ISSN 0972-8546

PLANT SCIENCE RESEARCH

Volume 46 , December, 2024

The Orissa Botanical Society

PLANT SCIENCE RESEARCH

Official Publication of

THE ORISSA BOTANICAL SOCIETY

Plant Science Research is the official publication of the Orissa Botanical Society and is published once a year. Subscription rate for non-members and institutions is Rs. 300/- in India and USD 30/- for overseas subscribers per annum. Life Members of the Society get the journal gratis. All communications regarding business matters should be addressed to Dr. Debaraj Parida, Managing Editor, Plant Science Research, Associate Professor, Department of Botany, Dhenkanal Autonomous College, Dhenkanal-759001, Odisha, India.
Email: odishabotanicalsociety1000@gmail.com

Plant Science Research 46 (1&2) : 1-4, 2024

Plant Science Research

ISSN 0972-8546

Medicago polymorpha L. (Fabaceae): An addition to the legume flora of Odisha, India

Prabhat Kumar Das and Pratap Chandra Panda[Ψ]

Centre for Biotechnology, Siksha 'O' Anusandhan (Deemed to be University), SPS Building, Kalinganagar, Bhubaneswar 751 003, Odisha, India

ARTICLE INFO

Article history:
Received : 16 August 2024
Revised : 22 September 2024
Accepted : 8 October 2024

Keywords:

Medicago polymorpha
Legume
New Plant Record
Weed
Invasiveness
Odisha

ABSTRACT

The occurrence of *Medicago polymorpha* L. (Fabaceae), commonly known as Bur clover or toothed medick and native to the Mediterranean Basin and Central Asia, is reported here as a new distributional record for the state of Odisha. The nomenclature, botanical description, notes on distribution, invasiveness, phenology, ecology and uses have been provided in this paper along with field photographs to facilitate easy identification.

1. Introduction

With about 770 genera and over 19,500 species (LPWG, 2017), the Leguminosae is the third largest angiosperm family in terms of species numbers after Asteraceae and Orchidaceae. Economically, the family is second in importance only to Poaceae and provides food, fodder, timber, medicine, firewood and ornamentals. Legumes are cosmopolitan in distribution, representing important ecological constituents in almost all biomes across the globe and occur in even the most extreme habitats (Schrire *et al.*, 2005). The family is an assemblage of species of all life forms such as trees, shrubs, vines and herbs. They constitute significant elements in terms of both species diversity and abundance in lowland wet tropical forests in Africa, South America, and Asia and savannas throughout the tropics ((Yahara *et al.*, 2013). In India, the family Fabaceae is represented by 174 genera, 1110 species and 256 intraspecific taxa (Sanjappa, 2020). The higher diversity of legumes are found in Peninsular India and North-Eastern India including eastern Himalaya corresponding to two hotspot areas in India (Sanjappa, 1991; Chavan *et al.*, 2013).

Bairiganjan *et al.* (1985) made a taxonomic inventory of Fabaceae (only Papilionaceae) of Odisha and listed 242 taxa belonging to 75 genera, which includes 12 new distributional records for the state of Odisha. Others like Bairiganjan *et al.* (1984), Panda *et al.* (1985& 1988); Dash & Panda (2013) and Das *et al.* (2020) have contributed to the taxonomy of legumes of Odisha in addition to the enumeration of legumes in several publications on floristic and ethnobotanical studies in the state (Haines, 1921; Saxena & Brahmam, 1994).

In connection with a study relating to the diversity of legumes in Eastern Ghats of India, we came across several populations of an interesting herbaceous species with small

[Ψ] *Corresponding author; Email:* pcpanda2001@yahoo.co.in

yellow flowers and spirally-coiled prickly pods in wastelands along Chandaka-Bharatpur Road, Bhubaneswar, Khurda District, Odisha (Fig. 1). On critical examination of its morphological characters, it could be identified as *Medicago polymorpha* L. (Fabaceae), which has not so far been reported to occur in Odisha state. In India, the species is so far reported from the states of Delhi, Haryana, Himachal Pradesh, Uttar Pradesh, Punjab, Rajasthan, Gujarat, Maharashtra, Tamil Nadu, Madhya Pradesh, Chhattisgarh, Jharkhand, Bihar, Assam and West Bengal (Yadav *et al.*, 2024). The present occurrence of the species from Bhubaneswar, Odisha is quite interesting from phyto-geographical point of view and also turned out to be new distributional record for the state of Odisha. The nomenclature, botanical description, phenology, notes on ecology, distribution and invasiveness of the species are provided in this paper along with colour photographs of the taxon. The voucher specimens have been deposited in the Herbarium of Centre for Biotechnology (CBT), Siksha O Anusandhan Deemed to be University, Bhubaneswar.

Medicago polymorpha L. Sp. Pl. 2: 779. 1753; Small & Jumphe, Can. J.Bot. 67: 3272. 1989; Mehregan *et al.*, Iran. Journ. Bot. 9(2): 217. 2002. *Medicago denticulata* Willd. Sp. Pl., ed. 4. 3: 1414.1802. *Medicago nigra* (L.) Krock. in Fl. Siles. 2(2): 244.1790.

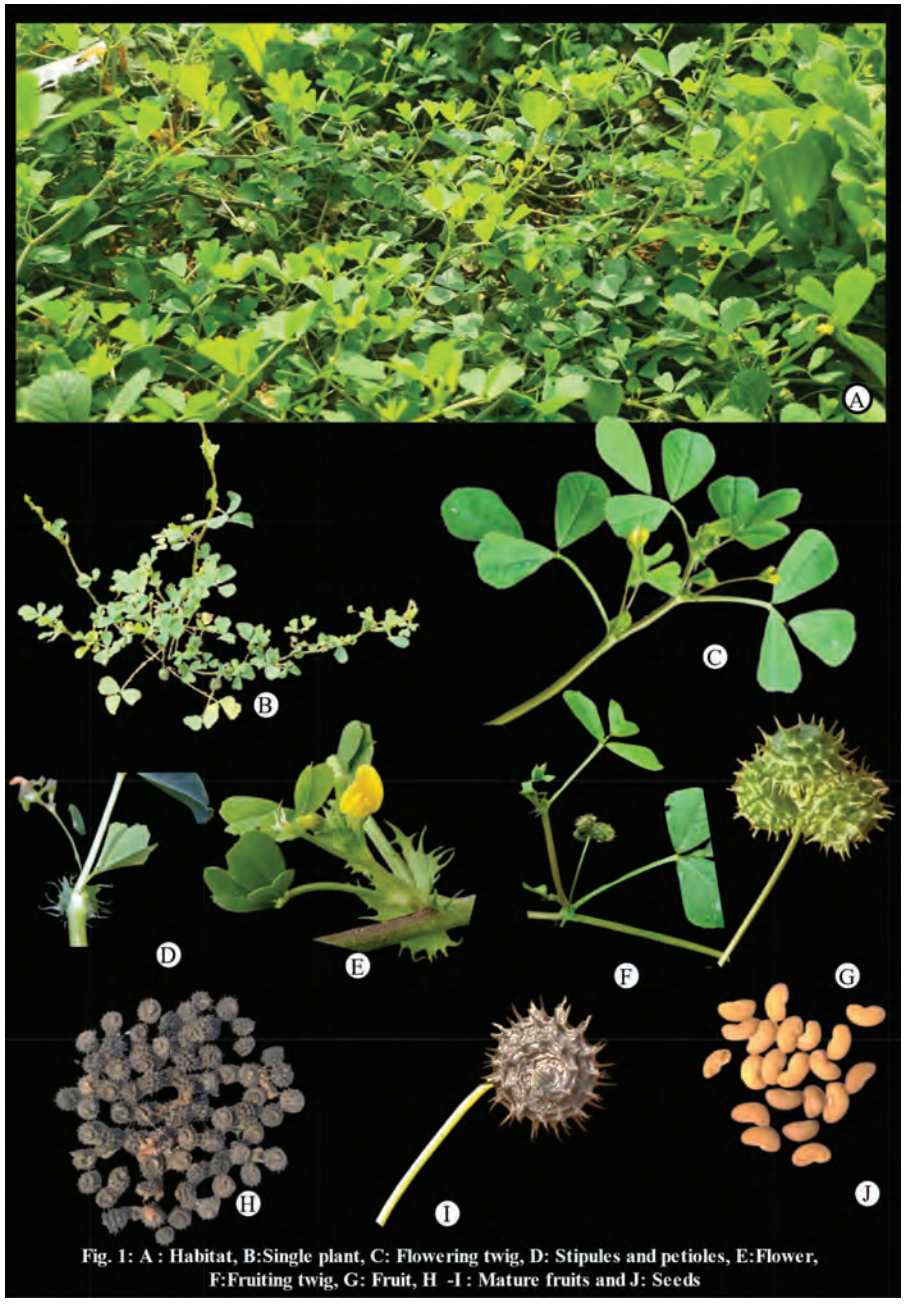


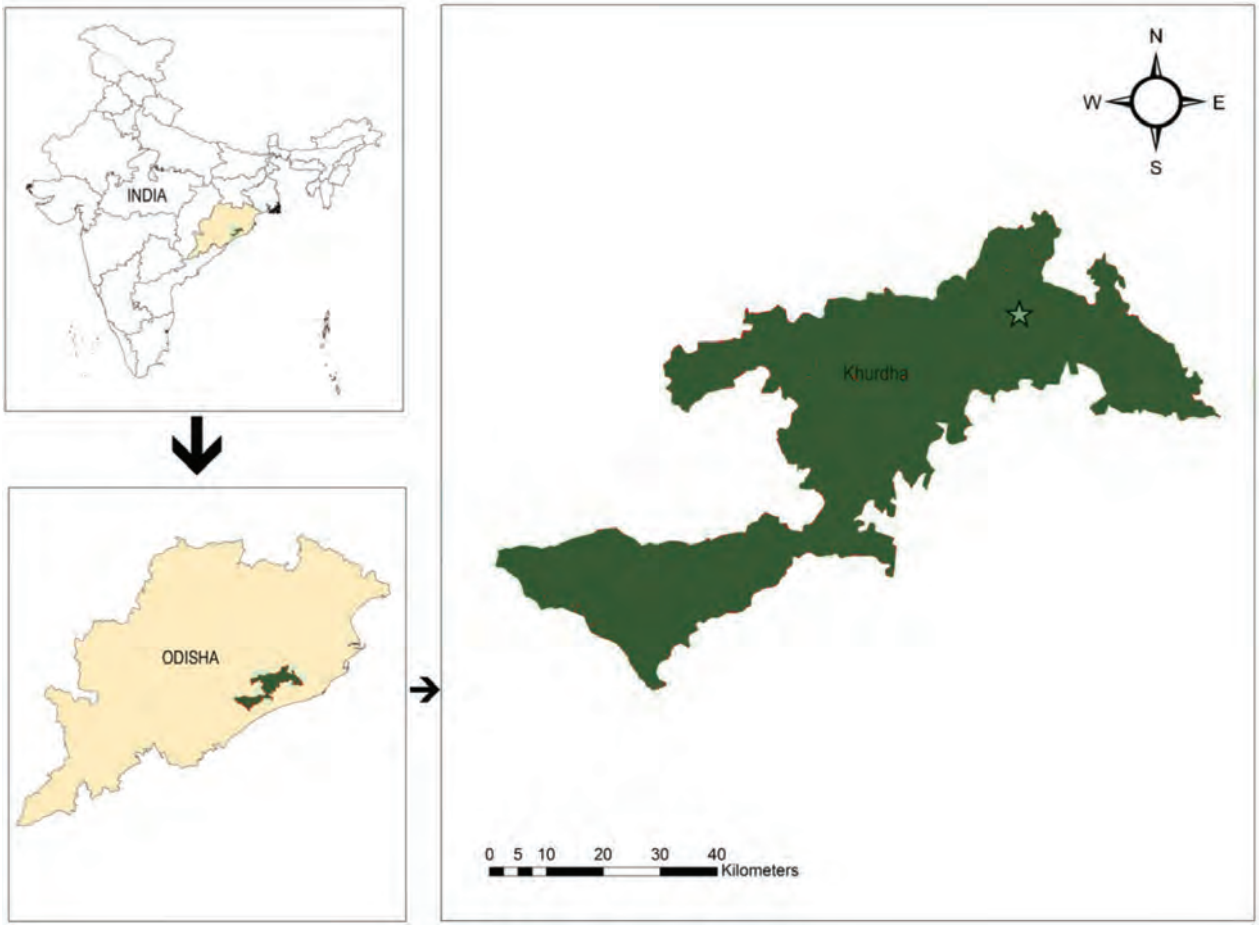
Fig. 1: A : Habitat, B:Single plant, C: Flowering twig, D: Stipules and petioles, E:Flower, F:Fruiting twig, G: Fruit, H -I : Mature fruits and J: Seeds

Vernacular names: Bur Clover, Bur medic, Toothed medic (Eng.); Maina, Chandausi (Hindi).

Botanical description: Prostrate or ascending herbs, up to 1 m long, much-branched at base, stem quadrangular, glabrescent. Stipules ovate-oblong, base auriculate, margin irregularly laciniate or deeply incised, apex acuminate; petioles elongated, up to 4 cm long; leaflets obovate or triangular-obovate, 2 × 1.5 cm, papery, upper surface sparsely hairy, glabrous below, base broadly cuneate, margin shallowly serrate on upper half, apex obtuse, truncate, or emarginate, apiculate. Flowers yellow, sessile or with small pedicels, 2-8 in axillary racemes; peduncles slender, 0.5-1.5 cm long, usually longer than leaves; corolla 3-4 mm; standard obovate, emarginate; stamens 10, diadelphus, filaments filiform; ovary sessile, many-ovules, style short and slightly incurved, stigma oblique. Pods greenish brown, 5-8 mm dia, silky, shining, spirally twisted with 1-3 tight coils, turning clockwise, radial veins connected near edge on coil face, with prominent spines or tubercles. Seed brown, reniform, about 2.0 × 1.5 mm, smooth.

Flowering: December to March. **Fruiting:** February-March

Specimens examined: INDIA, Odisha: Khurda District, Chandaka, 09.12.2023, P. K. Das & P.C. Panda 2561/CBT; Khurda District, Kantabada, Bhubaneswar, 09.12.2023, P. K. Das 2562/CBT. (Map - 1)



Map 1: Map showing the location of collection site of *Medicago polymorpha*

World Distribution: The native range of *Medicago polymorpha* is Macaronesia, Europe to Central Asia and W. Nepal, N. & NE. Tropical Africa to Arabian Peninsula but is found throughout the world. Besides it is introduced widely around the world.

Distribution in India: Delhi, Haryana, Himachal Pradesh, Uttar Pradesh, Punjab, Rajasthan, Gujarat, Maharashtra, Tamil Nadu, Madhya Pradesh, Chhattisgarh, Jharkhand, Bihar, Assam and West Bengal.

Habitat and Ecology: *Medicago polymorpha* grows in open waste places, roadsides and cultivated fields forming dense patches in association with *Cynodon dactylon*, *Oldenlandia corymbosa*, *Cyperus rotundus*, *Leucas aspera*. It is likely tobecome invasive in some areas where it is reported to occur by outcompeting local plants.

Uses: The species is a useful pasture plant and fodder for livestock. The decoctions, juices, infusions, powders, and pastes of various parts of *M. polymorpha* are used in traditional system of medicine (Yadav *et al.,* 2024). The leaves are cooked and consumed as a leafy vegetable (Abbasi *et al.,* 2013).

Acknowledgement

The authors are grateful to Prof. M. R. Nayak, President, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar for providing necessary facilities and encouragements.

References

Abbasi, A. M., Khan, M. A., Shah, M. H., Shah, M. M., Pervez, A. and Ahmad, M. (2013). Ethnobotanical appraisal and cultural values of medicinally important wild edible vegetables of Lesser Himalayas-Pakistan. J. Ethnobiol. Ethnomed. 9(1): 66.

Bairiganjan, G.C., Panda, P.C, Choudhury, B.P. & Patnaik, S. N. (1985). Fabaceae in Orissa. J. Econ. Tax. Bot. 7(2): 249-276.

