid, evidently exerts a deleterious impact on the morphology and physiology of plants (Patel *et al*., 2020). Arsenic (As) persists in the environment for an extended duration and accumulates to levels that can adversely affect the physicochemical qualities of soils, resulting in diminished soil fertility and reduced crop output (Garg *et al*., 2011). The primary target of arsenic stress is the plasma membrane, which is compromised due to membrane instability. The cause of this is an increase in reactive oxygen species (ROS) concentration, which leads to lipid peroxidation and the production of malondialdehyde (MDA). Elevated levels of lipid peroxidation was seen in rice (Gautam *et al*., 2020). Membrane damage results in an imbalance of nutrition absorption and stomatal conductance

(Chandrakar *et al*., 2018). The generation of MDA was diminished in the As + P therapy (Gomes *et al*., 2012).

The process commences with the seed, which serves as the fundamental stage for the growth of plants in all spermatophytes. Seed germination diminished markedly with the rise in arsenic content. Conversely, the administration of phosphate enhanced the proportion of seed germination. The immobilization of proteolytic enzymes may reduce the availability of carbohydrates and nutrients essential for seed germination (Seneviratne *et al*., 2019). Seeds cannot adequately absorb water and nutrients under arsenic treatment, which adversely affects germination (Yadu *et al*., 2019). During the germination process, complex carbohydrates, proteins, and lipids are hydrolyzed into simpler molecules by enzymes, supplying energy for the development of new cells and tissues. The reduction in alpha amylase and soluble proteins serves as regulatory variables for optimal seedling establishment. The As + P treatments enhance the levels of alpha amylase activity and soluble proteins. These findings align with the results of Choudhury *et al*., 2010; Srivastava *et al*., 2014; Azeem *et al*., 2017.

Morphological characteristics, such as radicle length, plumule length, and biomass, were adversely impacted by arsenic treatment. Plant biomass diminished as a result of tissue permeability and tissue degradation (Kamran *et al*., 2018). The biomass of *Lens culinaris* (lentil) was adversely affected by arsenic poisoning throughout its whole life cycle (Alam *et al*., 2019). Arsenic toxicity decreased seed moisture content and shortened of radicle and plumule length in *Vigna radiata* (Khan *et al*., 2024). Arsenic accumulated predominantly in the roots as arsenite in the *Phaseolus vulgaris* bean plant (Chandra *et al*., 2017). Roots are more adversely impacted than the shoot due to direct exposure to arsenic. The addition of phosphate alleviated plant stress by significantly enhancing morphological characteristics (Mitra *et al*., 2020).

Chlorophyll is the primary pigment utilized by plants in photosynthesis. Chlorophyll content decreased at an arsenic concentration of 200 µM in maize plants (Anjum *et al*., 2017). Arsenic may directly inhibit the activity of RUBISCO, and chloroplast 29 kDa ribonucleoproteins could be potential contributors to the reduced photosynthesis rate (Ahsan *et al*., 2010). Arsenic can alter the morphology of chloroplasts, resulting in partial degradation that reduces chlorophyll levels (Miteva and Merakchiyska, 2002). Phosphorus supplementation significantly enhanced chlorophyll content when administered alongside 150 mg/kg of arsenic in wheat plants (Al-Qahtani *et al*., 2025).

In nature, plants possess their own defense mechanisms to counteract reactive oxygen species (ROS) by synthesizing antioxidant enzymes. The native variety of Nayagarh exhibited increased SOD activity in arsenic-treated plants for their protection. SOD activity was seen substantially decreased at As + P treatments. The treatment of phosphorus with arsenic metal diminished the generation of reactive oxygen species (ROS) along with controlling the synthesis of the SOD enzyme and also increased photosynthesis. Conversely, catalase activity diminishes at elevated arsenic doses. Plants fed with phosphorus exhibit elevated catalase activity. Consequently, it suggests that the excess H_2O_2 generated by metabolic activities of stressed plantswere mitigated by the substantial synthesis of catalase enzyme in phosphate treatment facilities. Catalase activity is more evident than superoxide dismutase in the two barley genotypes, ZDB475 and ZDB160, during arsenic phosphate interaction (Zvobgo *et al*., 2019). The reduction in catalase levels following arsenic exposure was similarly documented in wild type *Arabidopsis thaliana* by Gupta *et al*., 2013. Our current findings align with the results given by Srivastava *et al*., 2013. However, the role of antioxidant enzymes needs more investigation for this particular kind.

The preceding information clearly indicates that arsenic adversely affects the general growth of plants. The utilization of phosphate is crucial for alleviating arsenic stress to sustain plant growth. Phosphate supplementation markedly ($p<0.05$) minimised the toxicity of arsenic. P and As engage in competitive reactions in soils, encompassing redox reactions and rhizosphere mechanisms that influence As bioavailability (Strawn *et al*., 2018). The mechanism underlying the interaction between arsenic and phosphorus in plants involves the reduction of arsenate uptake from the soil due to competition for strong adsorption sites (Wu *et al*., 2022). Phosphorus supplementation generated nitric oxide within the cell and enhanced the defensive mechanism to augment arsenic tolerance in mustard (Khan *et al*., 2021). The current study supported that *Vigna radiata* mitigates arsenic toxicity through phosphate supplementation. All signs clearly demonstrated that phosphate supplementation improved the situation.

Conclusion

The current study found that arsenic inhibits the growth and metabolism of *Vigna radiata* L. seedlings. This plant exhibits significant sensitivity to arsenic stress. The addition of phosphate mitigated arsenic stress and enhanced all growth indices. Consequently, phosphate may be employed as a fertilizer in regions susceptible to arsenic contamination. Its efficacy varies across different plants and is contingent upon the dosage. In the current

investigation, phosphorus (20 ppm) exhibited a protective effect against arsenic stress.

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Topographic Influence on Soil Properties in Agricultural Lands of Raighar Block, Nabarangpur District, Odisha

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ABSTRACT

Topography plays a critical role in shaping soil physico-chemical properties, which in turn influence fertility and agricultural productivity. This study investigates the soil variations across the landforms (Upland, Plain and Valley) on key soil parameters in Raighar Block, Nabarangpur District, Odisha. Soil samples were collected from representative sites across each landform and analysed for pH, electrical conductivity (EC), organic carbon (OC), bulk density (BD), texture (sand, silt and clay), nitrogen (N), phosphorus (P), and potassium (K). Results reveal significant variation in soil fertility indicators across topographic zones, with Valley soils exhibiting higher moisture and nutrient retention, while Upland soils showed signs of leaching and acidity. The plain soils, with moderate slope and better drainage, maintain balanced fertility and are suitable for a variety of crops. These findings underscore the need for landform-specific soil management and crop selection strategies to optimize agricultural outcomes in the region.

1. Introduction

Healthy soil is the foundation for the productivity and sustainable agriculture. Agriculture sustainability has been necessary for the present requirements and to meet the future challenges of food security. Land area is limited in the earth surface, so that we should use the limited area for agricultural purposes without expanding and destroying forest or natural resources. A healthy soil is needed for dynamic living system which delivers various ecosystem services, like enhancing plant productivity, balancing soil nutrient cycling and water quality, and greenhouse gases removing from the atmosphere. And in the other way , the diversity of soil microorganisms and their activities are the main focusing components for the soil health. The sufficient macro-nutrients of the soil and the way of sustainable agriculture produces high yielding without environmental degradation. Nutrient management and crop suitability are the crucial composition for sustainable agriculture and significantly enhance yields. Crop selection and application of nutrient requirements with respect to soil characteristics and topography must be focused for the eco-friendly agricultural activities. Soil fertility is a cornerstone of sustainable agriculture, and its spatial variability is often governed by topographic features. In undulating landscapes, elevation, slope, and drainage patterns influence the distribution and transformation of soil nutrients (Priyadarshini and Dash, 2024). Soil properties are inherently variable across landscapes due to topographic gradients, erosion, and deposition processes. Slope position affects water movement, organic matter accumulation, and nutrient cycling, making it a key factor in soil fertility assessment (Moges and Holden, 2008; Ewunetu *et al.*, 2025). Understanding these variations is essential for sustainable land management and precision agriculture. Raighar Block, located in the northern part of Nabarangpur District, comprises a mosaic of Upland, Plain, and Valley landforms.

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These micro-topographies affect soil formation processes, erosion intensity, and water retention, thereby influencing crop suitability and productivity (Dash *et al.*, 2022).

The aim of this research is to find out the current status of the soil with respect to different landforms, which will be helpful to the local people in choice based agricultural practices and nutrient management. With this, the documentation of the findings may help to the scientist and policy makers to carry out the research on climate resilient site specific crop varieties, and development.

2. Materials and Methods

The soil samples were collected from different topographic positions i.e., upland, plain, and valley, for analysing the physicochemical parameters like pH, EC, OC, BD, Moisture, Sand, Silt, Clay, N, P, and K.

2.1. Study Area

The study site was selected at Raighar block (Fig 1) of Nabarangpur district, a southern part of Odisha, which is tribal dominated, and economically poor area (876.61 km2), characterized by hot moist sub-humid climate. The land pattern includes hills (hill top, side slope, foot hill, isolated hillock), upland, middle land (plain) and low land (valley), and mostly the maize and paddy are cultivated in this region. It harbors three season mainly name as pre-monsoon (March-June), monsoon (July-Oct) and post monsoon (Nov-Feb). The temperature of summer season is about ~40^{0}C and the winter is about ~12^{0}C with annually rainfall in the monsoon season from 1030mm to 1569mm.

2.2. Soil Sampling

The field studies were conducted during the pre-monsoon season in the year 2025. The soil samples were collected from 0 to 15cm depth in polythene bags from different landforms of upper (Upland: 3 nos.), middle (Plain: 5nos.) and lower land (Valley: 2 nos.) and taken into the laboratory for the physico-chemical analysis.

2.3. Analysis of Physico-chemical Parameters

The soil physico-chemical properties were analyzed according to the standard soil testing protocol (Mishra *et al.*, 2019). The soil pH and EC were measured using digital meters, and BD was measured by core auger method. The texture (sand, silt and clay) and SOC were analyzed by Bououucos hydrometer method and Walkley-Black method respectively. The macro-nutrients (NPK: available nitrogen, available phosphorus and available potassium) were analyzed by Kjeldahl, Olsen's and Flame photometry methods respectively.

3. Results

In the average value pattern of soil physico-chemical properties, the results show the observations within the

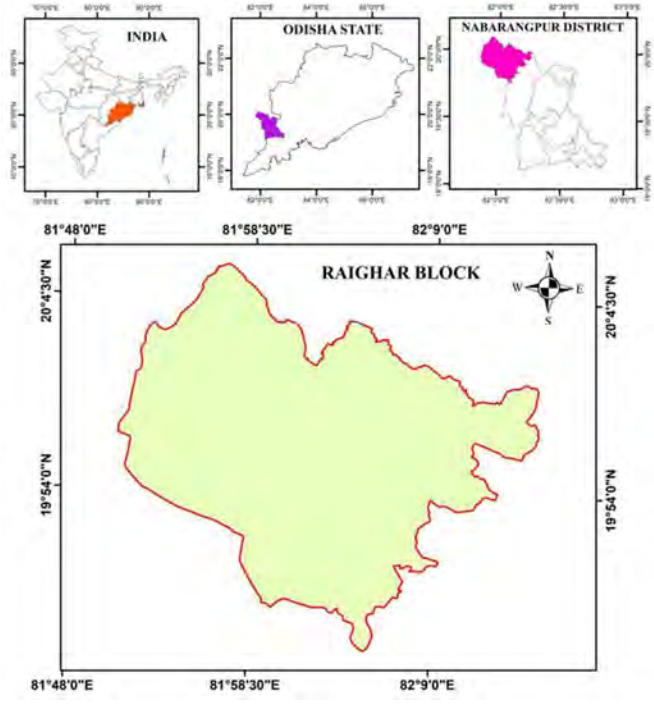

Fig 1: Study Site

landforms (upland, plain and valley) with value in each (Table 1), where the pH increased from Upland to Valley indicating reduced acidity downslope. The EC was higher in Plain and Valley, suggesting greater ionic concentration with high nutrient accumulation and the OC peaked in the Plain, possibly due to the vegetation and lowest in upland soils due to the erosion and sparse vegetation. The BD was highest in the Valley, indicating more compacted soils, and the moisture content followed a similar trend like the BD due to the water retention. In case of texture, the sand content decreased down slope, while silt and clay increased, reflecting finer particle accumulation in lower elevations. The macronutrients (N, P, and K) showed slight variation as balanced nutrient levels, with potassium (K) peaking in the plain supporting crops like paddy and vegetables. Nitrogen and phosphorus level were the highest concentrations in the lower slope likely due to the moderate leaching and better retention. In the Pearson's r coefficient Analysis (Graph 2), the strong positive correlations were observed among BD, Moisture, Silt, Clay, and macronutrients (N, P). Sand showed strong negative correlations with these variables. OC was inversely related to BD and nutrient levels (NP).