Bairiganjan, G.C., Panda, P.C. & Patnaik, S.N. (1985). Cytotaxonomic studies in *Sesbania* Scop (Fabaceae) in Orissa. J. Orissa Bot. Soc. 6(1): 22-30.

Chavan, S., Sardesai, M. M. and Pokle, D. S. (2013) *Alysicarpus sanjappae* (Leguminosae-Papilionoideae): a new species from Western Ghats of India. Kew Bull. 68 (1): 183 186.

Das, P. K., Kamila, P. K. and Panda, P. C. (2020). *Arachis pintoi* Krapov. & W. C. Greg. (Fabaceae): An addition to the legume flora of Eastern Ghats of India. NeBio 11 (4): 271-273.

Dash, P. K. & Panda, P. C. (2013). *Crotalaria hebecarpa* (DC.) Rudd. (Fabaceae)- a new plant record for Odisha. J. Econ. Taxon. Bot. 37 (1): 109-110.

Haines, H. H. (1921). The Botany of Bihar and Orissa, Part-II, Adlard and Son & West Newman Ltd, London.

LPWG (2017). A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. Taxon 66 (1): 44-77.

Panda, P. C, Choudhury, B. P. & Patnaik, S. N. (1985). The genus *Macrotyloma* (Wt. & Arn.) Verdc. (Fabaceae) in Orissa. J. Econ. Tax. Bot. 7(3): 631-633.

Panda, P. C., Mishra, R. C. & Das, P. (1988). Three additional legumes for the flora of Orissa. J. Econ. Tax. Bot. 12(2): 371-372.

Sanjappa, M. (2020). Fabaceae (Leguminosae). In: Mao, A.A. & S.S. Dash (eds.). Flowering Plants of India: An annotated checklist- Dicotyledons, Botanical Survey of India, Kolkata, pp. 300-446.

Saxena, H. O. and Brahmam, M. (1994). The Flora of Orissa, Vol. 1, Orissa Forest Development Corporation and RRL, Bhubaneswar.

Schrire, B.D., Lavin, M. and Lewis, G.P. (2005). Global distribution patterns of the Leguminosae: Insights from recent phylogenies. Biol. Skr. 55: 375-422.

Yadav, G., Yadav, V., Patel, A., Das, S., Goyal, M., Satpathy, S. and Patra, A. (2024). Comprehensive review on traditional uses, phytochemistry, pharmacological properties and metal nanoparticles of a leafy vegetable, *Medicago polymorpha* Girendra. Eur. J. Med. Chem. Rep.11: 100164.

Yahara, T., Javadi, F., Onoda, Y., Queiroz, L.P. de, Faith, D., Prado, D.E. et al.(2013). Global legume diversity assessment: Concepts, key indicators, and strategies. Taxon 62: 249-266.

Plant Science Research 46 (1&2) : 5-14, 2024

Plant Science Research

ISSN 0972-8546

Microalgae and Cyanobacteria for Sustainable Wastewater Treatment and Circular Bioeconomy: A Review

Sk Riyazat Khadim[1Ψ], Kumar Avishek[1], Abhishek Mohanta[2] and Debaraj Parida[1]

[1] P.G. Department of Botany, Dhenkanal Autonomous College, Dhenkanal, Odisha
[2] Department of Botany, Betnoti College, Betnoti, Mayurbhanj, Odisha

ARTICLE INFO

Article history:
Received : 18 August 2024
Revised : 25 September 2024
Accepted : 17 October 2024

Keywords:

Wastewater treatment
Pollution
Microalgae
Cyanobacteria
Bioremediation
Resource recovery
High-value compounds

ABSTRACT

It has been imperative to remediate water of different wastewater sources and recover resources out of them to ameliorate dwindling ecological footprint of consumable water. Biological treatment, the most conventional method to remediate wastewater, has lately added microalgae and cyanobacteria to the inventory because of their ability to recover and utilize organic and inorganic nutrients like N, P, and carbon, from the wastewater while rapidly producing biomass rich in high value compounds. They are also found to be great bioagents for sequestering and removing array of different pollutants like heavy metals, pesticides, dyes, antibiotics, etc. This review encompasses critical account of microalgae and cyanobacteria based wastewater treatment to reduce load of pollutants and facilitate economic as well as efficient disposal of leftover sludge. Discussions are also made through various mechanisms employed by such microorganisms to bioremediatenew-age pollutants present in wastewater. It also emphasizes on developing circular bioeconomy approach by commercializing them through high value compounds produced during the treatment process for long-term sustainability of the process.

1. Introduction

The primary goal of wastewater (WW) treatment is to radically cut down the amount of carbonaceous materials, nitrogen (N) and phosphorus (P) components before being released into the environment. Most of the developing nations including India rely on conventional method of treating WW in a two-phase manner; primary and secondary treatments. The soluble organic debris that eludes primary treatment is eliminated by secondary treatment with the help of heterotrophic microbes. However, the Activated Sludge Process (ASP) of secondary treatment depletes large amount of oxygen, and maintaining optimal O_2 level is energy consuming and cost intensive. Instead, it has been suggested to check effluent discharge strictly within permissible limits. Traditional waste removal techniques use several chemical, physicaland biological processes. Chemical treatment methods such as ozonation, coagulation, chloramination, flocculation, and chlorination work by altering the properties of the water by removing dissolved compounds, turbidity causing suspended particles, adjusting pH, all of which improve water quality, although, they are associated with obligatory cost escalation due to expensive dewatering and maintenances. On the contrary, biological treatment tactics exploit the metabolic activity of the microbes via which wastes and pollutants of WW are putrefied and turned to biomass, releasing various gases like CO_2, SO_2, CH_4 and N_2 and eventually, declining the BOD/COD levels in the effluent (Dalvi *et al.*, 2021). Biological treatments involve living microbes like bacteria, fungi, microalgae and cyanobacteria that create an efficient system for bio-decomposition and bleaching of wastes. In spite of adopting these multitude WW treatment processes, the nutrient and pollutant removal still remains inadequate and unable to prevent eutrophication in the downstream water bodies, although, majority of biological systems cater to cost-effective treatment of WW under both aerobic and anaerobic environments.

Ψ *Corresponding author; Email:* sheikhriyazat@gmail.com/riyazat.bot24@dhenkanalcollege.ac.in

As the world strives hard to avail and utilize water efficiently, effluent discharge standards have become more rigorous and consequently, compelled to consume immense energy and release huge amount of CO_2, thus posing concern of economic feasibility and long-term sustainability. In developed nations, an estimated 3% of total electricity generated by the country is burned away for WW treatment while simultaneously contributing to substantial CO_2 emission (Wang *et al.,* 2016). A vast majority of this energy is utilized only to run the O_2 transfer equipments. Nitrifiers and denitrifiers remove inorganic N under aerobic and anoxic conditions respectively; Phosphate is accumulated by microbes under anoxic and anaerobic condition; and heterotrophic organisms utilize the carbonaceous materials under aerobic condition. This exceptionally labyrinthine rotation of treatment parameters under a continuous spatio-temporal scale is further aggravated with higher O_2 demand for assimilating or converting inorganic N and P by respective microbes. Accordingly, removal of 1 kg of N and P from WW would consume enough electricity that would have led to emission of 2.8 and 3.4 kg CO_2 respectively (Staff, 2012). About 3% of total anthropogenic GHG emission is contributed by treatment of WW through conventional means. Moreover, production of huge amount of activated sludge in conventional systems further adds to its disadvantage, promotes GHG emission, laden waste with toxic heavy metals, thus, posing serious handling and disposal concerns. Thus, conventional systems, although operated with relatively greater success, only shifts the problem to secondary pollution, extensive energy consumption and continual GHG emission. Therefore, novel processes must be explored to minimize energy consumption and sludge production, while maintaining economic sustainability.

2. Why to choose microalgae and cyanobacteria?

Microalgae and cyanobacteria are one of the extremely diverse oxygenic photosynthetic organisms possessing a very high photosynthetic rate, short generation period and spectrum of adaptation to diverse biotic and abiotic stresses (Tiwari *et al.,* 2019). The microalgal biomass is usually rich in an array of commercially important compounds like pigments, proteins, secondary metabolites, neutral lipids etc. Mass cultivation of microalgae serves an invariable limiting factor for large scale nutraceutical, pharmaceutical and biofuel applications (Khadim *et al.,* 2018). Their intrinsic ability to act as environment friendly and a sustainable tool for WW treatment, potentially replacing energy-exhaustive, conventional treatment systems, however remains largely underutilized (Singh *et al.,* 2015). In recent decades, microalgae and cyanobacteria have been utilized to get rid of various toxic substances originating from industrial, agricultural, and municipal sources, while producing invaluable compounds of commercial importance (Fig. 1). These systems offer innate disinfection properties and are generally more efficient at mitigating nutrient pollution compared to traditional treatment methods. In contrast, microalgae and cyanobacteria serve as highly effective agents for detoxifying a range of pollutants due to their huge surface-to-volume ratios, which significantly enhance their biosorption capabilities. Advantageously, these microorganisms can adapt to their surroundings via switching across growth modes, utilizing photoautotrophic, mixotrophic, and heterotrophic processes (Mohanta *et al.*, 2023). They take up carbon (C), nitrogen (N), phosphorus (P), and micronutrients from environment, convert them into organic compounds and render the heavy metals non-toxic. The levels of essential nutrients like nitrogen and non-renewable like phosphorus found in WW are adequate to support microalgal growth, carbon neutrality and production of invaluable compounds like neutral lipids for biofuel applications (Riyazat Khadim *et al.,* 2023). This capability not only lowers overall treatment costs but also mitigates environmental impact. Environmentally, microalgae represent a sustainable solution due to their significant capacity for CO_2 fixation through photosynthesis. The primary products of photosynthesis can be further transformed into valuable compounds, making this approach economically appealing as well. Thus, utilization of these microorganisms can contribute to reducing global warming while minimizing pollution.

3. Microalgae and cyanobacteria in Wastewater Treatment

Microalgae and cyanobacteria-based bioremediation is seen as an effective alternative to traditional WW treatment systems, providing a dependable method for managing liquid and solid waste generated by conventional approaches, while also converting them into commercially important products. One promising strategy to lower production costs for microalgal biomass based compounds is to integrate their cultivation with other common WW treatment systems. Given their strong ability to absorb nutrients and produce significant biomass, researchers have lately been proactive towards integrating WW treatment system with mass cultivation of microalgae for their commercial viability and achieving a circular bioeconomy. Microalgal cultivation encompasses autotrophic, heterotrophic, and mixotrophic growth modes. Autotrophic microalgae fix inorganic carbon like CO_2 as their carbon source, thereby helping to lower atmospheric CO_2 levels; for instance, 0.45 kg of microalgal biomass can absorb about 0.82 kg of CO_2. Some microalgae

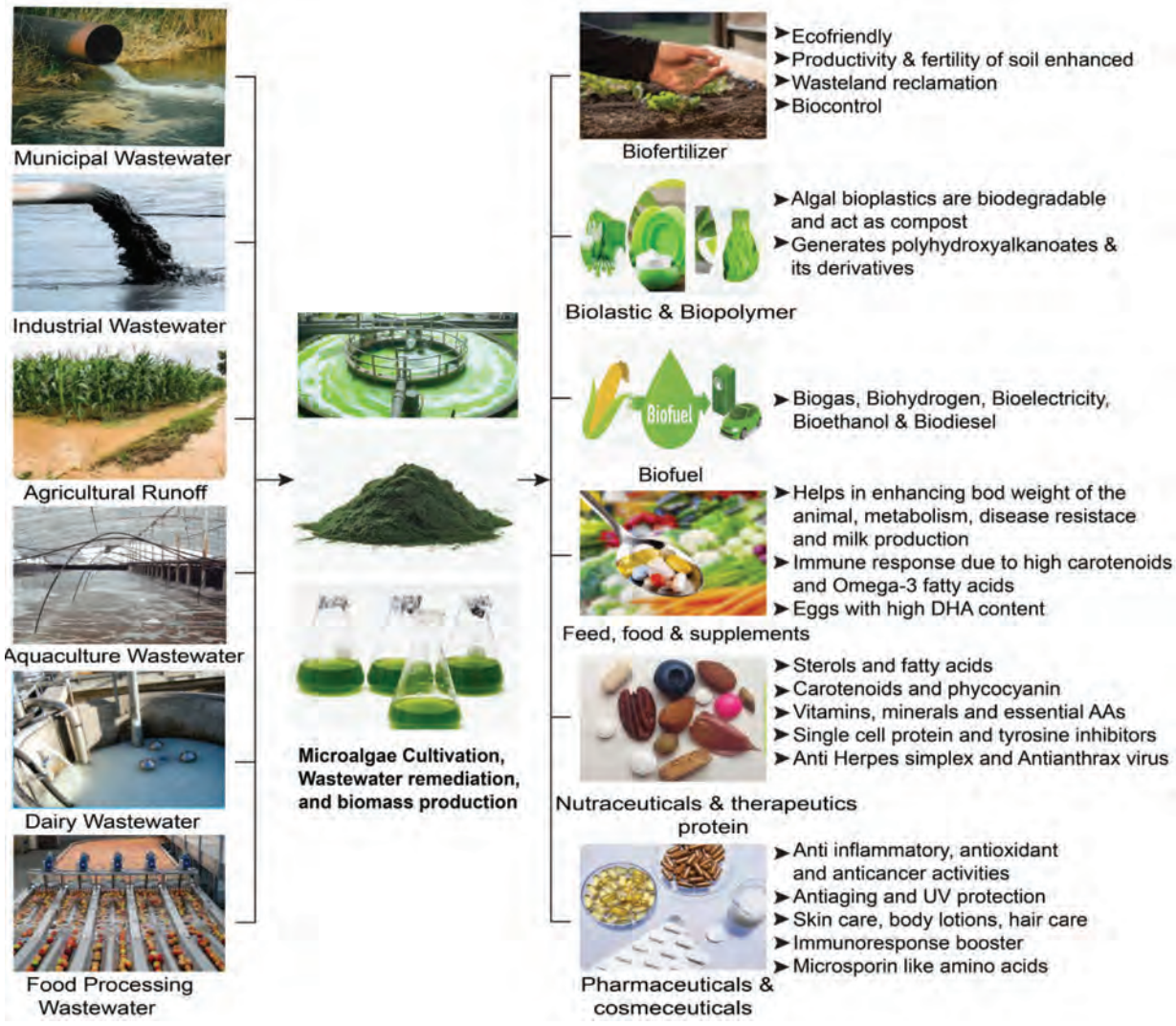


Figure 1: Microalgae and cyanobacteria mediated wastewater treatment with simultaneous production of commercially important high-value products.

are obligate heterotrophs that rely on organic carbon sources from industrial waste, such as glycerol, ethanol, acetate, and glucose. Many others are mixotrophic, capable of auxiliary fixation of CO_2 and organic carbon (Mohanta *et al.*, 2024). Additionally, microalgae are great assimilators of phosphorus and nitrogen, converting these nutrients into carbohydrates, proteins, fatty acids, and other valuable products (Khadim *et al.*, 2021). WW typically contains high levels of nutrients and incorporating microalgae into WW treatment can significantly reduce production costs and minimize the overall carbon footprint. Recent studies have increasingly focused on the microalgae-based treatment of WW originated from various sources, including industrial effluents, municipal, dairy, agricultural, aquaculture run-offs, etc.

3.1 Mechanism of microalgae and cyanobacteria-based environmental remediation

Microalgae and cyanobacteria can effectively remediate WW using any of the three major mechanisms; biosorption, biodegradation and bioaccumulation as in case of microalgae shown in Fig. 2.