Table 1

Average value of soil physico-chemical parameters on the basis of landforms

Soil Parameters	Upland	Plain	Valley	Observations
pH	4.94	5.40	5.79	Increasing trend from upland to valley
EC (dSm?¹)	0.048	0.086	0.084	Higher in plain and valley
OC (%)	0.44	0.51	0.42	Slight peak in plain
BD (g/cm³)	1.07	1.08	1.26	Highest in valley
Moisture (%)	11.37	9.80	13.67	Highest in valley
Sand (%)	83.67	77.00	73.75	Decreasing trend
Silt (%)	4.90	7.90	10.65	Increasing trend
Clay (%)	11.43	15.10	15.15	Higher in plain and valley
N (Kg/ha)	266.85	260.79	267.62	Relatively stable
P (Kg/ha)	12.62	13.38	14.81	Increasing trend
K (Kg/ha)	179.81	328.20	237.95	Peak in plain

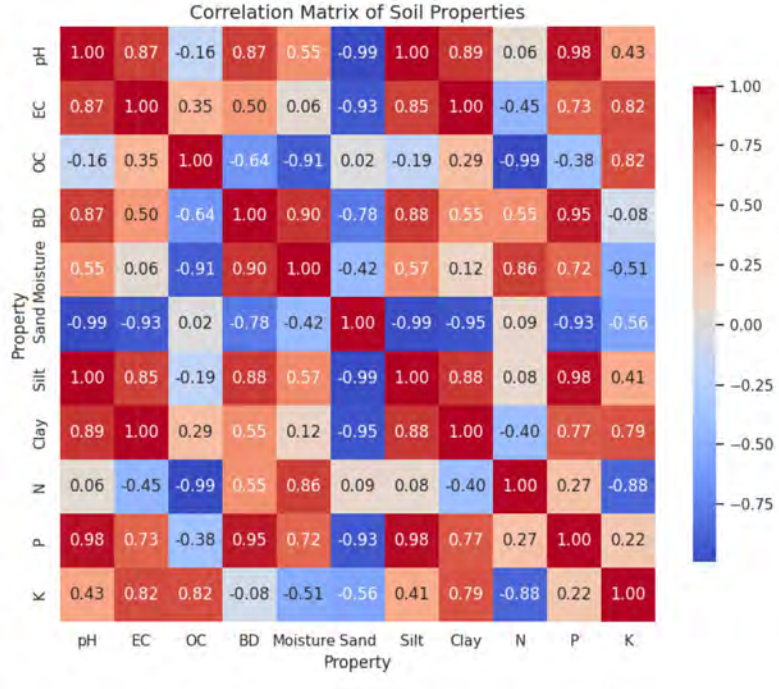

Figure 2: Correlation matrix heat map (Pearson's r coefficient)

4. Discussion

Human life on the planet "earth" depends upon its sustainability and of about 42% of total population in the world relies on agricultural production for their livelihood which derives the economic conditions of the developing countries (Jose *et el*., 2019). A greater understanding of sustainable land management is necessary for sustainable agriculture, because of the lack of proper planning of land use conversion process causes the natural resources loss and degradation of the local environment. To eradicating such problems the advanced technical concepts are needed regarding land use, use of pesticides, applying fertilizers, irrigation facilities and suitable selected crops.The data clearly shows that the topography governs soil fertility in Raighar Block. Upland soils, being more exposed and prone to erosion, exhibit lower nutrient content and higher acidity. This aligns with findings from similar top sequence studies in Odisha, where upland pedons showed reduced organic matter and nutrient retention (Priyadarshini and Dash, 2024; Dash *et al*., 2022). Plain soils, with moderate slope and better drainage, maintain balanced fertility and are suitable for a variety of crops. Valley soils, due to their low elevation and water retention capacity, accumulate nutrients and organic matter, making them ideal for intensive cropping systems (Dash *et al*., 2022). The downslope accumulation of finer particles and nutrients aligns with findings from Li *et al.* (2025) and Mathewos (2020), who reported similar patterns in tea plantations and Ethiopian watersheds. Increased BD and moisture in valleys suggest reduced aeration but enhanced nutrient retention. The inverse relationship between Sand and fertility indicators confirms that coarse-textured soils are less supportive of nutrient and water retention.The strong correlations between BD, moisture, and nutrient levels imply that soil structure and water availability are key drivers of fertility. The inverse relationship between Sand and other properties suggests that sandy soils are less fertile and retain less moisture.

These findings highlight the need for slope-specific soil management. Upland areas may benefit from organic amendments and erosion control, while valleys require attention to compaction and drainage.These results reinforce the importance of landform-specific soil management that the uplands arebest suited for drought-tolerant crops like millets and groundnut and Plains are Ideal for maize, pulses, and oilseeds, while the Valley regions are Suitable for paddy, leafy vegetables, and tuber crops.

5. Conclusion

Topographic variation significantly influences the soil physico-chemical characteristics. Upland soils are acidic and nutrient-poor, while Valley soils exhibit higher fertility potential due to increased moisture, finer texture, and nutrient accumulation. These findings highlight the importance of tailoring crop selection, land-use planning and soil management practices to landform characteristics. Future studies incorporating replicated sampling and statistical testing to validate these trends with GPS-based spatial analysis which can further refine these recommendations and support precision agriculture in the region.

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Applications of biostimulants in plant tissue culture: the recent studies, and future prospectives

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ABSTRACT

In vitro plant tissue culture represents a cornerstone of modern plant biotechnology, enabling large-scale clonal propagation, genetic modification, germplasm conservation and production of stress-resilient cultivars. Despite its wide utility, their dependence on synthetic plant growth regulators (PGRs) remains a critical limitation as their application often introduces somaclonal variation, limits reproducibility across genotypes and increases production costs. Emerging evidence indicates that biostimulants include natural extracts, and microbial products enriched with bioactive metabolites could provide an alternative or complementary strategy to conventional PGRs. These compounds not only modulate nutrient uptake, osmotic balance and stress responses in conventional cultivation systems but also harbor hormone-like molecules capable of influencing morphogenic pathways *in vitro*. Their integration into regeneration protocols offers potential advantages, including enhanced organogenic competence, improved rooting efficiency and greater acclimatization success. However, challenges such as compositional variability, lack of standardized formulations and limited mechanistic understanding constrain their adoption in micropropagation. Advances in multi-omics approaches and customized formulations offer new opportunities to clarify their action, and optimize applications. This review highlights the untapped potential of biostimulants as sustainable modulators of *in vitro* morphogenesis and outlines research priorities for their integration into scalable plant regeneration platforms.

1. Introduction

Plant tissue culture has become an indispensable tool for both fundamental research, and commercial horticultural practices, serving as the foundation for crop improvement, germplasm conservation and large-scale production of elite cultivars. It plays a pivotal role in micropropagation, genetic transformation, somatic embryogenesis and the conservation of endangered species, thereby supporting both scientific advancements, and practical applications in plant biotechnology. The efficiency of tissue culture protocols often varies across species, and genotypes. In vitro culture techniques, however, are highly dependent on the external supplementation of plant growth regulators (PGRs), which play a pivotal role in regulating morphogenesis, and developmental pathways. While these regulators are essential, their potential to induce physiological or epigenetic variations possess significant challenges. These drawbacks can compromise the reproducibility, scalability, and genetic fidelity of tissue culture-derived plants, highlighting the need for alternative or supplementary approaches that ensure both efficiency, and stability.

In recent years, biostimulants have emerged as promising alternatives or supplements to conventional PGRs in agriculture, and horticulture. Biostimulants are naturally derived compounds or beneficial micro organisms that, when applied to plants or their surrounding soil environment, promotes growth, development and resilience to stress, functioning independently of their nutrient contribution (U.S. Government, 2018). These natural or biologically derived products are known to enhance nutrient uptake, water-use efficiency and stress tolerance, thereby improving crop yield, and quality under greenhouse, and field conditions

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(Fiorentino *et al.,* 2025; Thakur *et al.,* 2025). Biostimulants are complex natural products that enhance plant growth, and morphogenesis primarily by stimulating endogenous physiological, and hormonal pathways, rather than functioning as defined plant growth regulators commonly used in tissue culture systems (Mateo-Cid *et al.,* 2025; Buttrós *et al.,* 2021). For example, humic acid has been shown to up-regulate auxin, and cytokinin biosynthesis genes in wheat and *Arabidopsis*, thereby stimulating endogenous hormone activities, while commercial seaweed extracts have been reported to modify cytokinin, and abscisic acid levels, and regulate the expression of hormone-related genes in *Arabidopsis*, illustrating that biostimulants act by indirectly modulating plant hormonal networks rather than functioning as direct plant growth regulators (Rathor *et al.,* 2024; Wally *et al.,* 2013). Despite their proven utility in conventional cultivation, such as in soil or hydroponic - based crop production systems under green house or field condition (Karaca, and Karagüzel, 2025; Buisset, and Scott, 2025), their potential role in plant tissue culture, and micropropagation remains underexplored. Integrating biostimulants into in vitro regeneration protocols may not only reduce reliance on costly synthetic regulators but also improve physiological, and genetic stability in regenerated plants. This review therefore aims to critically assess the role of biostimulants in plant tissue culture, with particular emphasis on their mechanisms of action, and potential applications in the production, and acclimatization of economically valuable plants.

2. Types of Biostimulants used in Micropropagation

The classification of biostimulants has advanced over decades, beginning with Filatov's (1951) four groups of biogenic stimulants. This was followed by further categorizations by Karnok (2000), and Du Jardin (2015), who developed a scientific framework of seven categories. Torre *et al.* (2016) later classified biostimulants into five categories. More recently, Zulfiqar *et al.* (2024) broadly divided them into two major groups: microbial, and non-microbial. The non-microbial biostimulants include plant, and seaweed extracts, humic substances, and protein hydrolysates, while microbial biostimulants comprise mycorrhizal, and non-mycorrhizal fungi, *Trichoderma* and plant growth-promoting rhizobacteria.

2.1. Seaweed extracts

Seaweeds, encompassing nearly 10,000 species across red, green, and brown groups (Khan *et al.,* 2009), are increasingly utilized in micropropagation due to their rich reservoir of bioactive metabolites. Brown algae such as *Ascophyllum nodosum, Ecklonia maxima* and *Durvillea potatorum* are widely exploited in commercial formulations (e.g., Kelpak®, Goëmar BM 86®, Algamino Plant®, Actiwave®) that mimics hormone-like activity, and stimulates plant morphogenesis (Francke *et al.,* 2022; Monder & Pacholczak, 2020). Seaweed-based formulations have demonstrated significant roles in stimulating shoot initiation, and multiplication. For example, *Ulva fasciata* powder (0.25%) enhanced shoot differentiation in *Sedum praealtum* (Mateo-Cid *et al.,* 2025), while *Sargassum tenerrimum* extract promoted shoot elongation, and chlorophyll accumulation in banana (Teraiya *et al.,* 2023). Similarly, strawberry micropropagation responded positively to seaweed-microalgae consortia that improved shoot proliferation, and root architecture (Chaouch *et al.,* 2023). Seaweed liquid extracts (SLEs) have proven effective in promoting robust rooting responses. *Sargassum ilicifolium* SLE significantly enhanced root induction in *Plumbago zeylanica*, outperforming traditional PGRs such as IBA, and GA_3 (Kakade *et al.,* 2022). Likewise, extracts from *Gracilaria edulis*, *Sargassum wightii* and *Gracilaria salicornia* accelerated rooting, and vegetative growth in tomato (Vinoth *et al.,* 2019; Satish *et al.,* 2015). For instance, the aqueous extract of *Kappaphycus alvarezii* demonstrated a positive effect, stimulating rooting, plantlet development, fresh mass gain,, and newly formed shoots of Epidendrum secundum plantlets, mainly at 50 mg L^{-1} concentration (De Araújo Amatuzzi *et al.,* 2020). Seaweed-derived metabolites also stimulate callogenesis, and organ differentiation. *Ascophyllum nodosum* extract improved callus proliferation, and phytochemical accumulation in *Rosmarinus officinalis* (Najim & Salim, 2025), while fucoidans isolated from *Turbinaria decurrens* enhanced germination, callus development, and organogenesis across several crops (Kaniyassery *et al.,* 2024). Beyond morphogenesis, algal extracts act as potent elicitors of phytochemicals, and antioxidants. In banana, *Sargassum* extract enhanced stress-related metabolite accumulation alongside growth promotion (Teraiya *et al.,* 2023). Similarly, *Ascophyllum nodosum*, and *Laminaria digitata* extracts exhibited species-specific, and dose-dependent modulation of secondary metabolite synthesis in red seaweeds (*Eucheuma denticulatum, Kappaphycus alvarezii*) (Borlongan *et al.,* 2023). Collectively, these studies emphasize that seaweed extracts serve as eco-friendly, and cost-effective biostimulants. By simultaneously supporting clonal propagation, improving rooting and shoot multiplication, and enhancing stress resilience, and phytochemical enrichment, marine algal extracts provide a sustainable alternative or supplement to synthetic plant growth regulators in micropropagation systems. Detailed data on the effect of different seaweed extract on the *in vitro* growth, and development of different plant species is provided in Table 1.

Table 1

The effect of different seaweed extract on the *in vitro* growth, and development of different plant species.