3.1.1 Biosorption

Biosorption refers to a passive process in which a biological material acts as a sorbent, binding and concentrating pollutants from water. Essentially, biosorption involves the transfer of substances from the liquid phase to the surface of a solid. This process encompasses physical, chemical phenomenon including surface complexation, ion exchange, precipitation, electrostatic interactions, adsorption

and absorption, as well as the mechanisms independent of metabolism (Chia *et al.,* 2020). For biosorption to occur, a biosorbent (the solid phase) and a target sorbate (the dissolved or dispersed substance) are necessary. The biosorbent can be living or dead microorganisms, or even their components. The strong affinity of the biosorbent for the target sorbate drives the attraction of the sorbate species, with the total capacity influencing the number of sorbate molecules that can bind. This process eventually makes equilibrium between the amount of sorbate adsorbed by the biosorbent and its concentration remaining in the liquid. The cell wall plays a crucial role in biosorption, with its chemical composition greatly influencing the process and the mechanisms involved (see Fig. 2). Additionally, pores being there on the surfaces of microalgae and surface charges facilitate biosorption. Cell wall being rich in various chemical groups such as hydroxyl, carboxyl, and sulfate, serve as binding sites and act as efficient ion exchangers, aiding in the metal ion complexation and adsorption of organics from contaminated water. Microalgae effectively capture both cationic and anionic forms of heavy metals due to their abundance of deprotonated carboxyl and sulfate groups, along with monomeric alcohols that enhance biosorption. Extracellular polymeric substances (EPS) produced by these organisms can further facilitate metal biosorption, with their effectiveness influenced by the structure of the EPS, solution properties, metal types, and operational conditions (Mota *et al.,* 2016). They demonstrated that EPS released by *Cyanothece sp.* can remove heavy metals from contaminated water through organic functional groups, specifically carboxyl and hydroxyl groups, rather than through ion exchange. At low pH, the EPS from *Nostoc linckia* showed the ability to biosorb Co (II) and Cr (IV), which was linked to interactions between the metal ions and the negatively charged functional groups present in the EPS (Mona & Kaushik, 2015). Additionally, the interaction of EPS from *Chlorella vulgaris* with cadmium reduced intracellular Cd^{2+} levels, enhancing the microalga's capacity to remove NH_{4^+} and $PO_{4^{3-}}$.

3.1.2 Biodegradation

Biodegradation is another most efficient method for removing pollutants from effluents, as it involves the breakdown of complex compounds into simple and safer chemical units. Unlike biosorption, where microorganisms function like a biological filter that concentrate pollutants and extract them out of WW, bioremediation focuses on breaking down specific pollutants. This can be done through absolute mineralization of pollutants into CO_2 and H_2O or by biotransforming the parent molecule via series of enzymatic events producing various metabolic intermediates. Microalgae and cyanobacteria-based biodegradation occurs both extracellular and intracellularly, or involving both the processes. Initially, extracellular decomposition takes place, followed by the intracellular breakdown resulting different intermediates. The extracellular decomposition relies primarily on the extracellular polymeric substances (EPS) secreted by the microorganisms. Recalcitrant pollutants must be added with other chemical substances or organic functional groups to create a cometabolic system and facilitate their biodegradation.

3.1.3 Bioaccumulation

In contrast to biosorption, bioaccumulation is a metabolically active process that involves the use of various accumulators within the cell. It requires energy and typically occurs at a slower rate. In bioaccumulation, living cells detoxify waste by taking up substances and subsequently either store or breakdown them. It is crucial for removing organic as well as inorganic pollutants, such as nitrates, sulfates, phosphates, pesticides, and heavy metals.

Microalgae and cyanobacteria have the ability to accumulate various pollutants alongside nutrients and trace elements. They can withstand low concentrations of pollutants thanks to their immense adaptive capabilities (Singh *et al.,* 2023). Additionally, microalgae exhibit significantly high tolerance for a wide range of pollutants from agricultural, aqua cultural, domestic, industrial and dairy sources, which significantly enhance their bioremediation potential. It has been reported that *Chlorella vulgaris* was able to enhance levofloxacin bioaccumulation from 34 to 101 mg/g cell weight upon 1% NaCl addition suggesting that a stressful environment can lead to membrane depolarization facilitating the bioaccumulation process (Xiong *et al.,* 2017). Regardless of the mechanism, both external and internal physicochemical parameter such as pollutant concentration, temperature, pH, and contact time, play crucial role in bioaccumulation. For example, the bioaccumulation of antibiotics across planktonic food web was notably affected by temperature (Trang *et al.,* 2021). In another study, *Scenedesmus obliquus* and *Chlamydomonas mexicana* were found to enhance bioaccumulation of carbamazepine with both longer cultivation time and higher carbamazepine concentrations (Xiong *et al.,* 2016).

4 Microalgae and cyanobacteria-mediated WW treatment: Applications

4.1 Domestic WW treatment

Of late, interest in microalgae and cyanobacteria-mediated WW treatment has grown big owing to less energy consumption, resilience of these microorganisms to thrive

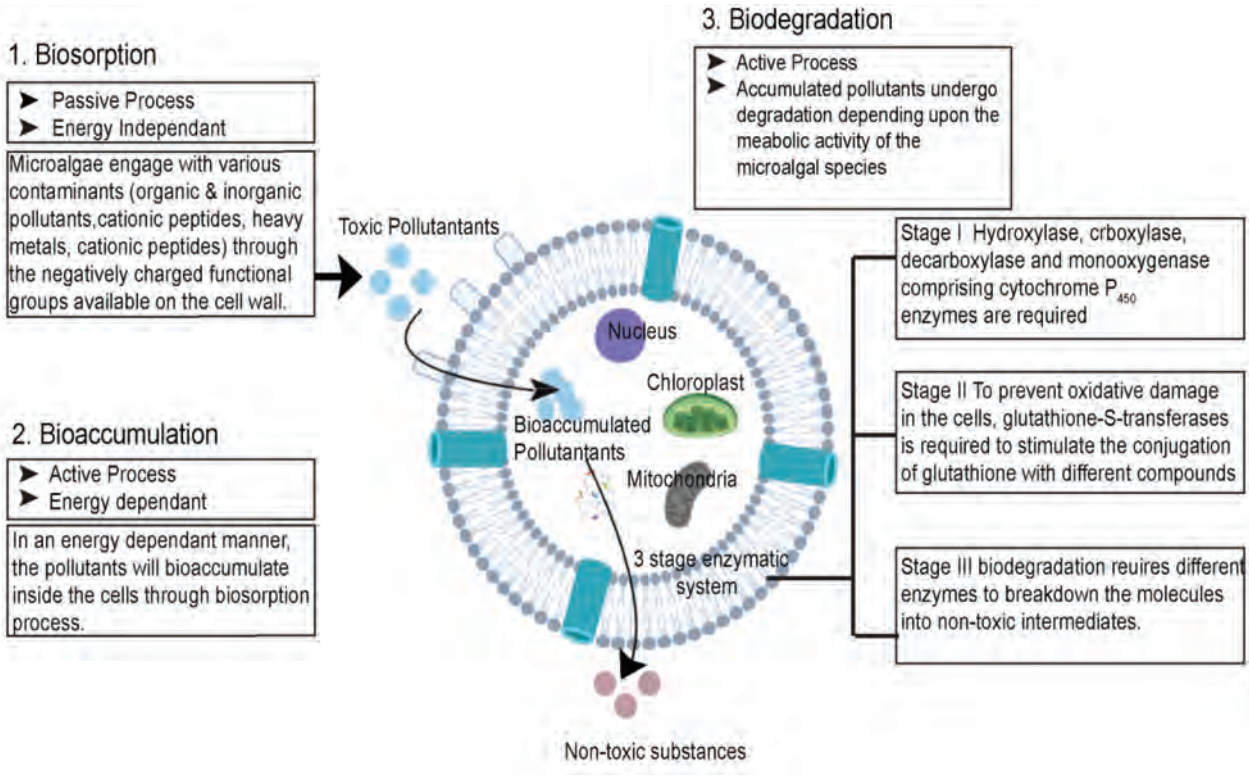


Figure 2: Employment of various bioremediation mechanisms by microalgae for pollutant removal in wastewater.

under diverse environmental and stressful conditions producing commercially important high-value compounds by converting and recovering WW nutrients. Domestic WW remediation using microalgae can help to meet increasingly stringent release and reuse norms since microalgae primarily rely on N and P for synthesis of proteins. A diverse array of microalgae species has been documented for their effectiveness in treating domestic WW, including *Botryococcus* sp., *Chlorella variabilis*, *C. vulgaris*, *C. sorokiniana*, *S. abundans* and *Scenedesmus obliquus* (Ling *et al.*, 2019; Sundar Rajan *et al.*, 2020; Tran *et al.*, 2021). Microalgae-based systems demonstrate a remarkable ability to remediate WW, effectively removing 45 to 65% of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (Al-Jabri *et al.*, 2020). In a study, the 17.59% removal of chemical oxygen demand (COD) was primarily attributed to the symbiotic relationship between microalgae and partner aerobic bacteria, where microalgal photosynthesis provided oxygen necessary for oxidizing organic matter by aerobic bacteria(Gonzalez-Camejo *et al.*, 2018; Gupta *et al.*, 2021) evaluated the consequence of light on NH4+-N competition and growth of microalgae in a microalgal-bacterial hybrid consortium. They found that increased light intensity enhanced NH_{4^+}-N assimilation by microalgae, promoting their growth over the oxidation of NH_{4^+}-N by nitrifying bacteria. (Al Momani & Örmeci, 2016) explored the potential of using *C. vulgaris*, *Neochloris oleoabundans*, and indigenous microalgal mixtures for their growth through removal and assimilation of organic carbon, nitrogen, and phosphorus from various effluents. They found that *C. vulgaris* and *N. oleoabundans* exhibited highest growth rates under C:N:P ratio of 24:5:1 in primary WW, while the mixed microalgae culture thrived well in secondary sludge with a C:N:P ratio of 4.4:1:1.5. These results call for thorough assessment of the nutrient compositions in various municipal effluents prior to microalgae treatment procedures. Similarly, many cyanobacterial species are found proficient in removal of industrial dyes and heavy metals (refer Table 1B and 1C).

4.2 Pathogen removal by microalgae

Microalgae present a promising way for the elimination or inactivation of pathogenic microorganisms via various mechanisms such as resource competition, pH alteration, increased oxygenation, sedimentation, and the production of microalgal toxins. Microalgae rapidly absorb nutrients and carbon of WW, thus starving bacteria for essential energy resources. Microalgal photosynthesis leads to an increased pH and elevated dissolved oxygen concentrations in WW that can inhibit pathogen growth. In a study (Mezzari *et al.*, 2017) found out that antibiotic-resistant pathogen *Salmonella typhimurium* was inhibited owing to altered pH and dissolved oxygen concentration augmented by microalgal photosynthesis within 48 hours of cultivation.

Recently, two microalgal species, *Mougeotia* sp. and *Hydrodictyon* sp., were found to effectively raise pH and DO levels in WW treatment ponds, which in turn improve the removal of total coliforms including *E. coli*, *Clostridium perfringens* and *Enterococci* (L. Liu *et al.,* 2020). Highest inactivation level for all but *Enterococci* was achieved at pH 10.5. *E. coli* as well as total coliforms showed reductions at both high (20 mg/L) and low (1 mg/L) DO levels, whereas medium DO level (8.6 mg/L) allowed them to survive. Again, Alsenani *et al.,* (2020) observed that extracts of several microalgae significantly inhibited proliferation of gram-positive bacteria, albeit, showed no inhibition for gram-negative bacteria, attributing the difference in antibacterial effectiveness to the distinct structures of the cell walls.

4.3 Industrial WW treatment

To treat industrial effluents, various treatment processes, including electrochemical treatments, activated sludge, membrane filtration, and advanced oxidation are employed, although with limited success, as these technologies come along only partial removal of heavy metals with high operation and maintenance costs, and the production of secondary wastes and noxious sludge as by-products. Microalgae and cyanobacteria-mediated bioremediation of industrial wastes offer eco-friendly, sustainable approach that can also be used for resource recovery as discussed below and shown in Table 1. A major class of pollutants present in industrial WW is pharmaceutical compounds (PCs), which include wide range of chemical species, often imparting negative consequences to local ecosystem by altering microbial diversities, reducing activity of soil microbes, and impacting denitrification rates. Mixotrophic microalgae are known to be capable of extracting and absorbing PCs from industrial WW (Table 1A). Mechanisms like bioaccumulation, biosorption, and biodegradation by these microorganisms play critical role in the removal of PCs from the environment, as illustrated in (Fig. 2.) (de Godos *et al.,* 2012) found that *Chlorella vulgaris* was able to sequester over 50% of tetracycline while cultured in high-capacity pond primarily through adsorption. *Chlorella* sp. was also found effective in bioaccumulation of trimethoprim, sulfamethoxazole, florfenicol, and carbamazepine via passive diffusion (Song *et al.,* 2019). Similarly (Zhang *et al.,* 2014) observed that approximately 85% of 17α-estradiol could be degraded by *Scenedesmus dimorphus* within seven cultivation days. *Chlorella sorokiniana* could completely eliminate ibuprofen and paracetamol through the use of its extracellular polymeric substance (EPS) matrix (de Wilt *et al.,* 2016).

Table 1A

Mechanisms of removal for pharmaceutical compounds (PCs) by microalgae.

Microalgae	PCs	Mechanism	Removal (%)	Reference
Desmodesmus subspicatus	17α-ethinylestradiol	Adsorption & biodegradation	68	(Maes *et al.,* 2014)
Chlorella pyrenoidosa	Norgestrel	Biodegradation	60	(Peng *et al.,* 2014)
Chlamydomonas reinhardtii	β-estradiol	Adsorption & biodegradation	100	(Hom-Diaz *et al.,* 2015)
Chlorella vulgaris	Metronidazole	Adsorption	100	(Hena *et al.,* 2020)
Chlorella sp.	Florfenicol	Bioaccumulation, biodegradation & adsorption	97	(Song *et al.,* 2019)

Table 1B

Abatement of dyes using microalgae and cyanobacteria

Dyes	Microalgae	The influent concentration of dye (mg L^{-1})	Removal efficiency (%)	Reference
Methylene blue	*Chlorella vulgaris*	100	83.04	(Chin et al., 2020)
Reactive Black 5	*Chlorella vulgaris*	200	80	(Ishchi & Sibi, 2020)
Direct Blue 71	*Chlorella vulgaris*	200	78	(Ishchi & Sibi, 2020)
Disperse Red 1	*Chlorella vulgaris*	300	84	(Ishchi & Sibi, 2020)

Malachite green	*Oscillatoria* sp.	5	93	(Gelebo *et al.*, 2020)
Safranin	*Oscillatoria* sp.	5	52	(Gelebo *et al.*, 2020)
Malachite green	*Haematococcus* sp.	100	67	(J. H. Liu *et al.*, 2018)
Acid Black 210	*Spirulina platensis*	125	98.55	(Al Hamadi *et al.*, 2017)
Acid Blue 7	*Spirulina platensis*	125	97.05	(Al Hamadi *et al.*, 2017)

Table 1C

Remediation of heavy metals (HMs) using microalgae and cyanobacteria.

HMs	HMs concentration (mg L^{-1})	Treatment method	Microalgae	Biomass concentration (g)	Reference
Cadmium	50	Biosorption	*Spirulina platensis*	1	(Gunasundari, 2017)
Chromium	50	Biosorption	*Spirulina platensis*	1	(Gunasundari, 2017)
Cadmium	50	Biosorption	*Anabaena sphaerica*	0.25	(Abdel-Aty *et al.*, 2013)
Lead	50	Biosorption	*Anabaena sphaerica*	0.25	(Abdel-Aty *et al.*, 2013)
Lead	100	Biosorption	*Pseudochlorococcum typicum*	4.52 µgchl a ml-1	(Shanab *et al.*, 2012)
Mercury	100	Biosorption	*Pseudochlorococcum typicum*	4.52 µgchl a ml-1	(Shanab *et al.*, 2012)
Lead	-	Biosorption	*Chlorella vulgaris*	2	(Ferreira *et al.*, 2011)
Nickel	-	Biosorption	*Chlorella vulgaris*	2	(Ferreira *et al.*, 2011)
Zinc	-	Fixed-bed column	*Chlorella vulgaris*	2	(Ferreira *et al.*, 2011)

4.4 Removal of Heavy Metals

Heavy metals (HMs) are a category of metals with atomic density exceeding 4000 kg/m³. Their non-biodegradable nature, widespread availability, toxicity, and tendency to accumulate make them a significant global issue, with potential health hazard risk to humans and animals. Remediation techniques for HMs from WW mediated through different microalgae can be categorized into three basic approaches: physical, chemical, and biological methods. Cell walls of microalgae and cyanobacteria possess the carboxyl, amino, sulfate and hydroxyl groups that serve as the primary binding sites for removal of HMs from WW. Freshwater microalgae are often used to effectively remove various HMs from WW. Several green algae, including *Chlamy domonas reinhardtii*, *Chlorella vulgaris*, *C. miniata*, and *Sphaero plea*, have proven successful in the removal of toxic HMs from WW (Gunasundari, 2017; Shanab *et al.*, 2012; Sibi, 2016).