Plant Species	Seaweed used	Optimal Conc.	Response	Reference
Solanum melongena L	*Gracilaria salicornia, Padina gymnospora, Padina boergesenii,* and *Gelidiella acerosa*	20-40 % sea liquid extract	High rate of shoot induction (96.2 %), proliferation (6 cm), and rooting (95.3 %) was found	Satish *et al.,* 2015
Solanum lycopersicum	*Ulva lactuca Linnaeus,* and *Padina gymnospora*		Polysaccharide-enriched extracts (PEEs), enhanced shoot, and radicle length, and improved dry weight at specific concentrations, while higher extract doses showed inhibitory effects.	Hernández-Herrera *et al.,* 2016
Solanum lycopersicum	*Gracilaria edulis* (with 2iP)	30% + 1.2 mg/l 2iP	95.8% shoot elongation; 14.7 cm shoot length.	Vinoth *et al.,* 2019
E. secundum	*Kappaphycus alvarezii*	50 mg/l	Enhanced rooting, fresh mass, and shoot formation	De araújoamatuzzi *et al.,* 2020
Plumbago zeylanica	*Sargassum ilicifolium*	Ms + liquid extract of *Sargassum ilicifolium*	2% extract for germination, 4% extract for shoot proliferation, and 2.5% extract for rooting, and metabolite production, consistently outperforming GA_3, BAP, and IBA.	Kakade *et al.,* 2022
C. maculata	*Ulva lactuca, C. scalpelliformis, S. wightii*	10-30%	Shoot elongation up to 14.56 cm.	Anbazhakan *et al.,* 2022
C. maculata	*Sargassum wightii*	20%	Best rooting: 7.33 roots/shoot.	Anbazhakan *et al.,* 2022
Fragaria × *ananassa*	*Algal consortium*	Ms medium+ TEO + mix of algae (100 ppm)	Improved root length with number along with shoot proliferation.	Chaouch *et al.,* 2023
Eucheuma denticulatum	*Ascophyllum nodosum,* and *Laminaria digitata*	0.05 mL LDE L^{-1}, and 0.5 mL ANE L^{-1}	High-quality, high-yielding of species.	Borlongan *et al.,* 2023
Musa spp.	*Stylidium tenerrimum*	-	Enhance the shoot elongation and phenol content	Teraiya *et al.,* 2023
Eggplant and Finger millet	*Turbinaria decurrens*	0.1-1.0 mg/l	Enhanced seed germination, seedling growth, biomass accumulation, callus proliferation and direct organogenesis in both plant treatments	Kaniyassery et al., 2024
Sedum praealtum	*Ulva fasciata* powder	0.25%	Improved area, and perimeter; promoted development, and regeneration.	Mateo-Cid *et al.,* 2025
Rosmarinus officinalis L	*Ascophyllum nodosum*	30 mL L^{-1}+ 2,4 - D (2mg/l)	Influence both callus proliferation, and the accumulation of secondary metabolites	Najim and Salim, 2025

2.2 Humic substances:

Humic substances (HS), formed from decomposed plant, animal, and microbial matter, represent the largest terrestrial reservoir of organic carbon, and play essential roles in ecological regulation, and plant physiology. Industrially, HS are obtained mainly from low-rank coal (leonardite, lignite), peat, or cellulose by-products such as lignosulphonates (Klavins *et al.*, 2021). Functioning as natural biostimulants, HS particularly humic acid (HA) are known for their auxin-like activity, influencing hormonal signalling, assimilate distribution and root architecture. Numerous studies report enhanced root length, diameter, lateral branching and root hair initiation, confirming HA's broad physiological, and biochemical effects (Canellas *et al.*, 2012; Pizzeghello *et al.*, 2002; Nunes *et al.*, 2019). Applications in micropropagation highlights HS potential. In date palm (*Phoenix dactylifera* L. cv. Quntar), combining 2.5 mg/L HA with 75 mg/L ZnONPs improved callus induction, shoot bud formation, nutrient uptake and rooting efficiency (Al-Mayahi*et al.*, 2021). In *azalea* microshoots, HA influenced ROS, phenolics, carbohydrate metabolism,, and gene expression, strongly upregulating POD1 for antioxidant activity, while auxins activated IAA- and ARF-related genes, suggesting complementary regulatory roles (Elmongy *et al.*, 2020). Similarly, in banana cv. Prata-Anã, fulvic acid (40 mg/L) produced the greatest increase in shoot, and root growth, underscoring its growth-promoting capacity under tissue culture (Buttrós *et al.*, 2021). In strawberry cultivars 'Elsanta', and 'Senga Sengana', soil-derived HA (1–5 mg·dm{ ³) enhanced rooting, and biomass accumulation more effectively than auxins, indicating its role in plant vigor, and micropropagation success. Moreover, HS alleviate abiotic stress; for instance, 15 mg/L humic, and fulvic acids improved salt tolerance in strawberry plantlets by enhancing chlorophyll content, nutrient uptake and defense responses (de Oliveira *et al.*, 2024). Beyond micropropagation, HA supplementation (10 µM) in *Withania coagulans* increased biomass, antioxidant enzyme activity (POD, SOD) and non-enzymatic antioxidant responses (DPPH, ABTS, FRAP), demonstrating enhanced growth, and oxidative stress tolerance (Khanum *et al.*, 2025). Likewise, in cotton (*Gossypium*), HA (0.1–1%) boosted seed germination, callus induction and biomass while conferring resistance against *Helicoverpa armigera*, with treated plants causing 60% larval mortality (Ghaffor *et al.*, 2025). Collectively, these findings confirm that humic, and fulvic acids act as potent natural biostimulants, not only promoting root, and shoot development but also enhancing stress resilience, and defense in diverse plant species, thereby holding significant promise for sustainable micropropagation systems. A detailed data on effect of humic acids on the *invitro* growth, and development of different plant species is provided in Table 2.

Table 2

The effect of humic substances on the *in vitro* growth, and development of different plant species.

Plant Species	Substance(s) Used	Observed Effect	References
Lilium Oriental	0.2, 2.0,, and 20.0 mg/L	A low HA concentration at 0.2 mg/L performed best in both root, and bulblet growth.	Wu *et al.*, 2016
Bacopa monnieri	1 ml/L humin + 9 g/l of agar	Humin alone significantly enhanced leaf development, explant survival, and plantlet weight in *Bacopa monnieri* compared to supplemented media.	Kashyap *et al.*, 2017
Zea mays	HA	Improved root traits (length, diameter, branching, root hair).	Nunes *et al.*, 2019
Azalea	HA, and auxins	Altered ROS, phenolic content, gene expression; enhanced POD1 gene by HA.	Elmongy *et al.*, 2020
Phoenix dactylifera (cv. Quntar)	2.5 mg/L HA + 75 mg/L Zn O nanoparticles	Enhanced callus induction, shoot bud formation, nutrient accumulation, rooting.	Al-Mayahi, 2021
Banana (cv. Prata-Anã)	2-40 mg/L HA, and Fulvic acids	40 mg/L fulvic acid gave max shoot height, and root length.	Buttrós *et al.*, 2021

Fragaria × *ananassa*	15 mg/L humic, and fulvic acids	salt-tolerant strawberry seedlings	de Oliveira *et al.,* 2024
Gossypium hirsutum	MS + auxin, and cytokinin+ humic acid (0.1%, 0.5%,, and 1%).	Enhanced callus induction, seedling growth, along with that 60% larval death of cotton bollworm in humic acid-treated plants indicating improved resistance.	Ghaffor *et al.,* 2025

2.3 Hydrolysed proteins and amino acids

Protein-based products can be divided into two major categories: protein hydrolysates, and individual amino acids. Protein hydrolysates (PHs) are biostimulants composed mainly of amino acids, and peptides, formed through partial hydrolysis of proteins from animal or plant sources. Animal-based PHs, often derived from by-products like leather, feathers, or fish waste, are typically processed via chemical hydrolysis, while plant-based PHs, from legumes or vegetable residues, are produced enzymatically. The composition, and bioactivity of PHs vary depending on the source, and hydrolysis method, with amino acids both free, and peptide-bound. Protein hydrolysates contain not only amino acids, and peptides but also bioactive compounds that enhance plant growth. Triacontanol, and IAA were also found in alfaalfa, and meat-based hydrolysates. Casein hydrolysate effectively supports tobacco growth as a nitrogen source, unlike casein protein, which is less efficient. Both forms significantly influenced auxin (phenylacetic acid), and cytokinin levels, indicating root system adaptation to nitrogen availability. Tobacco roots supplied with casein showed elevated free amino acid levels, while those grown without nitrogen had undetectable amounts, highlighting the role of casein-derived nitrogen in amino acid accumulation, and root development (Belonzníková *et al*., 2023). Al-Asadi *et al.* (2024) investigated the effects of dicamba (DIC), and casein hydrolysate (CH) on *in vitro* callus growth, shoot multiplication, and biochemical traits in date palm cv. Barhee. Their results demonstrated that both DIC, and CH were critical for successful regeneration. The highest callus biomass (287 mg) was obtained with 4.0 mg/L DIC combined with 1.0 g/L CH, whereas the greatest shoot response (86.67%), and shoot number per jar (15.07) were achieved with 4.0 mg/L DIC, and 0.5 g/L CH. Furthermore, shoots cultured on this optimized medium exhibited significantly elevated levels of potassium, phosphorus, calcium, and free amino acids, indicating enhanced nutritional, and biochemical status under the combined treatment. Munawar *et al.* (2024) demonstrated that supplementing MS medium with 2,4-D, and casein hydrolysate markedly improved callogenesis across several cultivars, including KSK-133, KSK-434, KS-282, PK-386, IR-6, and Super Basmati. Optimal callus formation was observed with 2 mg/L 2,4-D combined with 4 mg/L casein hydrolysate, highlighting a synergistic effect. These findings underscore the potential of combining hormonal signals with organic supplements to improve regeneration efficiency, a strategy that may be applicable across diverse rice genotypes in micropropagation protocols. Although these studies demonstrate strong beneficial effects of PHs in *in vitro*, and seedling models, direct applications in micropropagation protocols remain limited. Most research has focused on greenhouse or field settings. Future studies aimed at addressing this gap could focus on: 1) Testing PHs in explant initiation, callus induction, regeneration and rooting phases. 2) Monitoring hormone-responsive gene expression during tissue culture. 3) Designing tissue culture trials comparing traditional PGR-only media with media supplemented with PHs. While plant, and animal-derived protein hydrolysates have shown clear auxin- and gibberellin-like effects enhancing rooting, shoot biomass and gene expression in seedlings, and cuttings research on their application in micropropagation systems remains limited, presenting a promising area for future investigation.

2.4 Microbial inoculant:

In recent years, beneficial microorganisms have gained importance as biostimulants in sustainable agriculture, and horticulture. Unlike fertilizers, they do not directly supply nutrients but enhance uptake, produce phytohormones, modulate stress responses,, and stimulates root, and shoot growth. A wide range of rhizobacteria synthesize auxins, and cytokinins via diverse pathways. Over 80% of rhizospheric soil bacteria produce auxins, especially indole-3-acetic acid (IAA), the most active form in plants (Amara *et al.*, 2015; Souza *et al.*, 2015). Traditionally used in soil or greenhouse systems, plant growth-promoting rhizobacteria (PGPR), endophytic bacteria,, and mycorrhizal fungi are now being explored for *in vitro* plant tissue culture. Although sterility presents challenges, carefully selected strains have shown compatibility in promoting callus induction, shoot regeneration and rooting, thereby reducing reliance on synthetic PGRs. The use of microbial biostimulants in plant

tissue culture has shown promising outcomes for enhancing *in vitro* regeneration. For instance, inoculation of garlic meristems with beneficial strains (*Enterobacter cloacae, Burkholderia cepacian*) increased biomass, and chlorophyll content (Costa Júnior *et al.*, 2020), while *Azospirillum brasilense* promoted significant shoot, and root growth in potato microplants (Kargapolova *et al.*, 2020). Similarly, endophytes in *Bromus auleticus* enhanced callus formation, regeneration,, and seedling vigor (Regalado *et al.*, 2018). In banana, *Bacillus subtilis*, and *Pseudomonas fluorescens* reduced *Fusarium wilt,* and doubled biomass during *in vitro* rooting (Kavino, and Manoranjitham, 2018). Beyond live inoculants, microbial metabolites like auxins from *Pantoea agglomerans* strain C1 improved rooting efficiency in pear microcuttings compared to synthetic IBA (Luziatelli *et al.*, 2020). Moreover, microbes producing ACC deaminase alleviate stress by lowering ethylene levels, supporting root development under salinity or other stress conditions (Heydarian *et al.*, 2016). Together, these findings underscore that microbial inoculants, and their metabolites can serve as natural, eco-friendly alternatives to synthetic growth regulators, improving both efficiency, and resilience in micropropagation systems. Detailed data on the effect of microbial inoculant on the *invitro* growth, and development of different plant speciesis provided in Table 3.

Table 3

The effect of microbial inoculant on the *in vitro* growth, and development of different plant species.

Plant Species	Microbial Agent	Key Effects	References
Camelina sativa	*Pseudomonas putida* UW4	Improved growth, and salt tolerance.	Heydarian *et al.*, 2016
Bromus auleticus	*Epichloe endophyte* (E^+)	Increase germination, callus induction, regeneration, biomass (*in vitro* & *ex vitro*)	Regalado *et al.*, 2018
Musa spp. (Banana)	*Bacillus subtilis* (EPB56, EPB10), *Pseudomonas fluorescens* (Pf1)	Decrease *Fusarium wilt,* Increase growth even in low nutrient media.	Kavino and Manoranjitham, 2018
Solanum tuberosum (Potato)	*Ochrobactrum cytisi* IPA7.2	Increase Shoot/root growth, leaf area, biomass.	Burygin *et al.*, 2018
Solanum tuberosum (Potato)	*A. brasilense* strains Sp245, and SR80	Increase Shoot length, and root length of microshoot.	Kargapolova *et al*, 2020

3. Challenges and Future Perspectives

Despite their potential, integrating biostimulants into micropropagation faces key challenges. Natural biostimulants often lack standardization, with variable compositions influenced by source, and processing methods, leading to inconsistent results (Yakhin *et al.*, 2017). Their molecular mechanisms remain poorly understood, limiting targeted application (Calvo *et al.*, 2014). Regulatory oversight is minimal, raising concerns over quality, and efficacy. Moreover, most research focuses on soil-based systems, with limited data on tissue culture contexts, highlighting the need for systematic studies. Looking ahead, applying multi-omics approaches (e.g., transcriptomics, proteomics, metabolomics) can clarify biostimulant action, and refine their uses. Customized formulations could support specific micropropagation stages like callus induction, shoot multiplication, or rooting. Synthetic biology may also enable the design of microbial systems producing optimized bioactive compounds. Additionally, integrating biostimulants into automated, high-throughout tissue culture systems can enhance consistency, and scalability, driving innovation in plant biotechnology.

4. Conclusion

Biostimulants represent a promising frontier in the advancement of micropropagation technologies. Their natural origin, multifaceted benefits and compatibility with sustainable agricultural practices make them attractive alternatives or complements to synthetic PGRs. By enhancing shoot proliferation, rooting and stress resilience, biostimulants can help optimize *in vitro* propagation protocols for a wide range of crops. However, for biostimulants to be fully integrated into commercial tissue culture systems, further research is essential. Studies exploring formulation standardization, dosage optimization and mechanistic insights will be critical. With growing

interest, and technological advancements, biostimulants are poised to play a vital role in the future of plant biotechnology, and clonal propagation.

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Effect of Pb on growth & biochemical properties of hyperaccumulator plant, *Vigna mungo*

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ABSTRACT

Heavy metals like Cr, Pb, Co, Cd etcaffect plant growth and metabolism when present at a higher concentration than required. Lead (Pb) is a global challenge in terms of agricultural soil contamination. Phytoremediation of heavy metals is an important method among various methods available for removing soil contaminants like Pb. In phytoremediation a hyperaccumulator plant is used that can accumulate heavy metals in such concentrations which may be many times greater than normal requirements. The present study was carried out to find the effects of Pb on *Vigna mungo* a hyperaccumulator plant. In this study, *Vigna mungo* plants were exposed to different Pb concentrations that ranged from 50 to 1000mg/kg of soil. Reduction in biomass, chlorophyll content and increase in carotene content was observed with increase in Pb concentration. Reduction in terms of plant growth parameters such as plant height, root length, shoot length, plant biomass (dry weight and fresh weight) was also observed. Lipid peroxidation products in leaves of treated plants has increased with high concentration of Pb indicating the membrane damage.