4.5 Removal of Dyes

The mounting applications of dyes across various industries have led to their widespread discharge into ecosystems, resulting in unpleasant aesthetic impacts and negative environmental consequences. Phycoremediation of dyes exploit algal cell wall functional groups that assist in removal of dyes. Several factors are crucial for effective dye removal, such as pH, contact time, temperature, adsorbent level and initial dye concentration. Phycoremediation uses microalgae and cyanobacteria to efficiently eliminate or biotransform pollutants such as dyes, xenobiotics and HMs from WW, CO_2 from polluted air and thus, contributing to environmental amelioration efforts. Removal of dyes using microalgae offers several advantages, being a simple, ecofriendly and cost-effective process with higher adsorption efficiency. *Chlorella vulgaris* was found to remove more than 83% of methylene blue, 80% of Reactive Black 5, 78% of Direct Blue 71, and 84% of Disperse red 1 from effluents (Chin *et al.*, 2020; Ishchi & Sibi, 2020). Similarly, *Oscillatoria* sp. was able to extract about 93 and 52% of malachite green and safranin from WWs at an initial dye concentration of 5 mg/L (Gelebo *et al.*, 2020). Another study showed extraordinary dye removal potential of *Spirulina platensis* by obtaining 98.55 and 97.05% removal efficiency for Acid Black 210 and Acid Blue 7 respectively in WWs with 125 mg/L dye concentration (Al Hamadi *et al.*, 2017). Moreover, (Brahmbhatt & Jasrai 2016) have demonstrated efficient dye

removal ability of several microalgae and cyanobacterial species employed to remove blue and red dyes.

5. Conclusion and Future Prospects

Microalgae and cyanobacteria possess extraordinary ability to eliminate toxic pollutants and reuse valuable resources from array of diverse WW sources. Phycoremediation does not malign ecosystem with secondary pollution and the produced biomass can be used for circular economy by commercializing the high value compounds obtained from their biomass. In addition to the described processes for WW treatment, many new technologies such as co-culturing them with bacterial or fungal species, synergistic application with microalgae and cyanobacterial biomass-derived nanoparticles are coming up to improve economy and efficiency of the process. However, several bottlenecks still needs to be addressed like translating pilot scale outputs into industrial scale applications, calibration of suitable strain with optimum environmental parameters for particular WW type, economic separation of their high value microalgal biomass from the treated water source, simplifying the operation with sufficient funding for research. Hence, WW treatment through microalgae and cyanobacteria and their biorefinery application mandates further research.

References

Abdel-Aty, A. M., Ammar, N. S., Ghafar, H. H. A., & Ali, R. K. (2013). Biosorption of cadmium and lead from aqueous solution by fresh water alga *Anabaena sphaerica* biomass. *Journal of Advanced Research*, *4*(4), 367–374.

Abdelfattah, A., Ali, S. S., Ramadan, H., El-Aswar, E. I., Eltawab, R., Ho, S.-H., Elsamahy, T., Li, S., El-Sheekh, M. M., & Schagerl, M. (2023). Microalgae-based wastewater treatment: Mechanisms, challenges, recent advances, and future prospects. *Environmental Science and Ecotechnology*, *13*, 100205.

Al-Jabri, H., Das, P., Khan, S., Thaher, M., & AbdulQuadir, M. (2020). Treatment of wastewaters by microalgae and the potential applications of the produced biomass—a review. *Water*, *13*(1), 27.

Al Hamadi, A., Uraz, G., Katýrcýoðlu, H., & Osmanaðaoðlu, Ö. (2017). Adsorption of azo dyes from textile wastewater by *Spirulina platensis*. *Eurasian Journal of Environmental Research, 1*(1), 19–27.

AlMomani, F. A., & Örmeci, B. (2016). Performance of *Chlorella vulgaris, Neochloris oleoabundans* and mixed indigenous microalgae for treatment of primary effluent, secondary effluent and centrate. *Ecological Engineering*, *95*, 280–289.

Alsenani, F., Tupally, K. R., Chua, E. T., Eltanahy, E., Alsufyani, H., Parekh, H. S., & Schenk, P. M. (2020). Evaluation of microalgae and cyanobacteria as potential sources of antimicrobial compounds. *Saudi Pharmaceutical Journal*, *28*(12), 1834–1841.

Brahmbhatt, N. H., & Jasrai, R. T. (2016). The role of algae in bioremediation of textile effluent. *Methods*, *21*, 28.

Chia, W. Y., Tang, D. Y. Y., Khoo, K. S., Lup, A. N. K., & Chew, K. W. (2020). Nature's fight against plastic pollution: Algae for plastic biodegradation and bioplastics production. *Environmental Science and Ecotechnology*, *4*, 100065.

Chin, J. Y., Chng, L. M., Leong, S. S., Yeap, S. P., Yasin, N. H. M., & Toh, P. Y. (2020). Removal of synthetic Dye by *Chlorella vulgaris* microalgae as natural adsorbent. *Arabian Journal for Science and Engineering*, *45*, 7385–7395.

Dalvi, V., Naaz, F., Nigam, H., Jain, R., Samuchiwal, S., Kalia, S., Kumar, R., Mathur, M., Bano, F., & Malik, A. (2021). Removal of pollutants from wastewater via biological methods and shifts in microbial community profile during treatment process. *Wastewater Treatment Reactors*, 19–38.

de Godos, I., Muñoz, R., & Guieysse, B. (2012). Tetracycline removal during wastewater treatment in high-rate algal ponds. *Journal of Hazardous Materials*, *229*, 446–449.

de Wilt, A., Butkovskyi, A., Tuantet, K., Leal, L. H., Fernandes, T. V, Langenhoff, A., & Zeeman, G. (2016). Micropollutant removal in an algal treatment system fed with source separated wastewater streams. *Journal of Hazardous Materials*, *304*, 84–92.

Ferreira, L. S., Rodrigues, M. S., De Carvalho, J. C. M., Lodi, A., Finocchio, E., Perego, P., & Converti, A. (2011). Adsorption of Ni^{2+}, Zn^{2+} and Pb^{2+} onto dry biomass of *Arthrospira* (Spirulina) *platensis* and *Chlorella vulgaris*. I. Single metal systems. *Chemical Engineering Journal*, *173*(2), 326–333.

Gelebo, G. G., Tessema, L. H., Kehshin, K. T., Gebremariam, H. H., Gebremikal, E. T., Motuma, M. T., Ayele, A., Getachew, D., Benor, S., & Suresh, A. (2020). Phycoremediation of synthetic dyes in an aqueous solution using an indigenous *Oscillatoria sp.,* from Ethiopia. *Ethiopian Journal of Science and Sustainable Development*, *7*(2), 14–20.

Gonzalez-Camejo, J., Barat, R., Pachés, M., Murgui, M., Seco, A., & Ferrer, J. (2018). Wastewater nutrient removal in a mixed microalgae–bacteria culture: effect of light and temperature on the microalgae–bacteria competition. *Environmental Technology*, *39*(4), 503–515.

Gunasundari, E. (2017). Adsorption isotherm, kinetics and thermodynamic analysis of Cu (II) ions onto the dried algal biomass (*Spirulina platensis*). *Journal of Industrial and Engineering Chemistry*, *56*, 129–144.

Gupta, S., Nayak, A., Roy, C., & Yadav, A. K. (2021). An algal assisted constructed wetland-microbial fuel cell integrated with sand filter for efficient wastewater treatment and electricity production. *Chemosphere*, *263*, 128132.

Hena, S., Gutierrez, L., & Croué, J.P. (2020). Removal of metronidazole from aqueous media by *C. vulgaris*. *Journal of Hazardous Materials*, *384*, 121400.

Hom-Diaz, A., Llorca, M., Rodríguez-Mozaz, S., Vicent, T., Barceló, D., & Blánquez, P. (2015). Microalgae cultivation on wastewater digestate: â-estradiol and 17á-ethynylestradiol degradation and transformation products identification. *Journal of Environmental Management*, *155*, 106–113.

Ishchi, T., & Sibi, G. (2020). Azo dye degradation by *Chlorella vulgaris:* optimization and kinetics. *Int. J. Biol. Chem*, *14*(1), 1–7.

Khadim, S. R., Mohanta, A., Maurya, P., Singh, P., & Asthana, R. K. (2021). Strategies for increased production of lipids and fine chemicals from commercially important microalgae. In Natural Bioactive Compounds, 165-186. Academic Press.

Khadim, S. R., Singh, P., Singh, A. K., Tiwari, A., Mohanta, A., & Asthana, R. K. (2018). Mass cultivation of Dunaliella salina in a flat plate photobioreactor and its effective harvesting. *Bioresource Technology*, *270*, 20–29.

Ling, Y., Sun, L., Wang, S., Lin, C. S. K., Sun, Z., & Zhou, Z. (2019). Cultivation of oleaginous microalga *Scenedesmus obliquus* coupled with wastewater treatment for enhanced biomass and lipid production. *Biochemical Engineering Journal*, *148*, 162–169.

Liu, J. H., Zhang, L., Zha, D. C., Chen, L. Q., Chen, X. X., & Qi, Z. M. (2018). Biosorption of malachite green onto *Haematococcus pluvialis* observed through synchrotron Fourier transform infrared microspectroscopy. *Letters in Applied Microbiology*, *67*(4), 348–353.

Liu, L., Hall, G., & Champagne, P. (2020). The role of algae in the removal and inactivation of pathogenic indicator organisms in wastewater stabilization pond systems. *Algal Research*, *46*, 101777.

Maes, H. M., Maletz, S. X., Ratte, H. T., Hollender, J., & Schaeffer, A. (2014). Uptake, elimination, and biotransformation of 17á-ethinylestradiol by the freshwater alga Desmodesmus subspicatus. *Environmental Science & Technology*, *48*(20), 12354–12361.

Mezzari, M. P., Prandini, J. M., Kich, J. D., & da Silva, M. L. B. (2017). Elimination of antibiotic multi-resistant salmonella typhimurium from swine waste water by microalgae-induced antibacterial mechanisms.

Mohanta, A., Khadim, S. R., Singh, P., Laxmi, & Asthana, R. K. (2023). A Study on Mixotrophic Approach for Maximizing Lipid Production in a Hypersaline Microalga, *Dunaliella salina*. *Bio Energy Research*, 16(4), 2512-2528.

Mohanta, A., Prasad, N., Khadim, S. R., Singh, P., Singh, S., Singh, A. & Asthana, R. K. (2024). Optimizing light regimes for neutral lipid accumulation in *Dunaliella salina* MCC 43: a study on physiological status and carbon allocation. World Journal of Microbiology and Biotechnology, 40(3), 82.

Mona, S., & Kaushik, A. (2015). Chromium and cobalt sequestration using exopolysaccharides produced by freshwater cyanobacterium *Nostoc linckia*. *Ecological Engineering*, *82*, 121–125.

Mota, R., Rossi, F., Andrenelli, L., Pereira, S. B., De Philippis, R., & Tamagnini, P. (2016). Released polysaccharides (RPS) from *Cyanothece sp.* CCY 0110 as biosorbent for heavy metals bioremediation: interactions between metals and RPS binding sites. *Applied Microbiology and Biotechnology*, *100*, 7765–7775.

Peng, F.-Q., Ying, G.-G., Yang, B., Liu, S., Lai, H.-J., Liu, Y.-S., Chen, Z.-F., & Zhou, G.-J. (2014). Biotransformation of progesterone and norgestrel by two freshwater microalgae (*Scenedesmus obliquus* and *Chlorella pyrenoidosa*): transformation kinetics and products identification. *Chemosphere*, *95*, 581–588.

Riyazat Khadim, S., Mohanta, A., Singh, P., Maurya, P., Kumar Singh, A., Kumar Singh, A., & Asthana, R. K. (2023). A study on *Dunaliella salina* under selected nutrient manipulation with reference to the biomass, lipid content along with expression of ACCase and RuBisCO genes. BioEnergy Research, 16(1), 622-637.

Shanab, S., Essa, A., & Shalaby, E. (2012). Bioremoval capacity of three heavy metals by some microalgae species (Egyptian Isolates). *Plant Signaling & Behavior*, *7*(3), 392–399.

Sibi, G. (2016). Biosorption of chromium from electroplating and galvanizing industrial effluents under extreme conditions using *Chlorella vulgaris*. *Green Energy & Environment*, *1*(2), 172–177.

Singh, B., Bauddh, K., & Bux, F. (2015). *Algae and environmental sustainability* (Vol. 7). Springer.

Singh, P., Singh, S., Maurya, P., Mohanta, A., Dubey, H., Khadim, S. R. & Asthana, R. K. (2023). Bioaccumulation of selenium in halotolerant microalga *Dunaliella salina*

and its impact on photosynthesis, reactive oxygen species, antioxidative enzymes, and neutral lipids. Marine Pollution Bulletin, 190, 114842.

Song, C., Wei, Y., Qiu, Y., Qi, Y., Li, Y. & Kitamura, Y. (2019). Biodegradability and mechanism of florfenicol via *Chlorella sp.* UTEX1602 and L38: experimental study. *Bioresource Technology*, *272*, 529–534.

Staff, I. E. A. (2012). *CO2 emissions from fuel combustion*. OECD.

Sundar Rajan, P., Gopinath, K. P., Arun, J., Grace Pavithra, K., Pavendan, K., & AdithyaJoseph, A. (2020). An insight into carbon balance of product streams from hydrothermal liquefaction of *Scenedesmus abundans* biomass. *Renewable Energy*, *151*, 79–87.

Tiwari, A., Singh, P., Riyazat Khadim, S., Singh, A. K., Singh, U., Singh, P. & Asthana, R. K. (2019). Role of Ca^{2+} as protectant under heat stress by regulation of photosynthesis and membrane saturation in *Anabaena* PCC 7120. *Protoplasma*, 256, 681-691.

Tran, D., Do, V., Quang Trung, N., & Le Truong, G. (2021). Simultaneous removal of pollutants and high value biomaterials production by *Chlorella variabilis* TH03 from domestic wastewater. *Clean Technologies and Environmental Policy*, *23*.

Trang, N. T. H., Tang, D. Y. Y., Chew, K. W., Linh, N. T., Hoang, L. T., Cuong, N. T., Yen, H. T., Thao, N. T., Trung, N. T. & Show, P. L. (2021). Discovery of á-glucosidase inhibitors from marine microorganisms: Optimization of culture conditions and medium composition. *Molecular Biotechnology*, *63*(11), 1004–1015.

Wang, H., Yang, Y., Keller, A., Li, X., Feng, S., Dong, Y., & Fengting, L. (2016). Comparative Analysis of Energy Intensity and Carbon Emissions in Wastewater Treatment in USA, Germany, China and South Africa. *Applied Energy*, *184*. https://doi.org/10.1016/j.apenergy.2016.07.061

Xiong, J.-Q., Kurade, M. B., Abou-Shanab, R. A. I., Ji, M.-K., Choi, J., Kim, J. O., & Jeon, B.-H. (2016). Biodegradation of carbamazepine using freshwater microalgae *Chlamydomonas mexicana* and *Scenedesmus obliquus* and the determination of its metabolic fate. *Bioresource Technology*, *205*, 183–190.

Xiong, J.-Q., Kurade, M., & Jeon, B.-H. (2017). Biodegradation of levofloxacin by an acclimated freshwater microalga, *Chlorella vulgaris*. *The Chemical Engineering Journal*, *317*, 1251–1257.

Zhang, Y., Habteselassie, M. Y., Resurreccion, E. P., Mantripragada, V., Peng, S., Bauer, S., & Colosi, L. M. (2014). Evaluating Removal of Steroid Estrogens by a Model Alga as a Possible Sustainability Benefit of Hypothetical Integrated Algae Cultivation and Wastewater Treatment Systems. *ACS Sustainable Chemistry & Engineering*, *2*(11), 2544–2553.

Plant Science Research 46 (1&2) : 15-21, 2024

Plant Science Research

ISSN 0972-8546

Preliminary Hairy Root Induction in *Lawsonia inermis* Using *In-Vivo* Explants: An Assessment of Key Parameters for Transformation

Aryaputri Bedaprabha[1], Bhaswatimayee Mahakur[1] and Durga Prasad Barik[1Ψ]
Department of Botany, Ravenshaw University, Cuttack-753003, Odisha, India

ARTICLE INFO

Article history:
Received : 22 August 2024
Revised : 5 October 2024
Accepted : 25 October 2024

Keywords:

Lawsonia inermis
Agrobacterium rhizogenes
In-vivo explant
Acetosyringone
Hairy root culture

ABSTRACT

Lawsonia inermis L. or Henna belongs to the family Lythraceae and is highly important in various fields, including cosmetic, traditional and modern medicine. Hairy roots were successfully induced in *L. inermis in-vivo* explants using two *Agrobacterium rhizogenes* strains, MTCC 532 and MTCC 2364. The highest transformation efficiency was observed in MTCC 2364 strain, with a 35.55% success rate in leaf explants and 24.44% in internodes with average of 2.62 and 2.51 root numbers respectively. In contrast, MTCC 532 failed to induce hairy roots in these explants.The induction process was significantly enhanced by the presence of 100 µM acetosyringone in the subculture media; no root initiation was observed in media lacking acetosyringone. Optimization of infection parameters revealed that the most effective results were achieved with a co-cultivation period of 48 hours and infection times of 45 minutes for leaf explants and 70 minutes for internode explants. Additionally, the bacterial suspension's optical density (OD_{600}) played a crucial role in root induction. Maximum transformation frequency was observed with an OD_{600} of 0.75 for leaf explants and 0.8 for internodes. These findings underscore the importance of selecting appropriate bacterial strains and optimizing infection conditions, particularly co-cultivation time, infection duration and acetosyringone concentration, to achieve high hairy root induction rates in *L. inermis*. The given approach can be used as a guide to creating an industrial-scale hairy root production system that focuses on the bioactive substances present in *Lawsonia inermis* roots. This method may optimize the production process and raise the yield and quality of these compounds, which are critical for use in the medical, cosmetic, and pharmaceutical sectors. Using this methodology to scale up the hairy root culture provides an effective and sustainable way to address the growing demand for these essential natural products.

1. Introduction

Lawsonia inermis L., commonly known as henna, belongs to family Lythraceae is a tropical plant native to North Africa, the Indian subcontinent, and the Middle East. It has been cultivated for centuries for its leaves, which produce a red-orange dye widely used for body art and hair coloring. The active compound responsible for the dyeing property is lawsone (2-hydroxy-1, 4-naphthoquinone) (Lekouch *et al.,* 2001), a naphthoquinone that binds to keratin that is present in skin and hair, resulting in a natural stain. Beyond its cosmetic applications, *Lawsonia inermis* holds significant medicinal value in traditional and modern medicine. It has been used for its antifungal, antibacterial, anti-hepatic and anti-inflammatory properties, and is often applied to treat wounds and digestive issues. Modern research has expanded into studying its pharmacological potential, including its antioxidant and antimicrobial activities, making it a subject of interest in both therapeutic and cosmetic fields. Henna has been used as a non-toxic medicinal agent in Ayurvedic and Unani medicine for treating skin diseases, cancer, burn wounds, gonorrhoea, hysteria, insomnia treatment, inflammation, TB, Jaundice, tumors, and so many other diseases. It can be used alone or in combination with other compounds (Patel & Patel, 2017; Sen *et al.,* 2023; Muheyuddeen *et al.,* 2023). The use of phytochemicals of this plant and their derivatives in contemporary drug development techniques may yield a novel molecule for certain medicinal actions. It is important

Ψ *Corresponding author; Email:* dpbarik@ravenshawuniversity.ac.in

to note that lawsone possesses strong anti-inflammatory and antioxidant properties, which may be a key component of several phytochemicals with anticancerous properties (Pradhan *et al.,* 2012). The normal roots of henna is lacking lawsone whereas transformed roots with lawsone used for the treatment of Jaundice (Bakkali *et al.,* 1997).

Recently, an additional method for producing secondary metabolites of commercial significance has been the use of root cultures acquired by transformation with the Gram-negative bacteria *Agrobacterium rhizogenes*. Through conjugational transfer into the plant genome and expression, a specific section of root-inducing plasmid DNA of *A. rhizogenes* (pRi T-DNA) facilitates interaction with plant cells. Due to this, "hairy root" conditions develop, which are characterized by two auxin biosynthesis genes (iaaM, iaaH), being synthesized and inducing adventitious root growth at the infection site. These genetically altered roots develop quickly in *in-vitro* culture, and they also show a lot of lateral branching, negative geotropism, cytogenetic stability, and the capacity to grow and biosynthesize continuously in the medium without the use of growth regulators (Sahu *et al.,* 2015). This technique will provide opportunities for the large-scale, sustainable production of beneficial phytochemicals from *Lawsonia inermis*.

For the first time, *in-vivo* explants of *Lawsonia inermis* were used to optimize various parameters in order to maximize the transformation efficiency and to create a repeatable procedure for the production of hairy roots. To successfully induce and develop hairy root cultures of *Lawsonia inermis* using *A. rhizogenes*, several parameters influencing genetic transformation need to be carefully assessed and standardized. These include the kind and source of the explant, the bacterial strains, the acetosyringone pre-culture, the infection period, the bacterial cell density, and the co-cultivation period. These cultures can potentially to be a viable and long-lasting source of phytochemicals with significant medicinal value.

2. Materials and methods

2.1. Explant collection and surface sterilization

Plant *Lawsonia inermis* in Ravenshaw University's department garden. The leaves of *L. inermis* were verified and assigned specimen number 2539/CBT by Dr. P. C. Panda, Biotechnology Department, Siksha "O"Anusandhan University, Bhubaneswar, Odisha. From that donor plant, valuable explants were collected and employed in the infection process. Explants from the mother plant, such as leaves and internodal segments were removed and surface sterilized to discard the dust and microorganisms. Explants were treated further for 25 minutes with a 2% labolene therapy after 30 minutes of treatment under running tap water. The explants were then carefully cleaned with distilled water four to five times to get rid of any dust particles. This was followed by a 28-minute treatment with 1% antifungal bavistin and another four to five rinses with distilled water. The explants were transferred to the Laminar air flow, followed by 1 min surface sterilization in 0.1% $HgCl_2$, 4-5 times rinsing with double distilled water.

2.2. Bacterial culture preparation and maintenance

A bacterial culture was prepared with 1.3 g of nutrient broth (Himedia, India) in 100 ml of distilled water with a pH of 7.4±0.2 was used to resurrect the *A. rhizogenes* cultures MTCC 532 and 2364 (ordered from CSIR-Institute of Microbial Technology IMTECH, MTCC, Chandigarh). To promote bacterial growth, the revived bacterial culture was stored in the incubator (Shaker and Incubator, N. Biotek, NB 205 QF) and kept at 26±1°C for a period of 24 to 48 hours. Hairy root induction was carried out using the optical density (OD) of both bacterial cultures, MTCC 2364 and MTCC 532, which were maintained in the range of 0.2 to 1 (Fig. 2) following a 24- to 48-hour incubation period. After the strains were revived in nutrient broth, the cultures were maintained for further use on a nutrient agar medium made with 1.8 g/100 mL bacteriological grade agar and 1.3 g/100 mL nutrient broth (Himedia, India). Streaked plates were kept in a refrigerator at 4-5°C. (Fig.1 C& D).

2.3. Plant Material Preparation and Infection Process

Leaf segments (0.5 cm × 0.5 cm) with well-matured midrib and internodal segments (1.0 cm) were chosen from young, healthy plants. To avoid contamination, the explants were surface sterilized according to the above-described standardization procedures (e.g. using labolene, sodium hypochlorite, or commercial sterilization agents, Fig. 1A &B). After being sterilized, the explants were sliced to the proper size and submerged in the *Agrobacterium rhizogenes* solution that had been made. To authenticate consistent infection, the explants were gently shaken while soaking in the bacterial mixture for different infection time intervals from 10 to 90 minutes at room temperature, which was only to standardize the best infection time with the highest hairy root induction response.

Following infection, the explants were soaked in sterile tissue paper and inoculated on solid co-cultivation medium with the opposite side of the piercing onto ½ MS (Murashige & Skoog 1962), and ½ MS + acetosyringone (19.6 mg/mL; Himedia, India) medium to see the effect of culture medium. The pH of this ½ MS medium was adjusted to 5.8±0.01 and gelled with 0.6% agar. The ideal co-cultivation period was 44–48 hours at 26°C in an incubator. (Fig. 1 E & F)

Following 44–48 hours of co-cultivation, the explants were transferred into a fresh flask containing the same concentration of media that had been inoculated before along with antibiotics, such as cefotaxime (Himedia, India). This was done to kill the excess bacterial growth while protecting the plant tissue. Several rounds of antibiotic treatments were needed, depending on the strain of bacteria, to eradicate it. They were then maintained in a dark culture chamber at 24°C to induce hairy roots.

2.4. Statistical Analysis

Every value is shown as the average across three biological replicates. There is a considerable difference in the mean values within columns with various superscript alphabets. ANOVA was used to analyze the data using Duncan's multiple range test ($p < 0.05$).

3. Results

3.1. Effect of various factors in Hairy root induction

Two distinct strains of *A. rhizogenes* were used to generate hairy roots from leaf and internode segments. It was discovered that strain MTCC 2364 was superior to other strains from the leaf and internodal segments for inducing hairy roots. When infected with strain MTCC 2364, leaf explants showed the highest transformation efficiency (35.55%) with 2.62 % hairy root, whereas internodal segments showed a poor transformation efficiency (24.44%) with 2.51 % hairy root (Table 1). The frequency of transformation from leaf explants was greater than that from internodal segments for strain 2364 that was employed and strain 532 showed poor transformation efficiency towards both explants. Since leaf explants infected with strain 2364 showed the greatest amount of root induction, these were used in further studies to examine the impact of other conditions on root induction.

Numerous variables were examined, including infection time, co-cultivation time, acetosyringone in the infection medium, and bacterial density. Root induction occurred more frequently in leaf and internodal explants when 100 µM acetosyringone was added to the media used for bacterial pellet re-suspension (Table 1). In contrast, media without acetosyringone didn't respond to any of the explants. Root induction was impacted by the bacterial density of the solution used to infect leaf explants (Fig. 1G & H). The largest frequency of hairy root induction was seen in leaf explants at 35.55% at an infection time of 45 minutes and in internode explants at 24.44% at 70 minutes of infection time, when a bacterial suspension with an OD_{600} of 0.75 and 0.8 was employed respectively, from six different densities of bacterial suspension (OD_{600} 0.2, 0.4, 0.6, 0.75, 0.8, and 1.0) used for the infection (Fig. 2).

3.2. Growth calculation

The growth of hairy roots can be calculated by measuring the transformation efficiency of explants to different parameters and it is always calculated in percentage.

Transformation efficiency (TE) = Number of explants that responded to the strain/ Total no. of explants infected with the strain×100 (Brijwal & Tamta 2015, Table 1).

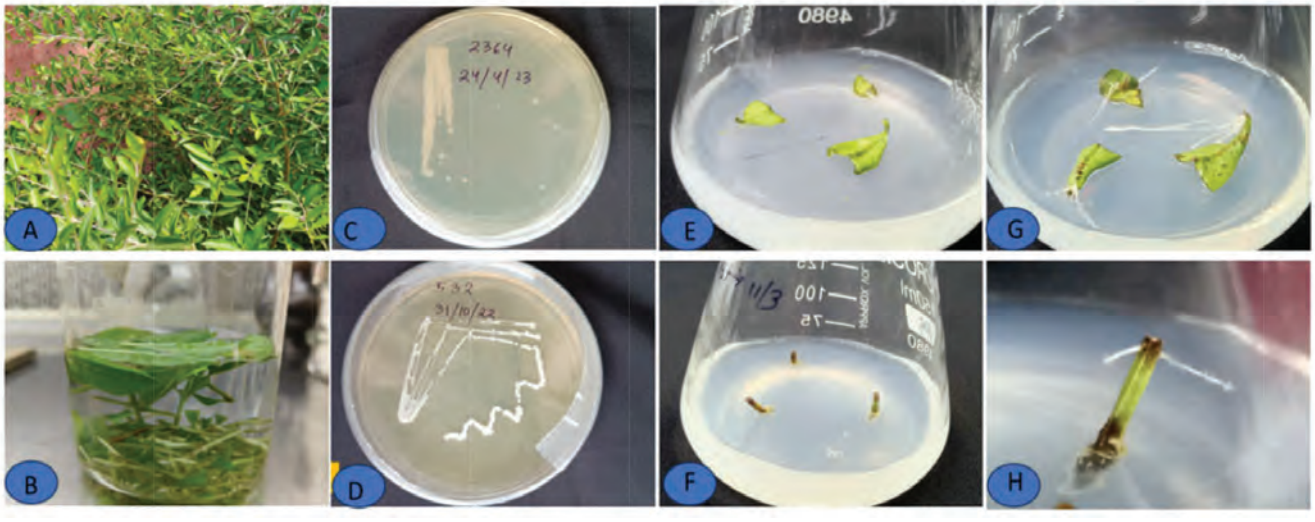


Figure-1: A: Source mother plant, B: Surface sterilized *in-vivo* explants, C: MTCC 2364 streak plate, D: MTCC 532 streak plate, E: *In-vivo* internode explants infected by MTCC 2364, G: Induction of hairy root from leaf explants, H: Induction of hairy root from internode explant

Table 1

Transformation efficiency of *in vivo* leaf & internode using MTCC 2364

Sl. No.	Infection time (Min)	LEAF		INTERNODE	
		Transformation efficiency (%)	Induced hairy root (Average)	Transformation efficiency (%)	Induced hairy root (Average)
1	10	0	0	0	0
2	15	0	0	0	0
3	20	8.88^{ef}	1.16^{cdef}	0	0
4	30	22.22^{b}	1.41^{bc}	2.22^{ef}	0.66^{ef}
5	45	35.55^{a}	2.62^{a}	11.10^{d}	1.66^{bcd}
6	60	17.77^{cb}	1.83^{b}	22.22^{ab}	2.29^{ab}
7	70	15.55^{cd}	1.38^{bcd}	24.44^{a}	2.51^{a}
8	80	11.10^{de}	1.33^{bcde}	17.78^{bc}	1.72^{abc}
9	90	6.66^{efg}	1^{cdefg}	4.44^{e}	1^{cde}

Data was compiled from five flasks for each replication and three explants per flask. The experiments were done three times (3×5×3 = 45). Each value represents the average of three replications. The mean values indicated by distinct letters vary substantially at $p < 0.05$ (DMRT).