1. Introduction

Presence of toxic trace metals in soil and their uptake into the plant have damaging effect on plant growth and productivity (Clemens & Ma, 2016; Emamverdian *et al.*, 2015). Such elements at higher concentration alter the rate of plants physiological processes such as photosynthesis, water absorption, protein synthesis, and nutrient uptake (Wani *et al.*, 2012; Kamal and Xavier., 2023; Chukwu and Gulser., 2025). Such alterations in plant physiology causes oxidative stress via formation of free radicals (Abd Elgawad *et al.*, 2020; Niekerk 2024).

There are physical, chemical and biological methods available for decontaminating the soil (Enva, 2025). Physical and chemical methods damage the soil properties, are expensive and need high labor hence these are not preferred. On the other hand, biological process is economical and reestablishes soil fertility. In biological method, plants which can easily uptake high concentration of toxic heavy metals are used (Zhang *et al.*, 2022). These plants absorb toxic elements in a rate much higher than other plants and distribute them in different tissues and organs of the plant such as root, stem, leaf and flower (Bhat *et al.*, 2025). The plants are able to mine metals from soils with very high concentration. Phytominingis carried out by growing such plantsin metal contaminated areas, then metals in their tissues are harvested (Akinbile *et al.*, 2025). Therefore, the ability of plants to extract metals from the contaminated soils are being studied by researchers around the globe.

Rapid industrialization and urbanization led to contamination of soil with heavy metalswhere, soil function as sinks for metal contaminants (Alloway, 2013). These contaminants enter the food chain after their assimilation into the plant tissues and in due course they make their way to humans (Zhang *et al.*, 2024). These contaminants cause various diseases in human. Hence, remediation of contaminated soil is necessary specially the agricultural soil.

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Through phytoremediation, both heavy metals as well as organic pollutants can be removed from the soil. Phytoremediation does not affect soil fertility, and arecost effective, hence is widely accepted by the farmers (Ali *et al.*, 2013; Fiorentino *et al.*, 2018; Oubohssaine and Dahmani, 2024).

Heavy metals when present in higher concentration interferes in seed germination process. Saravanan. *et al.*, (2019) have reported decrease in growth of plant (weight and length) with increase in the concentration of the Cr (VI) ion and assigned it to effect of chromium on the seed germination. Damaged chlorophyll, declination in photosynthesis, reduced biomass, amendments in water balance, nutrient assimilation and programed cell death are the impacts of heavy metals (Singh *et al.*, 2016). Several trace elements affect the biochemical process of plants differently. Some might hamper the growth while some the germination rate and some may show their effects in metabolic and physiological process of plant while some can cause all these (Miller, 2023). Chlorosis, poor root and stem growth are the symptoms of heavy metal toxicity (Miller, 2023). While high doses of heavy metals can cause death to several plant species (Ali and Gill, 2022).

Mechanism for metal tolerance in hyperaccumulator plants include reduction in rate of uptake of heavy metals and paucity in metal transport across cell membrane. Plants may also alter the toxicity of the concerned heavy metal so that the metal would not affect the natural processes of plant. Heavy metal induced oxidative stress in quashed by antioxidant defense system which provides heavy metal tolerance to plants (Mansoor *et al.*, 2023). Plants have the ability to synthesize the antioxidants to scavenge heavy metal stress induced harmful free radicals (Mansoor *et al.*, 2023) which is the best strategy for antioxidants mediated tolerance in plants. Antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) provide defense against oxidative stress in plants (Sharma *et al.*, 2022). Therefore, in the present study, there is an attempt made to evaluate the response of plant black gram (*Vigna mungo*) towards various concentrations of heavy metal Lead (Pb).

2. Materials and Methods

2.1. Soil Preparation:

Surface soil (upto 1 ft depth) was collected from an remotely located rural area with no known source of heavy metals (like vehicular movement, use of fertilizers etc). The collected soil was air-dried at room temperature, gently crushed, and passed through a 80 µm sieve to remove debris, stones, and plant residues. The pH of the soil was measured in triplicate using a pH meter prior to experiment. The sieved soil was sterilized by autoclaving it for 20 minutes. The soil was then allowed to equilibrate for two days under ambient laboratory conditions before lead treatment.

To study the effects of heavy metal on plant *Vigna mungo* different concentrations of Lead nitrate were applied. The different concentrations of recommended dose as well as control (no heavy metal) were freshly prepared with distilled water and calibrated thoroughly with the soil. The solution was made at a concentration of 10ppm (by mixing 10gm of Lead Nitrate with 1 litre distil water) and then diluted with distilled water to obtain required doses. Different doses from 0 (for control) to 1000mg/kg were then applied to 8 different pots. Each pot was filled with 5kg of soil. The lead solution was added gradually to the soil and mixed thoroughly to ensure uniform distribution. Control soil received an equal volume of distilled water without lead. The duration of exposure was 7 days under ambient condition. The average daily temperature was 30-35^{0}C.

2.2. Germination percentage:

Seven days after the transfer of seeds to the treated pots and germination percentage was calculated by using following equation (Talebi *et al.*, 2014).

Germination percentage (%) =(Total No. of seeds germinated / Total No. of seeds sown) × 100

2.3. Morphological and Biomass Analysis:

After 7 days of Pb treatment root length, shoot length significantly inhibit.

2.4. Photosynthetic pigment assay:

Photosynthetic pigment from heavy metal treated leaves and control leaves were extracted with 80% aqueous acetone. Then it was homogenized. After grinding, the mixture was centrifuged at 5000rpm for 15 minute and absorbance was determined spectrophotometrically (Biochrom Libra S22) at 663 nm and 645 nm against blank. Chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll contents (mg g^{-1} FW) were calculated following Arnon., 1949.

Chlorophyll a = (12.7 × OD663) - (2.69 × OD645) × V/ 1000 × 1 wt mg/g FW

Chlorophyll b = (29.9 × OD645) - (4.68 × OD663) × V/ 1000 × 1 wt mg/g FW

Total Chlorophyll = (20.7 × OD645) - (8.02 × OD663) × V /1000 × 1 wt mg/g FW

2.5. Lipid peroxidation assay:

Lipid peroxidation as say was carried out using spectrophotometric method (Heath and Packer, 1968). Leaf tissues of both control and treated plants were homogenized separately in 0.1% of TCA. Then the extracts were centrifuged at 15000 rpm for 15 min and the supernatants were collected. Supernatant was mixed with 20% TCA and 0.5% TBA in the ratio of 1:2:2 respectively. Then the mixture was heated in a boiling water bath at 950C for 15 minutes followed by immediate cooling and centrifugation at 10000 rpm for 10 minutes. The supernatant was collected and absorbance of the supernatant at 532nm and 600nm was recorded. The concentration of MDA was calculated using Beer-Lambert's equation.

3. Results and Discussion

3.1. Soil pH:

pH of the uncontaminated soil was in the range of 6.5-7.0. After addition of Pb into the soil the pH varied. Negligible change in pH was observed for Pb treatment upto 600 mg/kg of soil, however pH decreased to 6 for Pb treatment 800 mg/kg of soil and 1000 mg/kg of soil. This decrease in pH may be attributed to release of H^+ ions during Pb salt dissolution (Zhang and Van Gestel., 2017).

3.2. Germination percentage:

Seven days after sowing of seeds the germination rate was measured. The maximum germination (95%) was observed in control. Germination percentage of seeds declined with increase in the concentration of Pb.

3.3. Morphological Analysis:

The average plant length varied between 14.3 to 23.3 cm and the root length varied between 1.2 to 3.7 cm. Maximum root length (3.7 cm) and plant length (23.3) was observed in control plants and on the other hand, root length and plant length reduced with increase in levels of Pb exposure. Minimum root length (1.2 cm) and plant length (14.3 cm) was observed in plants grown under 1000mg/kg Pb in soil. Pb at higher concentration affects plant growth and also amends physiological processes (Emamverdian *et al.,* 2021; Singh *et al.,* 2023). The decrease of plant height with increase in Pb concentration may be ascribed to decrease in mitotic frequency and accumulation of Pb in cell wall components such as pectic substances & hemicelluloses.

Fig 3.1 Experimental set up

Fig 3.2 Seed germination

3.4 Biomass Assay:

The fresh and dry weight of 15 days older *Vigna mungo* seedlings revealed that treatment of Pb has affected the plant biomass immensely (Fig. 3.5 and 3.6). The minimum fresh weight (0.1212 gm) and dry weight (0.0011 gm) were observed at the concentration of 1000mg/kg of Pb, while maximum fresh weight (0.3906 gm) and dry weight (0.0034 gm) were observed in the control plants. Pb affected dry weight of seedlings manifested via poor growth of root and shoot. Such poor growth may have occurred due to reduction in cell division, cell differentiation and cell elongation in response to increased concentration of Pb (Kastori *et al.,* 1993). The decrease biomass of plants may be assigned to reduction in photosynthates (Joshi *et al.* 1999). Similar reductions in growth parameters and biomass under Pb stress have been consistently reported across different plant species, confirming that Pb toxicity adversely affects plant growth and productivity in a concentration-dependent manner (Emamverdian *et al.,* 2015; Singh *et al.,* 2023).

3.5 Chlorophyll and Carotenoid content

The total chlorophyll content was found to be maximum in the control plants and it was significantly

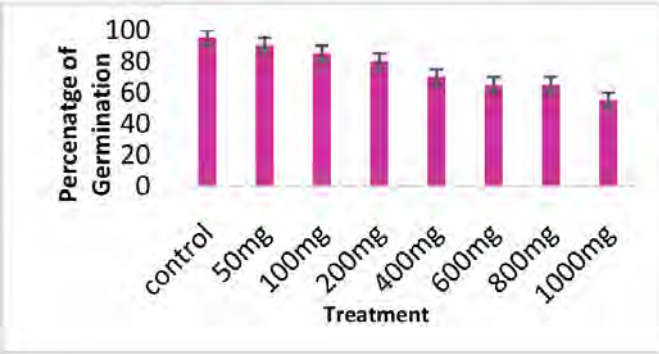

Fig. 3.3. Effect of Pb on germination

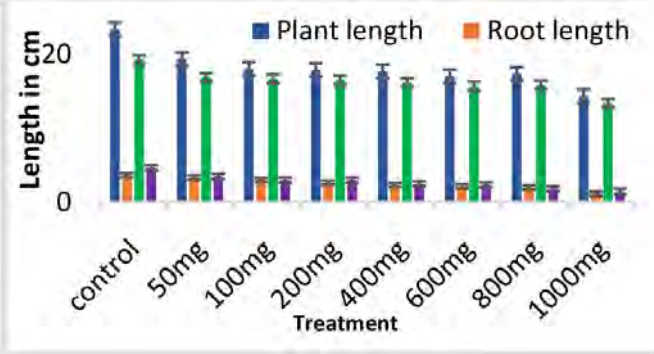

Fig 3.4 Effect of Pb on Plant Growth Parameter

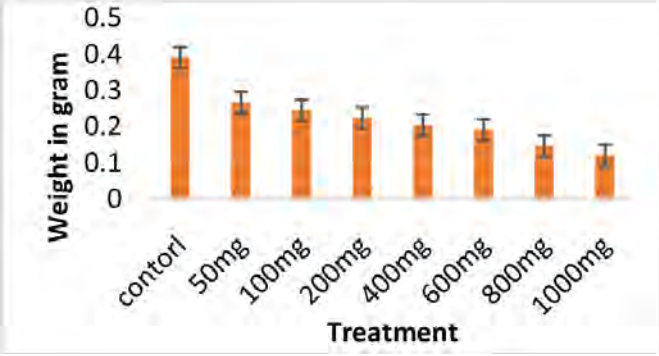

Fig. 3.5. Effect of Pb on fresh weight of plant

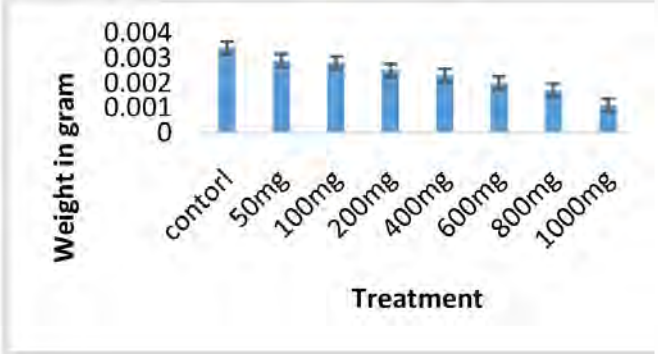

Fig. 3.6. Effect of Pb on dry weight of plant

decreased with increase in the concentration of Pb (Fig. 3.7). Minimum chlorophyll content was found in plants exposed to highest concentration of Pb (1000mg/kg) (Fig. 3.7). Reduction in chlorophyll biosynthesis may be attributed to inhibition in the activity of amino levulinic acid dehydrogenase and proto chlorophyllide reductase due to heavy metal stress. The carotenoid content was however elevated with increase in concentration of Pb and maximum carotene content was recorded in plants exposed to highest concentration of Pb (1000mg/kg) while the control plants showed least amount of carotene in their leaves (Fig. 3.8). Declination in chlorophyll a, chlorophyll b, and total chlorophyll have been consistently reported in plants grown in Pb-contaminated soils, often in a concentration-dependent manner (Baek *et al.*, 2012; Emamverdian *et al.*, 2021; Singh *et al.*, 2023). The carotene amount present in leaf of control plants was 4 times less than that of plants with the highest concentration of Pb. Carotene is naturally a stress scavenging molecule due to which the plants at high stress condition produces more carotene than in control plants. In response to heavy metal stress, plants often exhibit an increase in carotenoid pigments, which function as non-enzymatic antioxidants capable of scavenging ROS and mitigating oxidative damage (Baek *et al.*, 2012). Carotenoids

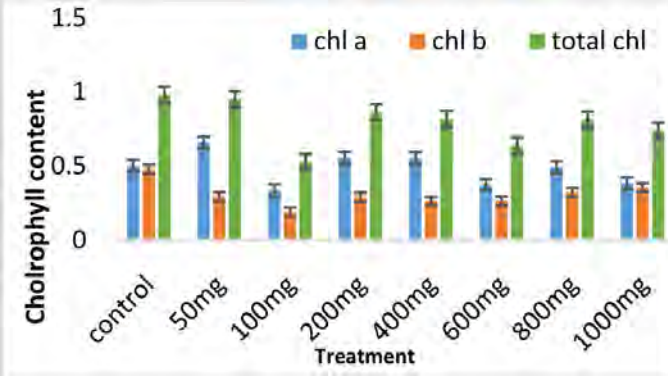

Fig. 3.7 Effect of Pb on chlorophyll content

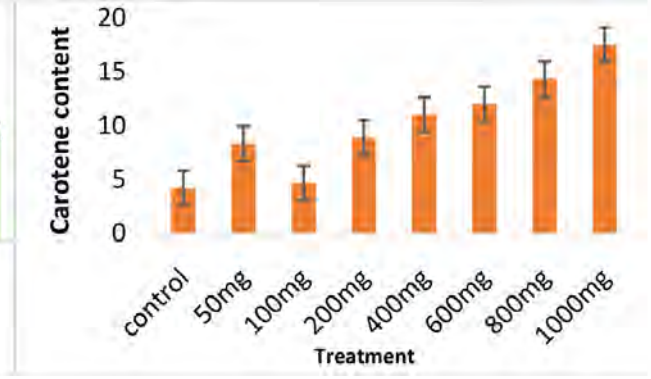

Fig. 3.8 Effect of Pb on Carotenoid content

play a crucial photoprotective role by stabilizing chlorophyll molecules and preventing photooxidative damage to the photosynthetic apparatus, particularly under stress caused by non-essential metals such as lead. Thus, enhanced carotenoid accumulation represents an important defense strategy that helps to maintain photosystem integrity under heavy metal-induced stress conditions. Our results also showed very little effect of Pb on the chlorophyll content.

3.6 Lipid peroxidation assay:

MDA (Malondialdehyde) content a product of lipid peroxidation is a bio-marker of oxidative stress and redox signaling. MDA content is widely used as an indicator of membrane damage in plants. The MDA content in the leaf tissues of control plants was found to be minimum and it was maximum at highest concentration of Lead i.e., 1000mg/kg as shown in (Fig. 3.9). This indicated that at more stress condition the plant produces more amount of MDA. Though MDA symbolizes damage in plants, but it also leads to activation of plant defense regulatory genes. Elevated MDA concentration may indicate stress acclimation processes rather than damage via regulation of defense gene expression. Similar to results of the present study, recent studies have consistently reported a concentration-dependent increase in MDA content in plants exposed to Pb, confirming that lipid peroxidation is a key physiological response to non-essential metal stress (Emamverdian *et al.*, 2015; Singh *et al.*, 2023).

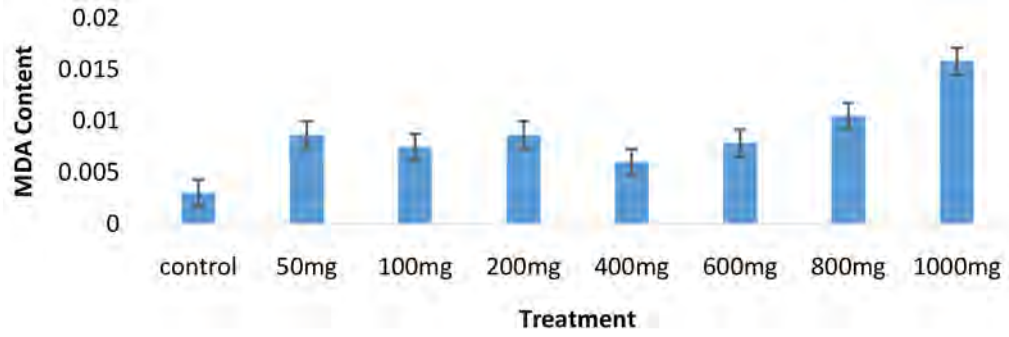

Fig. 3.9. Effect of Pb on MDA content

4. Conclusion

The heavy metal used in the present study (Pb) exhibited a negative impact on growth and physiology of *Vigna mungo* plant. Stress induced by Pb caused a series of systemic alternation in growth, biomass and photosynthetic pigments of the plant. Moreover, increased MDA concentration indicated the membrane damage which may consequently result in oxidative stress on plant. Such observation warrants further investigation in terms of soil metal bioavailability, uptake and transport into plant parts from the soil. Also stress indicators such as the proline content, ROS generation, etc. are needed to be investigated.

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Response of antioxidant enzymes and physiochemical changes during bioaccumulation of nickel in *Tagetes erecta* L. - a potential plant for phytoremediation

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ABSTRACT

The decorative plant known as African marigold (*Tagetes erecta* L.) is rich in phytochemicals with potential applications in industry and medicine. The tolerance of *T. erecta* to nickel (Ni) stress and its possible use in the rehabilitation of Ni-contaminated over burden soils were assessed in this study. Under regulated pot conditions, plants were exposed to graded doses of Ni (25-200 mg $NiCl_2$ kg^{-1} soil) for 60 days. Growth metrics, biochemical characteristics, growth and stress tolerance indicators, and antioxidant enzyme activities, such as catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX), were used to evaluate metal-induced stress responses. Concentration-dependent increases in nickel accumulation were seen in both roots and shoots. Increased proline levels and increased antioxidant enzyme activity suggested that Ni-induced oxidative stress had been effectively mitigated. In contrast to the control, plant growth and tolerance indices decreased with greater Ni concentrations. All things considered, the results show that *T. erecta* is a viable option for phytoremediation of Ni-contaminated soils, with the added benefit of enhancing landscape aesthetics.

1. Introduction

Nickel (Ni) is the most prevalent element on Earth, ranking 24th in terms of abundance with an approximate concentration of 0.008% (Anum *et al.,* 2019). Furthermore, substantial amounts of nickel are released into the environment when fossil fuels, liquid fuels, and petroleum derivatives are burned. Additionally, due to its resistance to corrosion and high temperatures, nickel is frequently employed in the metallurgical industry, particularly in the manufacture of stainless steel. It also stimulates the food industry (Bosiacki and Wojciechowska, 2012). The extensive use of nickel and similar compounds in industry has led to a rise in metal contamination (Denkhaus and Salnikow, 2002), which is detrimental to agricultural soil (Jamil *et al.,* 2014).

Ni is engaged in nitrogen metabolism, is regarded as a necessary nutrient for plants, and is a co-factor for enzyme activities, such as urease (Baccouch *et al.,* 1998). However, an excess of Ni can be detrimental to plants, causing growth inhibition, disruption of mineral nutrition, interference with sugar transport, suppression of photosynthesis, and imbalance in water relations (Amari *et al.,* 2014). After outgrowing untreated control plants at lower Ni concentrations, *Macrotyloma uniflorum's* protein content, root and shoot length, and production of antioxidant enzymes (SOD, CAT, and GPX) all decreased as a result of the increased Ni levels in the soil (Sahoo *et al.,* 2016). When exposed to up to 200 mmol of Ni concentration in hydroponic cultures, Tagetes erecta has been demonstrated to dramatically increase in dry weight, root length, proline content, and antioxidant activity; however, such Ni-exposures had a negative impact on the plantlets' shoot length, moist weight, and chlorophyll content (Bhurat *et al.,* 2017). Ni exposure has been shown to cause spindle abnormalities in *Allium cepa* roots, including multipolarity, sticky bridges in anaphase, early separations, chromosome

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clumping, and direct chromosomal damage, including chromosome breakage, erosion, and laggard chromosomes (Gantayat *et al.,* 2018). Ni-induced oxidative damage has led to increased cell abnormality index and proline content, as well as significant reductions in root length, mitotic index, total protein levels, and catalase (CAT) activity. It's interesting to note, nevertheless, that the stressed roots were shown to have reduced oxidative stress by increasing the tissue's guaiacol peroxidase (GPX) activity (Gantayat *et al.,* 2018).

African marigold or *Tagetes erecta*, is a widely prized ornamental plant in the Asteraceae family. The plant has a short life span, thick fibrous root architecture, quick propagation, and accelerated growth (Goswami and Das 2017). According to Madanan *et al.* (2021), *T. erecta* has shown a remarkable ability to accumulate heavy metals as Cr, Zn, Cd, Ni and Pb (Pradhan *et al.,* 2023). These characteristics led to the species' selection in the current study as a viable option for Ni phytoremediation. While several studies have shown how enzymatic and non-enzymatic antioxidant mechanisms scavenge reactive oxygen species (ROS) under heavy metal stress in a variety of plant species, nothing is known about how *T. erecta* responds to Ni stress. Moreover, no research has yet been done on how Ni buildup affects growth tolerance parameters in this species. In this study, we show that *T. erecta:* (i) Ni bioaccumulation in roots and shoots; (ii) Ni phytoextraction efficiency; (iii) Ni-induced changes in growth, biochemical components, and antioxidant enzyme activities; and (iv) tolerance capability in Ni-contaminated soil.

2. Materials and methods

2.1. Culture conditions and morphological study

We bought certified *Tagetes erecta* (var. Marvel, orange blossom) seeds from Indo American Hybrid Seeds (IAHS). To find out how Ni affected *T. erecta* growth, biochemical compound concentrations, antioxidant enzyme activities and tolerance, pot culture experiments were conducted in the Department of Botany's greenhouse at Utkal University in Odisha. Earthenware containers with a diameter of 30 cm were used to first germinate *Tagetes erecta* seeds. Four uniform and healthy seedlings were moved into comparable earthenware pots (30 cm in diameter) with a drainage hole at the bottom after 20 days. To stop nickel from leaching during irrigation, each pot was filled with 6 kg of well-homogenized soil and set on collection trays. The soil was amended with different amounts of nickel (25, 50, 100, 150, and 200 mg $NiCl_2$ kg^{-1} soil) ten days after implantation. The pots were kept in a greenhouse with frequent watering, and they were set up in a randomized block configuration. Every treatment was carried out in triplicate, and the results are shown as the average of three separate replicates. On the sixty-first day after Ni exposure, the plants were harvested.

2.2 Biochemical and antioxidative enzyme assay

After leaf tissue was extracted in 80% (v/v) acetone, the amount of chlorophyll was measured (Arnon, 1949). According to Bates et al. (1973), proline concentration was measured using 0.5 g of fresh leaf material extracted in 10 mL of 3% sulfosalicylic acid and centrifuged at 3000 rpm for 10 min. After hydrolyzing 100 mg of leaf tissue in 5 mL of 2.5 NHCl in a hot water bath for three hours, the supernatant was used to determine the total carbohydrate content (Somogyi, 1945). 0.5 g of fresh leaf tissue and 1.5 mL of 0.1 M sodium phosphate-EDTA buffer (pH 7.4) were homogenized in a cold mortar and pestle for antioxidant enzyme tests. To avoid proteolysis and phenolic interference, 10% (w/v) polyvinylpyrrolidone and phenylmethylsulfonyl fluoride were added before homogenization. The cleared supernatant was kept at -20°C until further examination after the homogenate was centrifuged at 14,000 rpm for 15 minutes at 4°C. By tracking the breakdown of H_2O_2, catalase (CAT) activity was measured (Aebi, 1984). The hydrogen peroxide-dependent oxidation of ascorbic acid was measured spectrophotometrically to estimate ascorbate peroxidase (APX) activity (Nakano and Asada, 1981). Using guaiacol as a substrate, the activity of guaiacol peroxidase (GPX) was measured at 25°C (Bergmeyer, 1974). Lowry's technique was used to measure the total soluble protein content (Lowry *et al.,* 1951).

2.3. Ni content analysis

Roots and shoots were collected at the end of the experiment, properly cleaned, and oven-dried for 48 hours at 65°C. Following the procedure outlined by Davies *et al.* (2002), the dried plant material was coarsely powdered and aliquots of 100 mg were acid digested using concentrated HNO_3 and H_2O_2. After the digests were filtered, Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES; Optima DV, Perkin Elmer Syngistix, USA) was used to measure the Ni contents.

2.4. Phytoextraction efficiency and tolerance indices

Because it does not take into consideration the availability of metals in the soil matrix, quantifying the concentrations of heavy metals in plant tissues is insufficient to appropriately assess the effectiveness of phytoextraction. The Bio-concentration Factor (BCF), Total Accumulation Rate (TAR), and Transportation Index (Ti) were thus computed using metal accumulation data in accordance with

the formulas suggested by Zurayk *et al.* (2002) and Ghosh and Singh (2005). Furthermore, the Translocation Factor (TF) was calculated using Chen et al.'s technique (2021). Equations developed by Wilkins (1957) were used to calculate stress tolerance indices related to different growth factors.

2.5 Statistical analysis

All treatments were carried out in triplicates and the data were presented as mean ± SE (Standard error of mean). All data were analyzed by analysis of variance (ANOVA) to check the variability and validity of results. Duncan's Multiple Range Test (DMRT) was used to compare the effects of Ni (Gomez and Gomez, 1984). Statistical significance was set at a 5% level of significance ($p \leq 0.05$).

3. Results

3.1 Bioaccumulation and phytoextraction of Ni

Concurrent with a rise in the concentration of Ni in the soil, all treatments demonstrated a significant buildup of metal in the tissues of the roots and shoots. However, compared to roots, shoot tissue had a much lower Ni concentration (Table 1). The 25 mg Ni kg^{-1} treatment caused the Translocation Factor (TF) and Transportation Index (Ti) to achieve their maximum values (0.38 and 37.59%, respectively), greatly surpassing the values seen in previous treatments. This suggests that an increase in the amount of Ni applied to the soil was hostile to metal translocation (Table 2). As the amount of Ni in the soil grew, both TF and Ti values trended downward. In contrast, the TAR and BCF values enhanced with higher Ni additions (200 mg Ni kg^{-1} soil treatment), peaking at 5.35 and 3.96 respectively (Table 2).