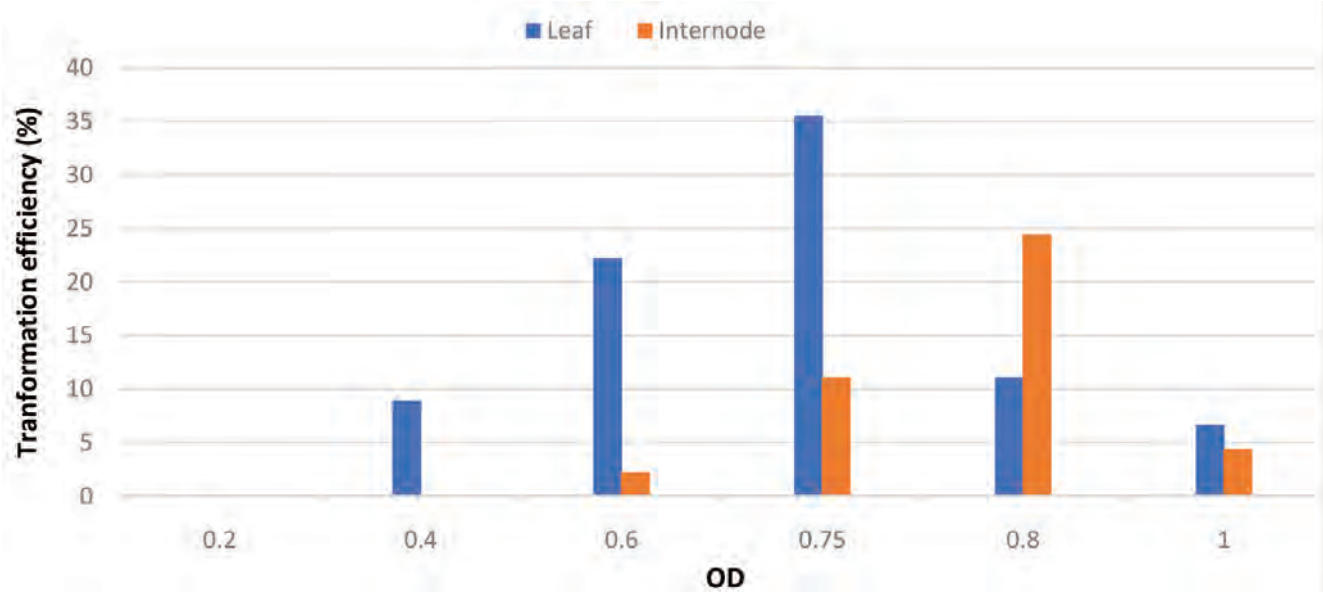


Figure 2: Effect of bacterial optical density (OD) on the transformation efficiency of different explants (leaf, internode) of *Lawsonia inermis.*

4. Discussion

Of the two strains of *A. rhizogenes* used in this hairy root induction experiment, MTCC 2364 and MTCC 532, the former was successful in inducing hairy roots, while the latter strain was found to be in effectual. This preliminary experiment as results indicate that *Lawsonia inermis in-vivo* explants exhibited variable responses to the various factors examined to induce hairy root culture. In support of our findings, a successful hairy root induction was informed in the *in-vivo* explants of *Rauwolfia serpentina* (Bhagat *et al.*, 2019).

4.1. Effect of different explants and strains

Leaf segments showed the highest frequency of root initiation amongst the explants, particularly when subjected to greater concentrations of *Agrobacterium rhizogenes* strain 2364, indicating that leaf tissues offer a favorable surface for bacterial adhesion and transformation, perhaps because of their surface area and cellular makeup. In previous

research, the highest transformation efficiency was observed in *in-vitro* leaves of *L. inermis* (Bakali *et al.,* 1997).

On the other hand, internode explants showed reduced induction rates, indicating that the tissue type is not as transformable or that the conditions (such as co-cultivation duration and bacterial concentration, infection time) were not right for these explants. Higher lignification may serve as a barrier to *Agrobacterium* penetration and subsequent root induction, which might account for the reduced induction in internode explants.This discovery aligns with previous research that conducted similar studies on different plant species with these strains (Bathoju *et al.,* 2017; Bhagat *et al.,* 2019). Other studies have reported that regardless of the *Agrobacterium* strains employed, Swain *et al.* (2010) suggested that stem internodes are noticeably more susceptible than leaf explants. Contrary to our findings, strain 2364 was found to be more successful in this experiment, and strain 532 showed poor induction efficiency towards the *L. inermis* but in some other plant species like *Plumbago rosea*, *Withania somnifera* strain 532 responded more effectively as compared to other used strains (Brijwal & Tamta, 2015).

4.2. Effect of co-cultivation time

Furthermore, it was shown that the co-cultivation time had a major impact on the production of hairy roots. Increased root initiation was seen in both leaf and internode explants after a longer co-cultivation period (e.g., 48 hours). However, this is due to the explants were exposed to the bacterial inoculum for a longer period of time, which allowed for higher integration of the Ri plasmid into the plant genome. On the other hand, very extended co-cultivation times were linked to tissue necrosis, suggesting that the best way to maximise root induction and preserve tissue viability is to balance the duration of bacterial infection.

4.3. Effect of optical density (OD)

This study depicts how different *Agrobacterium rhizogenes* culture optical densities (OD) affected *Lawsonia inermis* explant transformation efficiency. The findings unmistakably showed that optical density is essential for increasing transformation efficiency, which directly impacts the induction and growth of hairy roots (Fig 1). All explant types showed a higher frequency of hairy root development at higher optical densities (OD_{600} = 0.2 to 1.0), with OD_{600} = 0.75 to 0.8 showing the best transformation efficiency. This implies that there are enough viable bacteria present at the ideal bacterial concentration to infect a sizable area of the explant surface, facilitating efficient gene transfer and root induction (Shahabzadeh *et al*., 2014). Successful root initiation was probably made possible by the *Agrobacterium rhizogenes* maintaining its viability and infection capacity at this OD without inflicting undue tissue damage (Mahendran *et al.,* 2022). However, at optical densities exceeding OD_{600} = 1.0, a noticeable decline in transformation efficiency was observed. This reduction can likely be attributed to over-infection, where high bacterial loads induce tissue necrosis rather than promoting root initiation. Such conditions may lead to an excessive release of virulence factors from the bacteria, causing localized plant tissue damage that inhibits further root formation. Furthermore, over-proliferation of *Agrobacterium* at high optical densities could lead to increased plant defense responses, which might restrict the transformation process.On the contrary, lower optical densities (OD_{600} = 0.2 to 0.4) also showed reduced transformation efficiency, likely due to the insufficient bacterial population to effectively initiate infection across the explant. In these cases, fewer explants successfully developed hairy roots, which implies that the bacterial concentration was below the threshold required for optimal gene transfer and root induction.The findings align with previous studies, where intermediate levels of *Agrobacterium* density have been shown to promote successful transformation by balancing bacterial infection with plant tissue viability (Mahendran *et al*., 2022). Thus, OD_{600} = 0.75 to 0.8 was identified as the optimal density for inducing hairy root culture in *Lawsonia inermis*, providing the highest transformation efficiency without causing tissue damage or plant defense responses.

4.4. Effect of infection time

The duration of exposure, or infection time, between the *Agrobacterium rhizogenes* and explants, plays a pivotal role in the transformation efficiency and subsequent induction of hairy roots. In the current study, we investigated how varying infection times impacted the transformation efficiency in *Lawsonia inermis* explants, providing valuable insights into the optimization of this parameter. Our results demonstrate a clear correlation between infection time and transformation efficiency, with intermediate infection times (10-90 minutes) and found that 45 minutes of infection time yielded the highest frequency of hairy root formation across leaf and stem explants. At these durations, explants were exposed to *Agrobacterium* long enough to allow effective T-DNA transfer from the Ri plasmid into the plant cells, resulting in successful root initiation. The moderate infection time likely provided ample opportunity for *Agrobacterium rhizogenes* to attach to the explant surfaces, activate virulence genes and transfer T-DNA without causing significant stress or damage to the plant tissue.

These findings align with prior research, which has established that moderate infection times are critical for balancing bacterial infection with tissue health in *Plumbago zeylanica* (Kumar *et al*., 2023), and many other species. Excessively short infection periods lead to inefficient gene transfer, while prolonged infection causes plant cell damage. Therefore, an infection time of 45 minutes emerged as the optimal window for maximizing transformation efficiency while minimizing tissue damage.

4.5. Effect of acetosyringone

The phenolic compound acetosyringone plays a crucial role in enhancing the transformation efficiency of *Agrobacterium rhizogenes*-mediated hairy root culture (Gelvin 2000). In this study, the addition of acetosyringone significantly improved the rate of hairy root induction in *Lawsonia inermis* explants, highlighting its importance in optimizing the transformation process. Compared to acetosyringone-free medium, acetosyringone in co-cultivation media not only accelerated explant transformation but also shortened the time required for hairy root induction (Brijwal & Tamta 2015). It was reported in various reports that different concentrations of acetosyringone affect differently in each reported plant species.In the current hairy root experiment, ½ MS medium supplemented with acetosyringone successfully induced hairy root formation. In contrast, no hairy roots developed from any explants inoculated on ½ MS medium lacking acetosyringone.

5. Conclusion and Future Prospective

This research established a foundational understanding of the parameters that govern efficient hairy root induction in *Lawsonia inermis*. By optimizing these variables, we have identified the conditions that maximize transformation efficiency, providing a reliable protocol for future studies. However, additional work is needed to further refine these parameters, particularly for other explant types such as stems and internodes, which exhibited lower induction rates. Moreover, exploring alternative explants, media compositions, and co-cultivation conditions could broaden the applicability of this transformation system.

Looking forward, further research should focus on the molecular mechanisms underlying the differential responses of various explants to *Agrobacterium* infection. A deeper understanding of plant-bacterial interactions at the cellular level could provide insights into why some explants are more responsive than others. In addition, it would be beneficial to investigate the potential of *in-vitro* explants, which may offer more controlled conditions for transformation and root induction.

Moreover, scaling up hairy root cultures for metabolite production holds significant promise. *Lawsonia inermis* is a source of valuable secondary metabolites, and the optimized hairy root culture system developed here could serve as a basis for the large-scale production of these compounds. Future studies could explore bioreactor-based systems to enhance root biomass and metabolite yield, enabling commercial applications in pharmaceuticals, cosmetics, and biotechnology.

In conclusion, this study provides a comprehensive framework for the efficient induction of hairy root cultures in *Lawsonia inermis*, with promising applications in both basic research and industrial processes. By optimizing transformation parameters and exploring new avenues of research, we anticipate significant advancements in the use of hairy root cultures for metabolite production and genetic studies.

References

Bakkali, A. T., Jaziri, M., Foriers, A., Vander Heyden, Y., Vanhaelen, M., & Homes, J. (1997). Lawsone accumulation in normal and transformed cultures of henna, *Lawsonia inermis*. Plant cell, tissue and organ culture. 51: 83-87.

Bathoju, G., Rao, K., & Giri, A. (2017). Production of sapogenins (stigmasterol and hecogenin) from genetically transformed hairy root cultures of *Chlorophytum borivilianum* (Safed musli). Plant Cell Tissue and Organ Culture.131: 369-376.

Bhagat, P., Verma, S.K., Singh, A.K., Aseri, G.K., & Khare, N. (2019). Evaluation of influence of different strains of *Agrobacterium rhizogenes*on efficiency of hairy root induction in *Rauwolfia serpentine*. Indian Journal of Genetics and Plant Breeding.79(4): 760-764.

Brijwal, L., & Tamta, S. (2015). *Agrobacterium rhizogenes* mediated hairy root induction in endangered *Berberis aristata* DC. Springer plus. 4(1): 443.

Gelvin, G.B. (2000). *Agrobacterium* and plant genes involved in T-DNA transfer and integration. Annual Review of Plant Physiology and Plant Molecular Biology.51: 223-256.

Kumar, A., Kumari, A., Demiwal, P., Roy, P., & Sircar, D. (2023). Enhanced production of bioactive plumbagin in hairy root cultures and adventitious root cultures of *Plumbago zylanica* L. by a novel apocarotenoid elicitor, α-ionone. Industrial Crops and Products. 203: 117140. https://doi.org/10.1016/j.indcrop.2023.117140

Lekouch, N., Sedki, A., Nejmeddine, A., and S., G. (2001). Lead and traditional Moroccan pharmacopoein. The Science of the Total Environment. 39-34.

Mahendran, G., Verma, N., Singh, M., Shanker, K., Banerjee, S., Kumar, B., & Rahman, L. (2022). Elicitation enhances swerchirin and 1, 2, 5, 6-tetrahydroxyxanthone production in hairy root cultures of *Swertia chirayita* (Roxb.) H. Karst. Industrial Crops and Products. 177: 114488.

Muheyuddeen, G., Divy, S.R., Gautam, S.K., & Gupta, S.K. (2023). *Lawsonia inermis* L. Phytopharmacological Characteristics and Recent Advancement. Research Journal of Pharmacognosy and Phytochemistry. 15(1): 11-23.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 15(3): 473-497.

Patel, K.M., & Patel, P.R. (2017). Review on *Lawsonia inermis* Linn.: An Update. Phytochemistry. 9(13): 14. https://doi.org/10.5958/2231-5713.2017.00036.8

Pradhan, R., Dandawate, P., Vyas, A., Padhye, S., Biersack, B.,Schobert, R., Ahmad, A. and Sarkar, F.H. (2012). From body art toanticancer activities: perspectives on medicinal properties of henna.Current drug targets. 13: 1777-1798.

Sahu, L., Jena S., Swain S.S., Sahoo, S., & Chand, P.K. (2013). *Agrobacterium rhizogenes*-mediated transformation of a multi-medicinal herb, *Boerhaavia diffusa* L.: optimization of the process and anti-microbial activity against bacterial pathogens causing urinary tract infections. Frontiers in Life Science. 7(3-4): 197-209.

Sen, S., Borthakur, M.S., & Chetia, D. (2023). *Lawsonia inermis* Linn: A breakthrough in cosmeceuticals. Sciences of Phytochemistry. 2(1): 128-158.

Shahabzadeh, Z., Heidari, B., & Hafez, R.F. (2014). Induction of transgenic hairy roots in *Trigonella foenum-graceum*co-cultivated with *Agrobacterium rhizogenes* harboring a GFP gene. Journal of Crop Science and Biotechnology. 16: 263-268.

Swain, S.S., Sahu, L., Barik, D.P., & Chand, P.K. (2010). *Agrobacterium* × plant factors influencing transformation of 'Joseph's coat' (*Amaranthus tricolor* L.). Scientia Horticulturae. 125(3): 461-468.

Plant Science Research 46 (1&2) : 22-29, 2024

Plant Science Research

ISSN 0972-8546

Cyanobacteria and Algae: The Green Dynamo for Ecosystem, Health and Human Well-being

Debabrata Mohanty[1+], Anil Behera[2+], Archana Priyadarshini Jena[3] and Binayak Mohapatra[1Ψ]

[1] Department of Botany, Utkal University, Vani Vihar, Bhubaneswar 751004, Odisha, India
[2] School of Biotechnology, Gautam Buddha University, Greater Noida 201312, Uttar Pradesh, India
[3] Department of Biotechnology, Pondicherry University, Chinna Kalapet - 605014, Puducherry, India
[+]*Equal Contribution*

ARTICLE INFO

Article history:
Received : 25 August 2024
Revised : 27 September 2024
Accepted : 17 October 2024

Keywords:

Microalgae
Macroalgae
Immunity
Diabetes
Metabolite
Biofuel
Bioremediation

ABSTRACT

This review highlights certain important roles exhibited by algae in ecosystem management, environmental protection and human health. Algae are an invaluable and a significant renewable resource which produce diverse chemical compounds leading to biological activities that possess properties such as antimicrobial, antilipidemic, antitumor, antidiabetic, anticoagulant, antioxidant, and antiallergic. In addition to health benefits, they contribute significantly to protecting the environment apart from supporting the food chain as a primary producer. Besides they produce biofuels and bioplastics and provide an eco-friendly and cost-effective alternative for wastewater management and bioremediation. Algae and cyanobacteria are also nutrient-dense, making them valuable as a component of functional foods and an excellent source of vegan dietary protein. Recent advancements in biotechnology have expanded the potential industrial applications of algae-based products. Implementing novel strategies in their culture could address many contemporary challenges, such as energy, food, and water security. Their contribution is climate change recovery paves the way for a more sustainable future.

Introduction

The expanding global population has created a gap between the demand and supply of various day-to-day needs. Despite numerous advancements in the agricultural field and pharmaceutical industries, meeting the demands for food and healthcare remains challenging. Above all, these advancements have severe impacts on environmental health due to the utilization of large areas of land for agriculture leading to biodiversity loss, pollution of water bodies due to industrial effluents, increasing CO2 footprint, global warming, etc. Human health is also directly and indirectly affected by these environmental damages (Onyeaka *et al.*, 2021, Xu *et al.*, 2019). Advancements in the field of phycology have revealed the enormous potential of algae to find a sustainable solution to these challenges.