3.2 Effect of Ni on growth parameters

In the current investigation Ni promoted *T. erecta* plant growth at modest soil augmentations (Table 1). In comparison to untreated control plants, root and shoot lengths considerably increased at the lowest dose (25 mg Ni kg^{-1} of soil). However, compared to control plants, T. erecta plants with greater Ni levels (50-200 mg Ni kg^{-1} of soil) exhibited shorter roots and shoots. After 60 days of

Table 1

Bioaccumulation of Ni and effect on root and shoot length in *T. erecta**

Ni treatment (mg kg^{-1} soil)	Ni content in root (µg g^{-1} dry wt.)	Ni content in shoot (µg g^{-1} dry wt.)	Root length (cm)		Shoot length (cm)	
			0 Day	60 Day	0 Day	60 Day
0 (Control)	0.50±1.88 ^{f}	0.25±0.60 ^{f}	6.93±0.14 ^{a}	16.05±0.17 ^{b}	11.18±0.26 ^{a}	60.24±0.96 ^{b}
25	33.25±5.16 ^{e}	11.88±1.91 ^{e}	6.25±0.44 ^{a}	18.15±0.15 ^{a}	11.6±0.22 ^{a}	61.77±0.65 ^{a}
50	69.5±2.92 ^{d}	24.63±3.00 ^{d}	7.05±0.49 ^{a}	15.5±0.26 ^{b}	12.03±0.31 ^{a}	55.1±0.60 ^{c}
100	228.38±21.85 ^{c}	50.75±3.14 ^{c}	6.78±0.46 ^{a}	13.78±0.38 ^{c}	11.25±0.24 ^{a}	45.78±1.35 ^{d}
150	338.63±15.54 ^{b}	102.13±9.59 ^{b}	6.58±0.21 ^{a}	12.68±0.24 ^{c}	11.9±0.13 ^{a}	40.05±0.49 ^{e}
200	618.75±16.46 ^{a}	173.63±16.60 ^{a}	6.8±0.33 ^{a}	9.98±0.18 ^{d}	11.65±0.22 ^{a}	37.23±0.5 ^{f}

* Different alphabets (superscript) are significantly different ($p \leq 0.05$)

Table 2

Ni phytoextraction efficiency in *T. erecta**

Treatment (Ni) (mg kg^{-1}soil)	TF	T*i*, %	TAR	BCF
25	0.38a	37.59b	0.30e	1.81c
50	0.36b	35.99b	0.59d	1.88c
100	0.23d	22.67d	1.59c	2.79b
150	0.31c	31.03c	2.68b	2.94b
200	0.31c	30.55c	5.35a	3.96a

* Translocation Factor (TF); Transportation Index (T*i*, %); Total Accumulation Rate (TAR, mg kg^{-1} day^{-1}); Bio Concentration Factor (BCF); Different alphabets (superscript) are significantly different ($p \leq 0.05$)

treatment, plants grown in soil containing 200 mg Ni kg^{-1} had shorter roots (9.98 cm) than untreated control plants, which had an average root length of 16.05 cm (Table 1). At the lowest Ni dose (25 mg kg^{-1} of soil), a notable increase in biomass output was noted. At 60 days, fresh weights of roots and shoots at 25 mg Ni kg^{-1} of soil showed substantial increases (around 1.02 and 1.03 times, respectively) compared to the control. However, as Ni concentrations increased, biomass output significantly decreased (Table 3). In comparison to control plants, significant decreases in fresh weight (FW) and dry weight (DW) of roots and shoots were noted at higher Ni concentrations.

Table 3

Effect of Ni on root and shoot biomass in *T. erecta*

Ni treatment (mg kg^{-1} soil)	Root fresh weight (g $plant^{-1}$)		Shoot fresh weight (g $plant^{-1}$)		Root dry weight (g $plant^{-1}$)		Shoot dry weight (g $plant^{-1}$)	
	0 Day	60 Day	0 Day	60 Day	0 Day	60 Day	0 Day	60 Day
0 (Control)	4.75±0.16	20.34±0.42	5.20±0.09	44.21±0.47	0.38±0.01	2.42±0.13	0.50±0.02	7.62±0.37
25	4.87±0.10	21.67±0.26	5.96±0.33	45.12±0.06	0.41±0.02	3.58±0.05	0.51±0.02	8.80±0.17
50	5.10±0.32	17.23±0.19	4.86±0.22	43.10±0.51	0.39±0.01	2.4±0.09	0.57±0.02	7.55±0.32
100	5.21±0.20	16.14±0.07	5.45±0.17	35.70±0.25	0.42±0.02	1.94±0.03	0.58±0.03	5.76±0.21
150	5.09±0.32	14.96±0.17	5.79±0.19	28.99±0.05	0.42±0.02	1.63±0.09	0.60±0.02	4.92±0.18
200	5.37±0.22	13.13±0.16	4.69±0.29	25.96±0.56	0.44±0.02	1.49±0.07	0.49±0.03	3.30±0.16

3.3. Effect on chlorophyll, proline, carbohydrate and protein content

The lowest dose of nickel treatment (25 mg Ni kg^{-1}) increased the amount of chlorophyll in the leaves. Plants treated with 25 mg Ni kg^{-1} showed a considerable increase in total chlorophyll content, while plants subjected to 50–200 mg Ni kg^{-1} showed a further decrease (Table 4). Additionally, there was no difference in the carotenoid content between the plants exposed to 25 mg Ni kg^{-1} treatment and the untreated control plants. Proline content increased significantly in all treatments. Compared to the untreated control plant, plants grown with 200 mg Ni kg^{-1} had *ca.* 2.6-fold increased proline content (Table 4). The

Table 4

Effect of Ni on biochemical compounds and photosynthetic pigments in *T. erecta**

Ni treatment (mg kg^{-1} soil)	Proline content (mg g^{-1} FW)		Protein content (mg g^{-1} FW)		Total carbohydrate content (mg g^{-1} FW)		Total chlorophyll content (mg g^{-1} FW)		Carotenoid content (mg g^{-1} FW)	
	0 Day	60 Day	0 Day	60 Day	0 Day	60 Day	0 Day	60 Day	0 Day	60 Day
0	0.21± 0.03^{a}	1.13± 0.11^{f}	6.15 ± 0.24^{d}	9.32± 0.34^{c}	0.63± 0.03^{a}	1.34± 0.05^{a}	0.92± 0.133^{b}	1.176± 0.17^{b}	0.033± 0.001^{a}	0.042± 0.004^{a}
25	0.21± 0.03^{a}	1.30± 0.14^{e}	5.90± 0.27^{d}	10.82± 0.49^{c}	0.70± 0.05^{a}	1.46± 0.09^{b}	0.96± 0.133^{a}	1.239 ±0.17^{a}	0.033± 0.00^{a}	0.043± 0.004^{a}
50	0.23± 0.03^{a}	1.92± 0.07^{d}	5.81± 0.21^{d}	9.90± 0.35^{bc}	0.57± 0.03^{a}	1.14± 0.05^{c}	0.811± 0.058^{c}	0.998± 0.033^{c}	0.034± 0.00^{a}	0.035± 0.001^{b}
100	0.25± 0.02^{a}	2.52± 0.22^{c}	6.05± 0.24^{d}	8.43± 0.99^{c}	0.66± 0.05^{a}	1.07± 0.05^{c}	0.796± 0.061^{d}	0.821± 0.052^{d}	0.033± 0.001^{a}	0.026± 0.001^{c}
150	0.26± 0.02^{a}	3.10± 0.21^{b}	6.26± 0.24^{d}	7.28± 0.31^{c}	0.54± 0.02^{a}	0.83± 0.08^{d}	0.736± 0.013^{e}	0.746± 0.075^{e}	0.034± 0.002^{a}	0.017± 0.001^{d}
200	0.24± 0.01^{a}	3.38± 0.17^{a}	6.42± 0.19^{d}	6.89± 0.33^{c}	0.60± 0.03^{a}	0.78± 0.08^{d}	0.72± 0.02^{e}	0.838± 0.061^{d}	0.034± 0.002^{a}	0.014± 0.001^{e}

** Different alphabets (superscript) are significantly different ($p \leq 0.05$)*

lowest Ni augmentation (25 mg Ni kg^{-1}) was found to have the highest total carbohydrate and protein content, which subsequently declined at increasing augmentations (Table 4). Significant decrease (*ca.* 1.72-fold) in carbohydrate content was recorded in 200 mg Ni kg^{-1} as compared to control.

3.4 Modulations in antioxidative enzyme activities

Significant hyperactivity of CAT, APX and GPX in leaves was demonstrated by *T. erecta* plants in all treatments (Table 5). CAT activity was found to be the maximum (3.61 mol UA mg^{-1} protein) in plants treated with 100 mg Ni, which declined sharply following treatment at 150 mg kg^{-1}. Maximum APX activity (0.039 mol UA mg^{-1} protein) was observed in 150 mg Ni treatment, which then declined at higher concentration. In the current study, maximum GPX activity was recorded at 100 mg Ni kg^{-1} concentration; thereafter it showed a sharp reduction at higher concentrations (Table 5).

3.5 Ni stress tolerance indices

Under various Ni treatments the Tolerance Index (TI) values and individual stress tolerance indices (RLNSTI, SLNSTI, RFNSTI, SFNSTI, RDNSTI, SDNSTI) deteriorated with increasing doses of hexavalent Ni in soil except at the lowest dose of 25 mg Ni kg^{-1} soil (Table 6). At 60 d of treatment, the lowest and highest tolerance indices for the growth parameters were recorded at 200 mg Ni kg^{-1} and 25 mg Ni kg^{-1}, respectively.

Table 5

Effect of Ni on antioxidative enzyme activities in *T. erecta**

Ni treatment (mg kg^{-1} soil)	NCAT activity (mol UA mg^{-1} protein)		APX activity (mol UA mg^{-1} protein)		GPX activity (mol UA mg^{-1} protein)	
	0 Day	60 Day	0 Day	60 Day	0 Day	60 Day
0	1.53 ± 0.22^{e}	1.74 ± 0.16^{e}	0.018 ± 0.003^{e}	0.019 ± 0.005^{e}	0.0014 ± 0.0004^{c}	0.0011 ± 0.0006^{e}
25	2.51 ± 0.11^{d}	1.52 ± 0.12^{e}	0.019 ± 0.003^{d}	0.020 ± 0.009^{d}	0.0017 ± 0.0004^{b}	0.0015 ± 0.0003^{d}
50	2.90 ± 0.08^{a}	2.28 ± 0.12^{d}	0.025 ± 0.002^{c}	0.029 ± 0.009^{c}	0.0021 ± 0.0005^{b}	0.0023 ± 0.0004^{b}
100	2.65 ± 0.27^{b}	3.61 ± 0.21^{a}	0.037 ± 0.003^{b}	0.035 ± 0.002^{b}	0.0032 ± 0.0002^{a}	0.0031 ± 0.0005^{a}
150	1.69 ± 0.10^{c}	2.14 ± 0.22^{b}	0.042 ± 0.002^{a}	0.039 ± 0.005^{a}	0.0022 ± 0.0006^{b}	0.0025 ± 0.0005^{b}
200	1.30 ± 0.17^{d}	1.61 ± 0.06^{c}	0.029 ± 0.003^{c}	0.028 ± 0.003^{c}	0.0016 ± 0.0003^{c}	0.0019 ± 0.0009^{c}

* Different alphabets (superscript) are significantly different ($p \leq 0.05$)

Table 6

Tolerance indices of Ni treatment in *T. erecta**

Treatment (mg kg^{-1} soil)	TI, %	RLNSTI	SLNST	RFNSTI	SFNSTI	RDNSTI	SDNSTI
25	123.29^{a}	113.14 $\pm1.92^{a}$	102.70 $\pm2.60^{a}$	106.74 $\pm3.07^{a}$	102.13 $\pm1.35^{a}$	150.19 $\pm11.99^{a}$	116.33 $\pm5.17^{a}$
50	99.11^{b}	96.60 $\pm1.98^{b}$	91.65 $\pm2.82^{b}$	84.87 $\pm1.90^{b}$	97.52 $\pm1.14^{b}$	100.22 $\pm7.53^{b}$	99.92 $\pm7.12^{b}$
100	76.73^{c}	85.98 $\pm4.03^{c}$	76.08 $\pm2.93^{c}$	79.47 $\pm1.66^{c}$	80.81 $\pm1.58^{c}$	81.56 $\pm5.85^{c}$	75.93 $\pm3.30^{c}$
150	65.26^{c}	79.07 $\pm2.69c^{d}$	66.54 $\pm0.93^{d}$	73.67 $\pm1.28^{c}$	65.61 $\pm0.95^{d}$	68.65 $\pm7.98^{d}$	65.35 $\pm5.51^{d}$
200	47.73^{d}	62.20 $\pm1.81^{d}$	61.82 $\pm0.20^{d}$	64.65 $\pm0.83^{d}$	58.70 $\pm0.85^{d}$	61.92 $\pm2.45^{e}$	43.59 $\pm3.03^{e}$

*Tolerance Index (TI); Root length Ni stress tolerance index (RLNSTI); Shoot length Ni stress tolerance index (SLNSTI); Root fresh wt. Ni stress tolerance index (RFNSTI); Shoot fresh wt. Ni stress tolerance index (SFNSTI); Root dry wt. Ni stress tolerance index (RDNSTI); Shoot dry wt. Ni stress tolerance index (SDNSTI); Different alphabets (superscript) are significantly different ($p \leq 0.05$)

Discussion

Bioaccumulation of Ni and stress tolerance

All treatments showed a notable accumulation of metal in the root and shoot tissues of both *T. erecta* and *T. patula*, concomitant with an increase in Ni concentration in soil. However, significantly lower content of Ni was accumulated in shoot tissue compared to roots (Table 1). The results suggested that both the plants are efficient phytoextractors and phytostabilisers of Ni because they retain the metal in roots and reduce its mobility to shoot and soil. Bhurat *et al.* (2017) had reported *T. erecta* to withstand Ni concentrations of up to 200 mmol in hydroponic culture conditions. The Tolerance Index (TI) values and individual stress tolerance indices had a negative correlation with augmentations of Ni in soil. This may be attributed to the decreasing trend in growth parameters under metal treatments. Similar observations were reported earlier in *Cymbopogon flexuous* and *Zinnia elegans* under Cr treatment (Patra *et al.*, 2019; Panda *et al.*, 2020).