Algae are generally described as a group of simple, autotrophic oxygen-producing organisms that can be unicellular or multicellular lacking differentiated tissues. Simple forms of algae have existed for around 1.5 billion years. Historically, single-celled organisms capable of forming colonies, filamentous undifferentiated plant body and prokaryotes termed as "cyanobacteria" or "blue-green algae" currently classified under the kingdom Monera. The classification of eukaryotic algae, traditionally placed in the domain of Protists and including both unicellular and multicellular forms, is complex and remains uncertain. Most have a haploid life cycle, while others follow a haplodiploid cycle, alternating between gametophytic (haploid) and sporophytic (diploid) generations. These chlorophyll-bearing organisms are known to be primary producers of aquatic ecosystems thus have been utilized as sources of functional

[Ψ] *Corresponding author; Email:* binayakmoha97@gmail.com

food since early civilization (Michalak and Chojnacka, 2018; García *et al.*, 2017).

Microalgae are a highly diverse group of microorganisms. These organisms account for approximately half of the global photosynthetic activity and are typically found in the euphotic zone, particularly in the surface and subsurface layers where light penetration is sufficient for growth. Among them are a sizeable microscopic and non-motile group of organisms known as phytoplankton which mostly serve as primary producers in the food chain, supporting the survival of aquatic consumers. In aquatic ecosystems, primary production is closely linked to the availability of external energy, which facilitates the injection of nutrients into the euphotic zone (Anderson, 1996).

Interest in algae and cyanobacteria is increasing due to the diverse array of compounds found in them. They are not only rich in primary metabolites like proteins, carbohydrates, lipids (mainly polyunsaturated fatty acids), minerals, etc, but several secondary metabolites belonging to polyphenols, alkaloids, terpenes, and small peptide groups are also found in them, which are of high medicinal value. These organisms can be used to produce an extensive range of products, ranging from nutritional supplements for humans and livestock feed to organic chemicals used in pharmaceuticals, pigments, and various industrial and energy applications (Michalak and Chojnacka, 2014).

In this review, the essential roles of algae, cyanobacteria and microalgae in both environmental and health contexts are briefly discussed in the following subsections, as represented in Fig. 1.

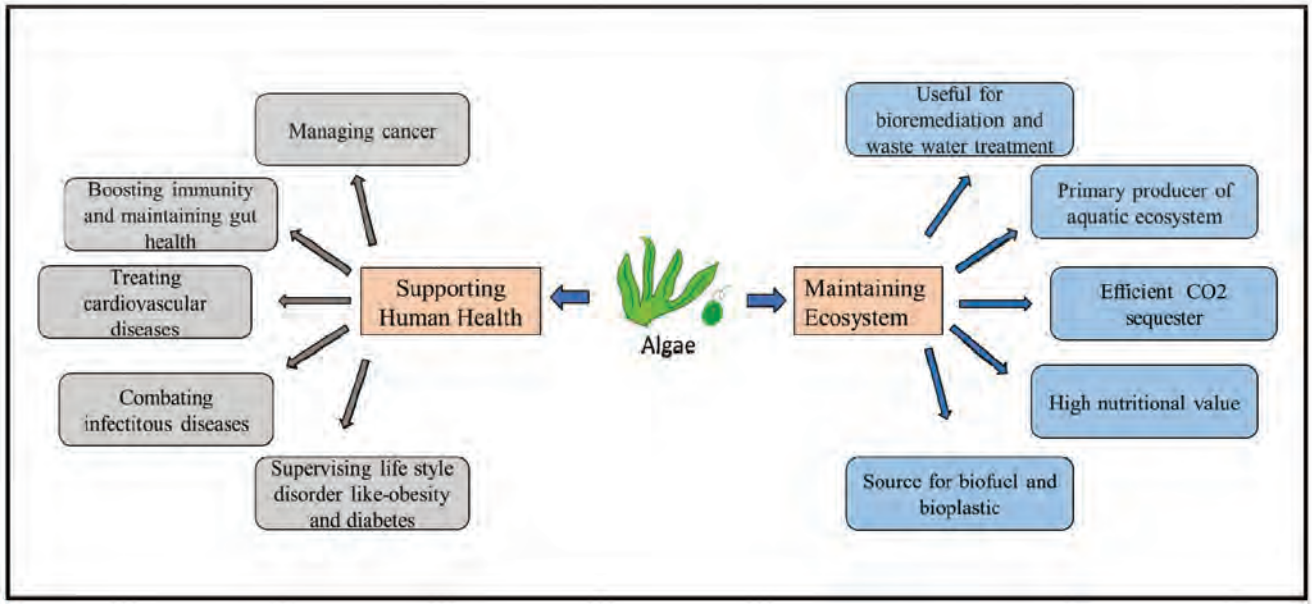


Figure 1: Schematic representation of various uses of algae and cyanobacteriain ecosystem maintenance and human health

1. The Role of Algae -Derived Products in Supporting Human Health

Both microalgae, macroalgae and cyanobacteria contain a diverse array of biologically active compounds, including different pigments, polysaccharides, organic minerals, etc. These compounds are associated with various potential health benefits, such as immunomodulatory, anti-inflammatory, antioxidant, antimicrobial, antidiabetic, anticarcinogenic, and prebiotic effects. For example, *Spirulina* and *Chlorella* have abundant vitamins, fatty acids, antioxidants, and minerals, whereas macroalgae *Ulva Armoricana* contains functional elements like sterols, alginic acid, and soluble fiber that have therapeutic potential. In the following section, we will explore their role in cardiovascular diseases, immunity, gut health, metabolic health (including obesity and diabetes), cancer, and infectious diseases.

1.1 Contributions to cardiovascular well-being

Cyanobacteria and algae are essential in promoting cardiovascular health due to their rich content of bioactive compounds. These organisms contain essential nutrients like carotenoids, γ-linolenic acid, etc., which can lower cholesterol levels and reduce inflammation in the body (Ku *et al.*, 2013). Microalgae-derived compounds like omega-3 fatty acids and phycobiliproteins can improve lipid profiles, reduce inflammation, and support cardiovascular health (Pereira *et al.*, 2024). Algal phytosterols, used in food

products, have grained attention because they are known to reduce cholesterol concentration of blood and prevent cardiovascular disorder. Taurine (2-aminoethanesulfonic acid), a specific peptide derived from microalgae and abundantly found in some red algae species, has recently gained popularity in beverages, foods, and nutritional supplements. The bioactive properties of this compound support cardiovascular health (Saide *et al*., 2021). Fucoidan, a long-chain sulfated polysaccharide found in a species of brown algae (e.g. *Laminaria japonica*), has recently been reported in a study to improve atherosclerotic cardiovascular disease by decreasing the production of oxygen radicals (Wang, Xin *et al*., 2016).

1.2 Immunomodulatory and gut health benefits of algal bioactive compounds

Several bioactive compounds derived from algae and cyanobacteria, like omega-3 fatty acids, polysaccharides (alginate, fucoidan, ascophyllan, and porphyrin), etc., have been shown to have potential immunomodulatory and gut health-promoting effects (Hwang *et al*., 2022). These compounds make algae a promising source of eco-friendly compounds for maintaining overall health by enhancing immunity. Algal species, such as *Tribonema sp*., *Gyrodinium impudicum*, and *Cystoseira indica*, contain sulfated polysaccharides (SPs). SPs having the ability to enhance the immune system due to their immunomodulatory activities, including stimulating the production of cytokines antibodies and activating T cells and other immune cells. Studies found that SPs treatment increases the up-regulation of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL10) levels in murine macrophage cells (Riccio G *et al*., 2019). The Food and Drug Administration (FDA) compound as taxanthin is mainly derived from the microalga *Haematococcus pluvialis* (Brendler *et al.*, 2019). In several studies, as taxanthin has also been found to enhance the immune response. In a study, it has been reported that astaxanthin increased natural killer cell (NK cells) cytotoxic activity and increased the total T and B cell subpopulations. Astaxanthin has been shown to enhance lymphocyte proliferation in *in vitro* mice models and elevate IL-2 and INF-α levels in *ex vivo* mice models. Similarly, cyanobacteria such as Spirulina can boost immune function through various mechanisms, including enhancing the activity of T cells, NK cells, and macrophages, which improves pathogens clearance. Additionally, it possesses anti-inflammatory properties that help to regulate the immune response.

Algal products like polysaccharides, phycobiliproteins, etc., may function as prebiotics, selectively stimulating the growth of beneficial gut bacteria. This, in turn, can strengthen the gut barrier, reduce inflammation, and enhance immune function (Pereira *et al.*, 2024). A study reported that the microalga *Chlorella vulgaris* can enhance immune function and serve as a prebiotic. When combined with bifidobacteria, *Chlorella vulgaris* also helps to lower triglyceride levels. In that study, *Chlorella* had a positive growth-promoting effect on the tested bifidobacteria and significantly increased the secretion of inflammatory cytokines. This combination of *Chlorella* sp. & bifidobacteria shows promise for developing new health-boosting functional foods (Hyrslova *et al*., 2021).

1.3 Exploring the anti-obesity and anti-diabetic potential of algal compounds

Inflammation and oxidative stress are critical factors in developing insulin resistance and metabolic syndrome. In obese animal models, some microalgae-derived compounds, such as fucoxanthin, have been studied for their potential to reduce body weight and adipose tissue accumulation (Pereira L *et al*., 2024). Fucoxanthin from algae exerts an anti-obesity activity by modulating the increase of ROS and the down-regulation of lipid metabolism genes. It also alters hepatic glucose-regulating enzyme activities. Recent studies suggest that fucoxanthin from *Phaeodactylum tricornutum* can prevent obesity and diabetes by inhibiting carbohydrate-hydrolysing enzymes and lipid accumulation. The algal compound astaxanthin has shown potential in protecting pancreatic α-cells from glucose toxicity. A study suggests that as taxanthin could be an effective supplement in preventing or aiding the recovery of lymphocyte dysfunctions in diabetic patients. Additional studies have also shown that as taxanthin prevents diabetic nephropathy by reducing oxidative stress and renal cell damage (Saide *et al.*, 2021). Algal polyphenols like flavonoids & alkaloids and fatty acids like eicosapentaenoic acid (EPA), arachidonic acid (AA), and docosahexaenoic acid (DHA) have potential anti-diabetic activities (Gundala *et al.*, 2018, & Saide *et al.*, 2021). Studies found that bioactive compound from microalgae, and cyanobacteria like zeaxanthin, phycoerythrin, α-carotene, α-glucan, etc., improves blood sugar levels (Selvan *et al.*, 2023).

1.4. Anticancer potential of algae and cyanobacteria derived Compounds

Antioxidant compounds, like polyphenols, and carotenoids from *Chlorella, Dunaliella,* and *Haematococcus*, have shown anticancer properties by controlling oxidative damage and inhibiting tumor cell growth (Ávila-Román *et al*., 2021). Red macroalgae *Peyssonnelia* species produce peyssonoic acids that exhibit antimicrobial effects and can inhibit the growth of human ovarian cancer cell lines (Lane *et al*, 2010). Astaxanthin can induce apoptosis

by regulating the activities of anti-apoptotic proteins and pro-apoptotic proteins in different cell types (Faraone *et al*., 2020). Brown alga Ochrophyta and Phaeophyceae-derived polysaccharides fucoidans have shown potential immuno modulatory and anticancer properties (Li *et al*., 2021).

1.5 Exploring the vital role of algae in combating infectious diseases

Various bioactive molecules and compounds derived from algae have shown potential in combating infectious diseases. Compounds such as carrageenan (from *Gigartina skottsbergii*), agar (from *Chlorella vulgaris* and other algae) laminaran (from *Fucus vesiculosus*, *Saccharina longicruris*, *Ascophyllum nodosum*) exhibit significant antiviral properties, particularly against certain viruses like HIV, influenza, and other viruses. Some marine metabolites from algae like griffithsin (from red algae, *Griffithsia*) ulvans (from green algae, *Ulva sp*.), phycocyanobilin, phycoerythrobilin, and folic acid have antiviral properties against the novel human coronavirus (Ahmed *et al.,* 2021). A study reported that hexane crude extracts from certain microalgae species, including *Rhodomonas sp.*, *Chlamydomonas mexicana*, *Nitzschia palea*, *Porphyridium cruentum*, *Aphanocapsa marina*, and *Isochrysis galbana*, inhibited at least 90% of the growth of Mycobacterium tuberculosis (Ruiz-Güereca *et al.,* 2019).

2. The Multidimensional Role of Algae to Maintain Ecosystem

The aquatic food chain begins with primary producers like algae and plays a very important role in sustaining life. Algae and cyanobacteria underpin the food chain by preparing food using energy from sunlight. The high nutritional value present in them makes it a dietary supplement and food additive across the globe to support human health. Moreover, these organisms have been increasingly utilized for applications such as biofuels and bioplastics reducing our reliance on pollutant-producing fossil fuels. Looking at global warming, green biofuels are the need of the hour. Algae also serve as the cleaning crews for terrestrial and aquatic systems to mitigate pollution by wastewater treatment and bioremediation. Additionally, they possess the unique ability to make wonders from waste such as nutrient recycling and waste management. Through all these contributions, algae not only maintains harmony in the ecosystem but also solves global sustainable challenges. The roles of algae with suitable examples are described below.

2.1. The foundation of aquatic life: Algae under pinning the food chain and acting as gaseous guardian

Primary producers of aquatic systems convert carbon dioxide and sunlight into food (carbohydrates) through the process of photosynthesis. Phytoplankton, a wide range of floating algae, and cyanobacteria found across aquatic bodies like rivers, oceans, and ponds being responsible to maintain the food chain. By carrying out photosynthesis using carbon dioxide, water, and light from the sun, these minute plants form the basis of food chains. Energy moves up through this chain as copepods and other zooplankton consume phytoplankton. In this way all life forms including fish depend on each other; for example, fish eat zooplankton. *Chlorella vulgaris* is an important source of protein, vitamins and minerals to small aquatic zooplankton like copepods.

In coastal and estuarine environments, microalgae are generally more efficient CO_2 consumers due to their high surface area/volume ratio and high metabolic rates, whereas macroalgae might contribute more to CO_2 assimilation in open ocean ecosystems due to their large biomass and habitat formation. Studies indicate that the cultivation of microalgae may produce an average of approximately 280 tons of dry biomass per hectare per year , making them substantially more efficient at fixing carbon dioxide than some terrestrial plants (Bhola *et al., 2014).* Furthermore, studies contrasting *Nannochloropsis gaditana* with *Chlorella vulgaris* have shown that the former is more capable of achieving very high biomass concentrations and rates of carbon fixation than the latter (Adamczyk *et al*., 2016).

2.2. Nature's tiny powerhouses: Unveiling the nutritional value of algae and cyanobacteria

Algae and cyanobacteria are extremely nutrient-dense organisms due to their ubiquitous nature. They are an abundant source of essential nutrients due to their short life cycle and high rates of photosynthesis. Nutritional value distribution is not class-specific. A wide range of vitamins, minerals, and antioxidants are distributed among all classes of these organisms. Most of them are reported to have a higher protein content (10-70%) suggesting their potential as a vegan substitute for protein, while carbohydrate content of nearly 20- 75% makes it an energy-rich diet. Though lipid content is comparatively less, it substantially eliminates the dependency on animal-based fatty acids. Thus, they are widely used as dietary supplements and functional food additives across the globe. Some examples of cyanobacteria and algae belonging to different classes along with their nutritional value are shown in Table 1.