Effect of Ni on growth and biomass

Elevated Ni concentrations in the soil led to reductions in root and shoot length, and fresh and dry biomass following initial increases observed at lower levels of metal exposure. Similar response to Ni treatments was reported in *Macrotyloma uniflorum* (Sahoo *et al.*, 2016). Additionally, Bhurat *et al.* (2017) reported decreased shoot length and fresh weight in *T. erecta* plantlets under Ni treatments after 21 days of exposure to the metal, which can be linked to a reduction in meristematic cells in shoot. However, dry weight and root length showed notable increases across all Ni treatments in *T. erecta* (Bhurat *et al.*, 2017). The observed increase in dry weight and root length could be linked to metal hyperaccumulation in the plantlets and the competition for nutrient uptake from the soil.

Effects on biochemical compounds

All treatments of Ni showed a significant rise in proline content. Proline content was 2.6-fold higher in *T. erecta* plants grown with 200 mg Ni kg^{-1} than in the untreated control plant. This is in agreement with results noted in *Macrotyloma uniflorum* grown under Ni stress in pot culture (Sahoo *et al.*, 2016). Under stress conditions, proline hyperaccumulation is crucial for osmoregulation, a fundamental need for plants to withstand nearly all types of abiotic stress. Additionally, the antioxidative properties of proline are known to safeguard plants from damage induced by heavy metals (Slater *et al.*, 2006). When plants are exposed to heavy metal stress, proline helps neutralize singlet oxygen and damage caused by free radicals, playing a key role in shielding proteins from denaturation (Bhurat *et al.*, 2017). Total carbohydrate and protein content was observed to enhance in the plants exposed to 25 mg Ni kg^{-1} as compared to that in control plants in *T. erecta*. However the metabolite's content decreased in higher Ni augmentations. These observations are in accordance with the findings of previous investigations conducted under Ni stress (Sahoo *et al.*, 2016; Bhurat *et al.*, 2017). Abiotic stresses, such as heavy metal exposure, induce reactive oxygen species (ROS) stress, which quickly target essential biomolecules, including proteins, carbohydrates, and nucleic acids within the cell, often resulting in irreversible damage (Juan *et al.*, 2021). Furthermore, chlorophyll content in the leaves of both the plants exhibited similar responses as carbohydrate content, with significant enhancement in low Ni augmentation (25 mg kg^{-1} soil). Thus it is suggested that low Ni augmentation enhanced plant growth and primary metabolite contents.

Antioxidative enzyme activities

The tested plant exhibited considerable increases in the activity of CAT, APX and GPX enzymes in their leaves across treatments of Ni. The significant increase in CAT, APX, and GPX activities in the leaves of the tested plants under Ni stress indicates that these enzymes helped to remove harmful H, O, in *T. erecta*, thereby aiding in ROS reduction. Similar upsurge in the activity of reactive oxygen species (ROS)-scavenging enzymes, such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), have been observed in numerous plants exposed to Ni (Gajewska *et al.*, 2006; Yan *et al.*, 2008). In response to oxidative stress, plants typically enhance the activity of antioxidant enzymes within the ascorbate-glutathione cycle, including catalase, peroxidase, superoxide dismutase, glutathione reductase, and ascorbate oxidase. These enzymes play a crucial role in safeguarding plant cells against the detrimental effects of free radicals (Yan *et al.*, 2008).

Conclusions

The escalating environmental concerns associated with heavy metal pollution have fuelled the exploration of sustainable and nature-based remediation strategies, with phytoremediation emerging as a forefront contender. On this context, the ornamental plant *Tagetes* stands out as a promising candidate, captivating researchers' attention for its inherent ability to thrive in contaminated soils while exhibiting substantial potential for heavy metal remediation. Thus, it is concluded that *T. erecta* may be recommended for propagation in Ni-contaminated sites. Further, these plants can impart aesthetic value to wastelands and phytoremediate the Ni-overburden soil effectively. Being non-edible crop species *T. erecta* would be more suitable

for phytoremediation. However, the detailed mechanism of Ni phytoremediation needs further elucidation in *T. erecta* and other plant species for the purpose of better understanding of the remediation process. Moreover it is a good source of many biologically active compounds and botanical nematicide. Thus *T. erecta* can be used with the multiple purpose of decontaminating the Ni-polluted sites while providing environment-friendly green spaces and a source of commercially valuable products.

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New record of *Smithia blanda* Wall. ex Wight & Arn. (Fabaceae) in Odisha

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ABSTRACT

Smithia blanda Wall. ex Wight & Arn. (Leguminosae), a species hitherto not known to occur in Odisha, is reported here as a new distributional record for the flora of the state. Nomenclature, botanical description,notes on distribution, ecology and phenology of the species have been provided in this paper along with colour photographs to facilitate easy identification.

1. Introduction

The genus *Smithia* Aiton, with about 21 species in the world, belongs to the tribe Dalbergieae DC. of the family Fabaceae and is distributed in the Old-World tropics, especially in Asia and Madagascar (Lavin *et al.*, 2001; LPWG, 2017; POWO, 2025). At present, 19 recognized species and a variety are reported to occur in India (Mao & Dash, 2020) including the recently described species *S. odishae* Sanjeet Kumar, R. S. Devi and R. Kr. Singh from Odisha. Of these, 10 species are endemic to different states of India (Sanjappa, 1992; Balan and Predeep, 2017; Devi *et al.*, 2023). South India, especially the Western Ghats, exhibit the maximum species diversity and endemism for this genus (Nayar *et al.*, 2014; Balan and Predeep, 2014). Most of the species of Smithia are herbaceous and inhabit open grasslands, forest clearings and in seasonally moist localities along rivers and hill streams. As many as four species of *Smithia* namely, *S. ciliata* Royle, *S. conferta* Sm., *S. sensitiva* Aiton, and *S. odishae* Sanjeet Kumar, Devi & R. Kr. Singh are reported from the state of Odisha (Haines, 1921; Mooney, 1950; Saxena & Brahmam, 1994; Devi *et al.*, 2023).

In course of plant exploration in Koraput district of Odisha in the month of July, 2025, the authors collected an interesting herbaceous legume species with pinnate leaves, yellow papilionaceous flowers and short pods from a shady moist hill slope along a stream near Bari village of Narayanpatana Block, Koraput District (18°43'17.81"N., 83°05'23.40"E; 1231 m alt.) (Map-1). The microhabitat of this species was characterized by high humidity, dense shade, rocky substratum with humus rich sub-soil and constantly wet environment. On critical examination of the morphological characters of the specimens and consultation of pertinent literature, the species was identified as *Smithia blanda* Wall. ex Wight & Arn. (Fabaceae). The voucher specimens have been deposited in the Herbarium of the Center for Biotechnology (CBT), Siksha O Anusandhan University, Bhubaneswar. This species has so far been reported from Assam, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Nagaland, Rajasthan, Sikkim, Tamil Nadu and West Bengal (Sanjappa, 1992; Balan & Predeep, 2017). The wild occurrence of *S. blanda* from Koraput district turned out to be an addition to the legume flora of Odisha and a new distributional record for the state.

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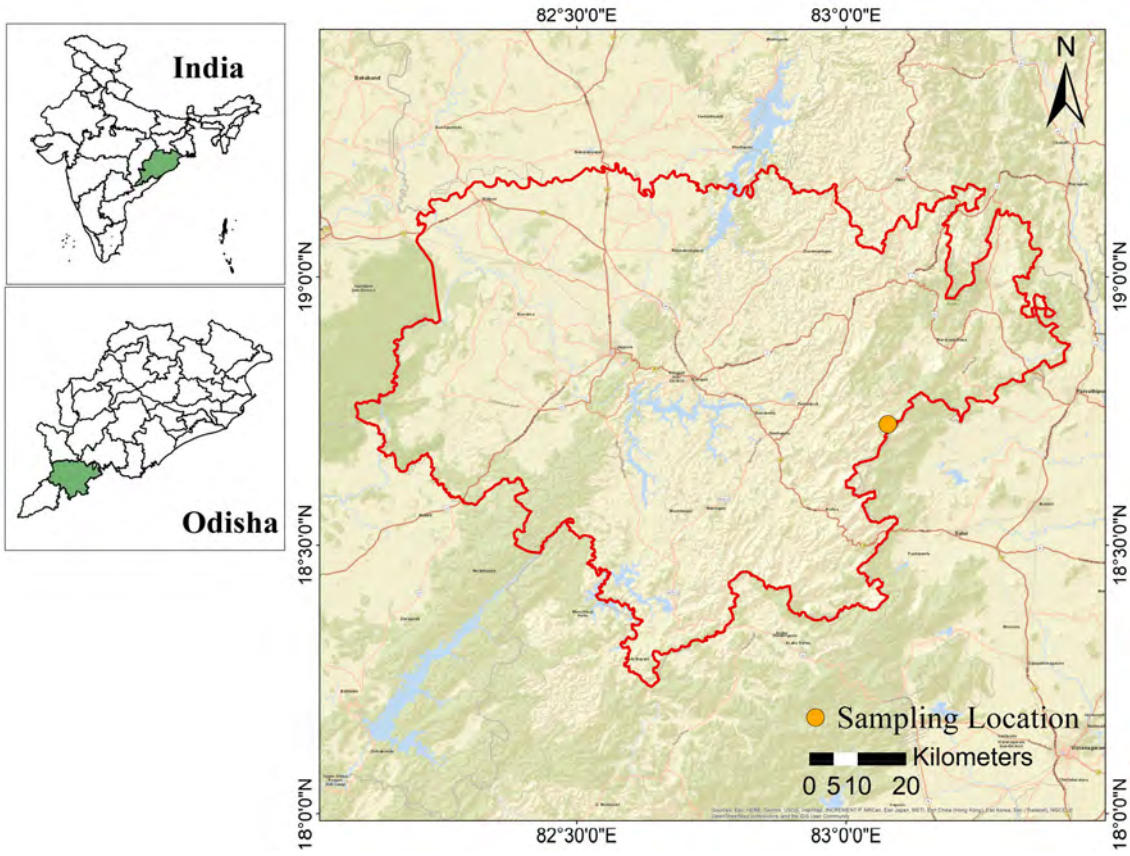


Map 1: Map showing the location of collection site of *Smithia blanda* in Odisha

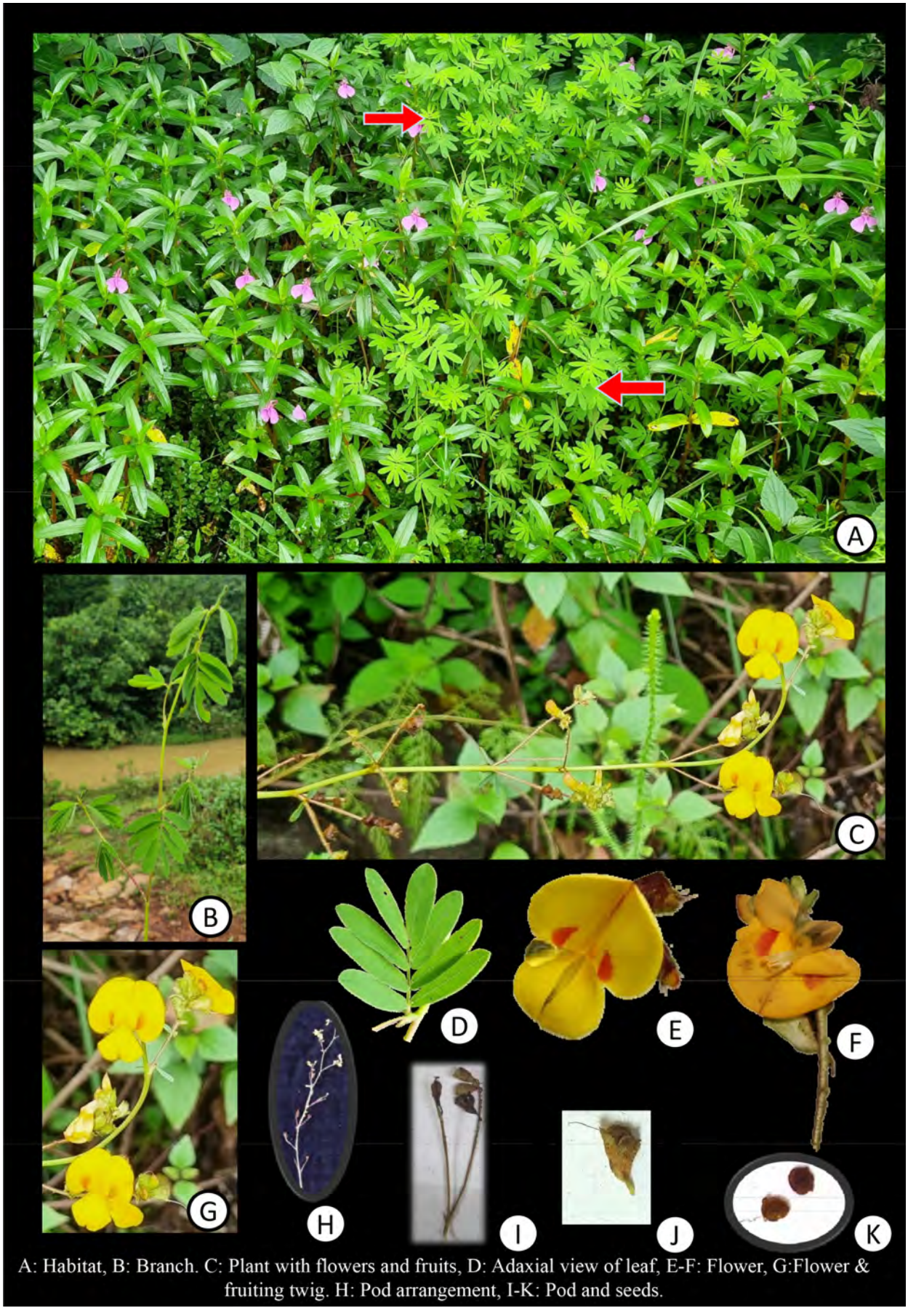
The nomenclature, botanical description, notes on distribution, phenology and ecology of the species are provided below along with colour photographs for ease of identification.

Smithia blanda Wall. ex Wight & Arn., Prodr. Fl. Ind. Orient. 1: 242. 1834; Balan & Predeep, Taiwania 62(2): 179. 2017; Sanjappa, Legumes of India 246. 1992; Baker in Hook. f., Fl. Brit. India 2: 151. 1876; Gamble, Fl. Madras 1: 330. 1918; Nayar *et al.*, Flowering Plants of W. Ghats, India 1: 455. 2014. *Smithia racemosa* Heyne ex Wight & Arn., Prod. Fl. Ind. Orient. 221. 1834. *Smithiablanda* Wall. ex Wight & Arn. var. racemosa (Heyne ex Wight & Arn.) Baker in Hook. f., Fl. Brit. India 2: 151. 1876, pro parte. *Smithia paniculata* Arn., Nov. Act. Phys.-Med. Acad. Caes. Leop.-Carol. Nat. Cur. 18: 330. 1836. *Smithia blanda* var. *paniculata* (Arn.) Baker in J. D. Hooker, Fl. Brit. India 2: 151. 1876. *Smithia yunnanensis* Franch., Pl. Delavay 170. 1889. *Smithia bodinierii* H. Lev., Fl. Kouy-Tcheou 242. 1914. (Fig. 1)

LECTOTYPE: India, Mont. Sillet [Khasi Hills, Meghalaya], 1823, F. De Silva, Wall. Cat. N. 5669 (Lectotype; K001121661!).

Description

Annual, diffuse or ascending herb, 15-45 cm tall. Stems slender, sparsely bristly-pubescent; stipules triangular, striate, ciliate, appendage 5-6 mm long. Leaves pinnate, 2-3 cm long; petioles up to 0.7 cm long; leaflets 3-5 pairs, obovate-oblong, obtuse at apex, oblique at base, glabrous above, sparsely pubescent beneath, margins ciliate. Panicles 4-5 cm long, terminal, corymbose; peduncles 2-3 cm long, hispid. Flowers bright yellow, 8-10 mm long; pedicels about 2 mm long, hispid; bracts elliptic, aristate, setaceous outside along keel, caducous; bracteoles obovate, acuminate, margins ciliate, setaceous outside, persistent. Calyx membranaceous, veins anastomosing; upper lip 4-5 × 3-4 mm,emarginate at apex, margins bristly, lower lip 3-lobed, lobes acute, sparsely bristly outside. Corolla papilionaceous; standard suborbicular, 6-7 × 6-7 mm,red-tinged towards claw; claw 2-2.5 mm long,wings 5-6 × 3 mm, obovate, shortly auricled; keels 5-6 × 2-2.5 mm, obliquely obovate, curved, ciliate at apex. Staminal sheath 4-5 mm long, curved; filaments about 2 mm long; stamens 10, diadelphous (5+5); united in the staminal sheath below. Ovary subsessile, curved; ovules 5-6; style 5-6 mm long, glabrous, filiform; stigma terminal,



A: Habitat, B: Branch. C: Plant with flowers and fruits, D: Adaxial view of leaf, E-F: Flower, G:Flower & fruiting twig. H: Pod arrangement, I-K: Pod and seeds.

pointed. Lomentum linear, 6-9 mm long, slightly exserted, twisted and folded back within the calyx, 3-5-jointed; joints reticulate, margins bordered. Seeds 2-4, brown, 1-1.5× 1-1.5 mm.

Flowering: July - August. **Fruiting:** August- October.

Specimen examined: INDIA, Odisha, Koraput district, Narayanpatana Block, near Bari village,18°43'17.81"N, 83°05'23.40"E, Alt. 1231m, 14.07.2025, P.K. Das 2873/CBT (Fig. 2)

Distribution: India [Assam, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Nagaland, Rajasthan, Sikkim, Tamil Nadu, West Bengal and Odisha (present report)], Bangladesh, Bhutan,China, Laos, Myanmar, Nepal, Sri Lanka and Thailand.

Habitat & Ecology: This annual herb grows in small patches along grassy slopes and seasonal streams in open forest areas at an elevation of 900-1200 m in the studied area. The common associates are Impatiens *oppositifolia* L., *Ageratina adenophora* (Spreng.) R. M. King & H. Rob., *Lycopodiella cernua* (L.) Pic. Serm., *Rotala rotundifolia* (Buch.-Ham. ex Roxb.) Koehne and Crotalaria prostrata Rottler ex Willd.

Notes: *Smithia blanda* morphologically differs from *S. conferta* and *S. sensitiva* by having flowers in corymbose panicles or racemes; lomentum joint being reticulate and bordered. Sometimes, the nature of indumentum on stems and leaves vary among different populations, it could be bristly, densely pubescent, puberulous or even glabrous. The red blotches on the standard petal of the flower towards claw may be absent or inconspicuous in some populations,

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Preliminary phytochemical screening and pharmacological activities of two commonly used leaves as food plates and food wrappers in Odisha

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ABSTRACT

India has a long history of using leaves and other plant parts for food plates and food wrapping. The advantage of using plant parts is because they are biodegradable, environmental friendly. Although, it is believed to add medicinal values to the food, scientific study should be done to ensure the usability and advancement of using plant parts over synthetically produced food plates and wrappers where harmful chemicals are used. The present study includes two of the plant leaves which has been extensively used as food plates and food wrappers i.e., *Phanera vahlii* (Wight & Arn.) Benth. and *Musa* × *paradisiaca* L. for their general phytochemical screening, antimicrobial activity against a human pathogenic bacterium namely *Salmonella typhi* responsible for typhoid fever and antioxidant activity. The phytochemical analysis shows that they have diverse metabolites like phenols, flavonoids, tannins, terpenoids, saponins, and alkaloids. The leaf extracts of both the plants showed significant antibacterial and antioxidant activity. Therefore, using the leaves of above plants hold its scientific significance for food plates and food wrapping.

Introduction

The lives on earth depends upon two basic components like materials (from soil) and energy (from sun) for their growth, development and sustenance. Among the lives, the humans always expand their habits to live a better life by developing sophisticated innovations in food, clothes and shelter. The food among the three bears an outmost priority because it provides the key component of life i.e., materials and energy. The food includes a series of events starting farming, harvesting, storing, transporting and ending with the serving of the food for intake. The storing, transporting and serving of foods needs special containers, wrappers and plates/pots. Traditionally, the storage and serving of foods has been done using leaves and other plant parts. They include Plantain leaf, Bastard teak leaf, *Ricinus communis* leaf, *Calotropis* leaf, Castor leaf, Secrete milky sap leaf, Lotus petals, Tahitian screwpine leaf, *Stereospermum suaveolens* leaf etc. (Hegde *et al.*, 2018). In Odisha, Sal (*Shorea robusta*) leaves, Siali (*Phanera vahlii*) leaves, Palasa (*Butea monosperma*) leaves, Saguan (*Tectona grandis*) leaves has been used for various food related purposes (Kora, 2019). Besides these, the leaves or plant parts of *Colocasia esculenta, Curcuma longa, Ficus religiosa, Ficus bengalensis, Musa* × *paradisiaca* etc. has been used extensively thought the Odisha in various cultural rituals as well as daily food serving. Now a days, the use of modern food plates/pots has increased a lot which releases carcinogenic toxic substances (Today, 2017). To address these problems, attentions have been made of using plant parts for eating and wrapping foods. Therefore, it becomes imperative to test the scientific advantages of traditional methods over modern one. Here, we have chosen two plant leaves which are commonly used in Odisha as food plates and food wrappers to investigate their general phytochemicals and pharmacological activities like antibacterial and antioxidant properties.

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Table 1
Phytochemical screening of *P. vahlii* and *M. paradisiaca*

Phytochemicals	*P. vahlii*		*M. paradisiaca*		Method used
	Methanolic extract	Acetone extract	Methanolic extract	Acetone extract	
Phenol	+	+	+	+	Shah & Yadav, 2015
Flavonoid	+	+	+	+	Garg & Garg, 2019
Tannin	+	+	+	+	Manzo *et al.*, 2017
Terpenoid	+	+	+	+	Patel *et al.*, 2016
Alkaloids	+	-	+	+	Aziz, 2015
Saponin	-	-	+	+	Singh *et al.*, 2019

The fresh leaves of *Phanera vahlii* (Wight & Arn.) Benth. and *Musa* × *paradisiaca* L. are collected from the Mayurbhanj district of Odisha, India. The plants were taxonomically authenticated at Department of Botany, Ravenshaw University, Cuttack, Odisha. Around 10 g of shed dried leaf powder of *P. vahlii* and *M. paradisiaca* were taken for the Soxhlet extraction using two different solvents i.e., methanol (polar) and acetone (mid polar) separately. The extract solutions were then lyophilized and the solutions were made using dimethyl sulfoxide (DMSO) for further study.

The phytochemical study revealed that all the major phytochemical are present and they may enter human body through food during eating. The phytochemicals contribute to the overall health of human beings (Kumar *et al.*, 2023).

The anti-oxidant activity was tested using DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay by taking Ascorbic acid as a standard and by following the method of Ara and Nur, 2009. The samples of different grade were prepared and mixed with and 1 ml of freshly prepared DPPH solution (0.15 mM in methanol). The absorbance (at 517 nm) of these reaction mixtures was taken after 30 minutes of incubation and plotted in a graph. Although, all the extracts have shown good anti-oxidant activity, the methanolic extract of *P. vahlii* indicated best among all treatment groups and is closer to the standard Ascorbic acid curve (Figure 1).

The anti-bacterial assay was estimated by the agar well diffusion method (Nalawade *et al.*, 2016) against the bacteria *Salmonella typhi*. *S. typhi* is the causative organism of typhoid fever, which spreads through contaminated water and food. A bacterial culture equivalent to 0.5M McFarland solution was for the purpose. The zones of inhibition were measured after a period of 24 hours incubation at 37 °C. Both the extracts have shown significant antibacterial activity against the target and the leaf extracts of *M. paradisiaca* have shown more effect that the other.

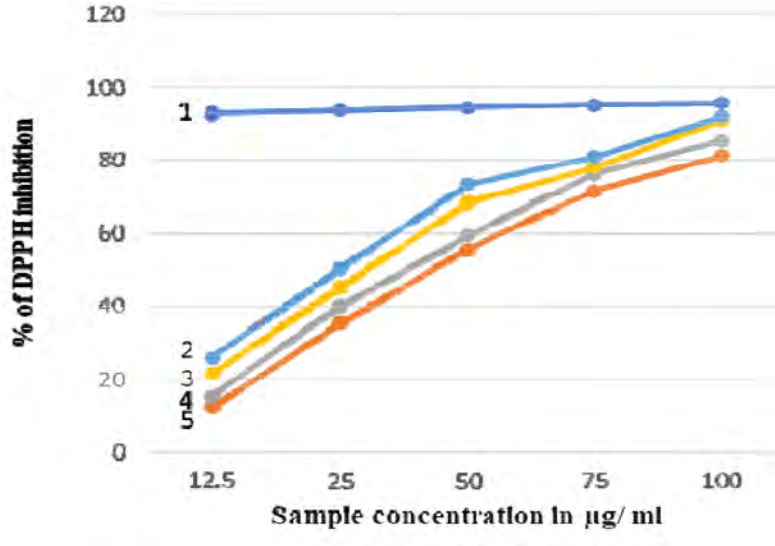

Fig. 1: DPPH inhibition (%) by Ascorbic acid (1), Methanol extract of *M. paradisiaca* (2), Acetone extract of *M. paradisiaca* (3), Methanol extract of *P. vahlii* (4) and Acetone extract of *P. vahlii* (5)

Table 2

Inhibition zones on the agar well containing the target bacteria

Agar well No.	Plant Extract Conc. (mg/ml)	Inhibition zone (mm)			
		Acetone *P. vahlii* (Fig 2a)	Methanol *B. vahlii* (Fig 2b)	Acetone *M. paradisiaca* (Fig 2c)	Methanol *M. paradisiaca* (Fig 2d)
A	10	18	18	19	19
B	8	17	17	18	18
C	4	15	16	18	18
D	1	15	16	18	18
E	DMSO	6	6	6	6

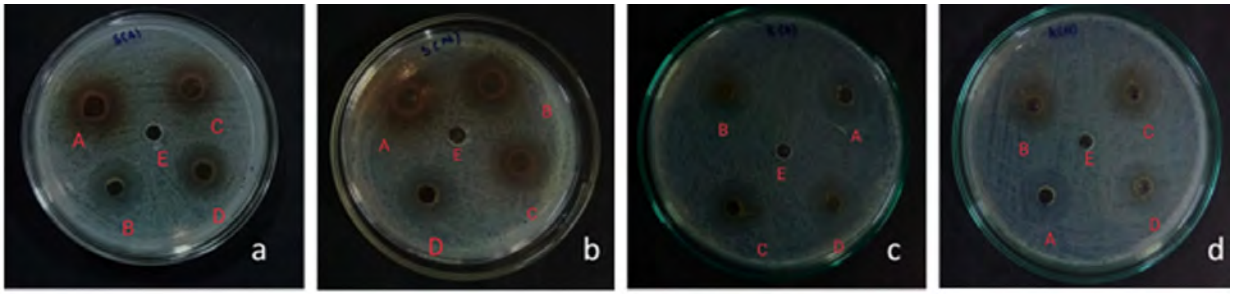

Figure 2. Inhibition zones of the wells on agar plates loaded with different concentration of plant extracts

Therefore, the use of these leaves as food plates and food wrappers are found to be medicinally significant and environmentally friendly. However, more studies are required to test other medicinal aspects with more microbial targets.

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