Table 1

Nutritional contents of some algae and cyanobacteria

Sl No.	Algae name	Protein %	Lipid %	Carbohy –drate %	Antioxidant	Vitamins	Minerals	Reference
1	*Chlorella*	51-58	14-22	12-17	Vitamin C, E, polyphenols	A,B1, B2, B3, B6, B12, C, E, K	Fe,Ca	Widyaningrum *et al.*, 2020
2	Nori (*Porphyra*)	7-50	0.12-2.8	40-76	Carotenoids, flavonoids	B12,A, C, E, K	I,Ca, P,Mg	Turan *et al.*, 2012
3	Wakame (*Undaria pinnatifida*)	10-16	01-2	30-40	Fucoxanthin, vitamins C and E	A, C, E, B2, B6, B12,B3	I,Ca.Fe,Na, Zn, K	Taboada *et al.*, 2012
4	Dulse (*Palmaria palmata*)	10-35	0.3-3.8	30-45	Carotenoids, Vitamin C	A, B2, B12, E C	Cl, K, Na,I	Morgan *et al.*, 1980
5	Kombu (*Laminaria japonica*)	05-9	01-2	40-65	Carotene, Vitamin C, K	B1,B2,B6, C, E, K	K,Ca,Na, P,Fe,I	OHNO *et al.*, 2011, Kim, 2011
6	Sea Moss (*Chondrus crispus*)	10-13	0.4-6	45-50	Phenol, Flavonoids, antioxidants, carotene, C and E	C, E	Na,K,P,Zn, Mg,Mn,I,Ca	Park *et al.*, 2024, Cao *et al*., 2016
7	Spirulina	60-70	5-10	16-20	Carotenoids, Phycocyanin	B1,B2,B6,B3,B12 *C,K*	Fe, Ca, Mg, K,Mn	Gaur *et al*., 2024,

2.3. Fuelling the future: Algae and cyanobacteria - A sustainable source for biofuels and bioplastics

Clean burning fuels are emerging for their demand due to the increase in pollution and both algae and cyanobacteria can be a promising source to meet the demand. Lipids produced by algae are converted to biodiesel; thus acting as a renewable source replacing conventional fossil fuel. Looking at the high volume of oil content in algae especially microalgae like *Nannochloropsis*, *Botryococcus braunii*, and *Crypthecodinium cohnii*, *Neochloris oleoabundans* are the best source to generate biodiesel (Chisti, 2007). The pigmented organisms also can address the food-fuel dilemma, particularly as sugarcane and other conventional crops were previously utilized for fuel, which could lead to food scarcity. However, algae provide a sustainable solution to this issue. Other benefits of using algae and cyanobacteria include the ability to grow on unproductive land, a high surface area-to-volume ratio, a fast life cycle, a weaker cell wall compared to terrestrial plants and most importantly reduced land usage.

Furthermore, these organisms could be used to make bioplastics, biodegradable substitutes for petroleum-based plastics which causes India to produce nearly 3.3 million tons of plastic materials annually for manufacturing industries (Chinglenthoiba *et al*., 2023). Biomass generated through mass culturing of *Chlorella pyrenoidosa* makes an eco-friendly alternative to plastic products. (Das *et al*., 2018). The quick and mass absorption of nutrient by cyanophytes from eutrophic and polytropic surface waters also exhibit a great potential of these organisms in this context.

2.4. Cleaning Crews of the Water World: Algal power in bioremediation and wastewater treatment

In contrast to the bioindicator function of algae, recent developments in science and technology have revealed another important role: the removal of contaminants from wastewater through bioremediation. Diatoms are well-employed to monitor pollution levels of air and water in terrestrial or aquatic environments. The presence and abundance of certain diatom species, such as *Actinocyclus normanii*, and *Epithemia gibberula* denote the quantity of nutrient or pollutant present in the aquatic system (Smol and Stoermer, 2010). Algae are remarkably effective at eliminating excess nutrients and contaminants from wastewater. Some species, such as *Scenedesmus dimorphus* and *Chlorella vulgaris*, are particularly good at absorbing

phosphorus and nitrogen, which are the causes of eutrophication, a situation in which excessive algal growth lowers oxygen levels in water bodies. In addition to cleaning wastewater, this bioremediation process also produces useful algal biomass as a byproduct, which can be used for downstream processes (Hu *et al*., 2021, Ferreira *et al*., 2008).

Discussion

The tremendous potential of algae and Cyanobacteria in nearly every field of biotechnology is coming to the forefront and capturing researchers' attention as a sustainable alteration of various resources for the future. Despite their remarkable chemical diversity and wide range of medicinal properties, large scale cultivation faces significant challenges that demand serious attention.

Although Various methods have been employed to carry out the increased synthesis of desired compounds by genetic engineering, mutagenesis etc, limitations are observed during both upstream and downstream processing during the scale-up. Numerous risks are associated with both closed and open cultivation of algae and Cyanobacteria. Maintaining water quality is highly essential as any pesticides or herbicides present can harm the growth of algae, similarly, poor culture conditions in open culture can lead to an increased chance of contamination which not only decreases the growth rate but also compromises the product quality, making the downstream processing more difficult (Novoveská, L *et al.*, 2023). In addition to that, higher operational costs and the need for trained professionals, make the transition from lab-scale to industrial-scale cultivation more difficult, especially in developing countries like India (Davis *et al*., 2011). Environmental risks associated with the large-scale cultivation such as emission of greenhouse gasses, use of genetically modified organisms etc poses serious threats (Novoveská, L *et al.*, 2023). To mitigate these risks, it is essential to implement and adhere to proper optimization parameters. Besides that, a large number of algal and Cyanobacteria species are unexplored which limits identifying the true potential of this organism. Therefore, there is a need for more bioactivity screening, genome sequencing, and species transformation to enrich the qualitative properties of these organisms.

Conclusion

In the current scenario, there is a growing interest in utilizing natural products in environmental and human activities, particularly those related to health and well-being. Natural sources are providing valuable insights into various sectors of the bio-based industry. Algae and cyanobacteria, in particular, have been applied across numerous fields, notably medicine and pharmacology. Research into the propagation, procuration, preliminary treatment, and conversion of algal biomass is driving the development of novel algal-based products. In the current era, where more people are adopting the vegetarian based diet, algae and algal based products are gaining more attention. Certain algae such as Chlorella and Dunaliella are classified as generally safe (GRAS), meaning that they are "safe to consume" by the U.S. Food and Drug Administration (FDA). Therefore, there is a need to overcome the limitations associated with large scale production of the compounds to meet the ever increasing demand. Despite its potential as a sustainable alternative of biofuel, it is difficult to get the approval from the regulatory bodies due to quality control issues. Mitigating these challenges along with expanding the industry by introducing system biology and synthetic biology, will open up new opportunities to harness algae and Cyanobacteria to maintain ecosystem, health and human well-being.

References

Adamczyk, M., Lasek, J., and Skawiñska, A. (2016). CO2 Biofixation and Growth Kinetics of Chlorella vulgaris and Nannochloropsisgaditana. Applied biochemistry and biotechnology. 179(7): 1248–1261.

Ahmed, N., Iqbal, H.M.N. and Xu, J. (2021) Algae-Derived Bioactive Molecules for the Potential Treatment of SARS-CoV-2. 1–16.

Anderson R. A. (1996) Algae. In: Maintaining Cultures for Biotechnology and Industry. Academic Press, San Diego. pp 29–64

Ávila-Román, J., García-Gil, S., Rodríguez-Luna, A., Motilva, V., & Talero, E. (2021). Anti-Inflammatory and Anticancer Effects of Microalgal Carotenoids. Marine drugs. 19(10): 531. https://doi.org/10.3390/md19100531

Bhola, V., Swalaha, F., Kumar, R.R., Singh, M. and Bux, F. (2014). Overview of the potential of microalgae for CO, sequestration. Int. J. Environ. Sci. Technol. 11: 2103-2118.

Brendler, T. and Williamson, E.M. (2019). Astaxanthin: How much is too much? A safety review. Phytother. Res. 33: 3090–3111.

Cao, J., Wang, J., Wang, S., and Xu, X. (2016). Porphyra species: A mini-review of its pharmacological and nutritional properties. J Med Food. 19(2): 111-119.

Chen, X., Song, L., Wang, H., Liu, S., Yu, H., Wang, X., Li, R., Liu, T. and Li, P. (2019). Partial characterization, the immune modulation and anticancer activities of sulfated polysaccharides from filamentous microalgae *Tribonema sp.* Molecules 24: 322.

Chingakham, C., Pukhrambam, B., Chanu, K.T., Devi, K.S., Meitei, N.J., Devika, Y. and Valiyaveettil, S. (2023). A review on microplastic pollution research in India. Regional Studies in Marine Science. 58: 102777. https://doi.org/10.1016/j.rsma.2023.102777.

Chisti, Y. (2007). Biodiesel from microalgae. Biotechnology advances.25(3): 294-306.

Davis, R., Aden, A. and Pienkos, P.T. (2011). Techno-economic analysis of autotrophic microalgae for fuel production. Applied Energy. 88(10): 3524-3531.

Del Mondo, A., Smerilli, A., Sané, E., Sansone, C., and Brunet, C. (2020). Challenging microalgal vitamins for human health. Microb Cell Fact.. 19(1): 201. doi: 10.1186/s12934-020-01459-1.

Faraone, I., Sinisgalli, C., Ostuni, A., Armentano, M. F., Carmosino, M., Milella, L., Russo, D., Labanca, F. and Khan, H. (2020). Astaxanthin anticancer effects are mediated through multiple molecular mechanisms: A systematic review. Pharmacological research, 155: 104689. https://doi.org/10.1016/j.phrs.2020.104689

Ferreira, A. and Gouveia, L. (2020). Chapter 28 - Microalgal Biorefineries, in: Jacob-Lopes, E.,Maroneze, M.M., Queiroz, M.I., Zepka, L.Q.B.T.-H. of M.-B.P. and P. (Eds.). Academic Press, pp. 771–798.

García, J. L., de Vicente, M., & Galán, B. (2017). Microalgae, old sustainable food and fashion nutraceuticals. Microbial biotechnology. 10(5): 1017–1024.

Gaur, K., Wal, A., Sharma, P., Parveen, A., Singh, P., Mishra, P., Wal, P., and Mishra, N. (2024). Exploring the nutritional and medicinal potential of Spirulina. Natural Resources for Human Health. 4: 277-286.

Gundala, N.K.V., Naidu, V.G.M. and Das, U.N. (2018). Amelioration of streptozotocin-induced type 2 diabetes mellitus in Wistar rats by arachidonic acid. Biochem. Biophys. Res. Commun. 496: 105–113.

Hu, D., Zhang, J., Chu, R., Yin, Z., Hu, J., Nugroho, Y.K., Li, Z. and Zhu, L. (2021). Microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus* co-cultivation with landfill leachate for pollutant removal and lipid production. Bioresource Technology. 342: 126003. https://doi.org/10.1016/j.biortech.2021.126003.

Hwang, J., Yadav, D., Lee, P.C. and Jin, J.O. (2022). Immunomodulatory effects of polysaccharides from marine algae for treating cancer, infectious disease, and inflammation. Phytother. Res. 36: 761-777.

Hyrslova, I., Krausova, G., Smolova, J., Stankova, B., Branyik, T., Malinska, H., Huttl, M., Kana, A., Doskocil, I. and Curda, L. (2021). Prebiotic and immunomodulatory properties of the microalga *Chlorella vulgaris* and its synergistic triglyceride-lowering effect with bifidobacteria. Fermentation 7: 125.

Korzeniowska, K., Górka, B., Lipok, J., and Wieczorek, P.P. (2018). Algae and Their Extracts in Medical Treatment. Algae Biomass Charact Appl. 73–87.

Ku, C.S., Yang, Y., Park, Y. and Lee, J. (2013). Health benefits of blue-green algae: prevention of cardiovascular disease and nonalcoholic fatty liver disease. Journal of medicinal food. 16(2): 103–111.

Meeranayak, U.F.J., Nadaf, R.D., Toragall, M.M., Nadaf, U. and Shivasharana, C.T. (2020). The role of algae in sustainable environment: A review. *J.* Algal Biomass Utln. 11(2): 28-34.

Michalak, I. and Chojnacka, K. (2018). Introduction: Toward Algae-Based Products. Algae Biomass Charact Appl 1–5.

Michalak, I. and Chojnacka, K. (2014). Algae as production systems of bioactive compounds. Eng Life Sci 14:2–3.

Morgan, K.C., Wright, J.L.C. & Simpson, F.J. (1980). Review of chemical constituents of the red alga. *Palmaria palmata* (dulse). Econ Bot 34: 27–50 https://doi.org/10.1007/BF02859553.

Mota, C.S.C., Pinto, O., Sá, T., Ferreira, M., Delerue-Matos, C., Cabrita, A.R.J., Almeida, A., Abreu, H., Silva, J., Fonseca, A.J.M., Valente, L.M.P., & Maia, M.R.G. (2023). A commercial blend of macroalgae and microalgae promotes digestibility, growth performance, and muscle nutritional value of European seabass (*Dicentrarchuslabrax* L.) juveniles. *Frontiers in nutrition*, *10*, 1165343. https://doi.org/10.3389/fnut.2023.1165343

Novoveská, L., Nielsen, S. L., Eroldoğan, O. T., Haznedaroglu, B. Z., Rinkevich, B., Fazi, S., Robbens, J., Vasquez, M., & Einarsson, H. (2023). Overview and Challenges of Large-Scale Cultivation of Photosynthetic Microalgae and Cyanobacteria. *Marine drugs*, *21*(8), 445. https://doi.org/10.3390/md21080445

Kim, Y.S., Kang, C.O., Kim, M.H., Cha, W.S., and Shin, H.J. (2011). Contents of water extract for *Laminaria japonica* and its antioxidant activity. KSBB Journal. Korean Society for Biotechnology and Bioengineering. https://doi.org/10.7841/ksbbj.2011.26.2.112.

Lane, A. L., Mular, L., Drenkard, E. J., Shearer, T. L., Engel, S., Fredericq, S., Fairchild, C. R., Prudhomme, J., Le Roch, K., Hay, M. E., Aalbersberg, W. and Kubanek, J. (2010). Ecological leads for natural product discovery: Novel sesquiterpene hydroquinones from the red macroalga Peyssonnelia sp. Tetrahedron. 66(2): 455–461.

Li, Y., Zheng, Y., Zhang, Y., Yang, Y., Wang, P., Imre, B.,

Wong, A. C. Y., Hsieh, Y. S. Y. and Wang, D. (2021). Brown Algae Carbohydrates: Structures, Pharmaceutical Properties, and Research Challenges. Marine drugs. *19*(11): 620. https://doi.org/10.3390/md19110620

Ohno, M., Dan, A., Yoshimoto, R., and Matsuoka, M., (2011). Morphology and quality of cultivated Laminaria japonica Aresch. in the temperate waters of Naruto Straits, Japan. Bull. Tokushima. Pref. Fish. Res. Ins. No. 7: 5 - 10

Onyeaka, H., Miri, T., Obileke, K., Hart, A. and Anumudu, C. (2021). Minimizing carbon footprint via microalgae as a biological capture. Carbon Capture Sci Technol 1:100007. https://doi.org/10.1016/j.ccst.2021.100007

Park, S.J., Sharma, A., and Lee, H.J. (2024). An update on the chemical constituents and biological properties of selected species of an underpinned genus of red algae: Chondrus. Mar. Drugs. 22: 47. doi: 10.3390/md22010047.

Pereira, L., Cotas, J. and Valado, A. (2024). Antioxidants from microalgae and their potential impact on human well-being. Explor Drug Sci. 2: 292–321.

Riccio G, Lauritano C. Microalgae with Immunomodulatory Activities. Mar Drugs. 2019 Dec 18;18(1):2. doi: 10.3390/md18010002. PMID: 31861368; PMCID: PMC7024220.

Ruiz-Güereca, D.A., Licea-Navarro, A.F. and Sánchez-Saavedra, M. del P. (2019) Evaluation of antimycobacterial activity from marine and freshwater microalgae. Rev Biol Mar Oceanogr. 54:82–90.

Saide, A., Martínez, K. A., Ianora, A., & Lauritano, C. (2021). Unlocking the Health Potential of Microalgae as Sustainable Sources of Bioactive Compounds. International journal of molecular sciences, 22(9), 4383. https://doi.org/10.3390/ijms22094383

Shalaby E. A. (2011). Algae as promising organisms for environment and health. Plant signaling&behavior. 6(9): 1338–1350.

Smol, J.P. and Stoermer, E.F. (2010). The Diatoms: Applications for the Environmental and Earth Sciences.2nd Edn. Cambridge University Press, 667 p.

Sruthy, K.D., Sathish, A. and Stanley, J. (2018). Production of biofuel and bioplastic from *Chlorella pyrenoidosa*. Materials Today: Proceedings.*Lin* 5(8): 16774-16781.

Taboada, M., Lence, R., and Miguez, M. (2012). Nutritional value of the marine algae wakame (*Undaria pinnatifida)* and nori (*Porphyra purpurea*) as food supplements. Journal of Applied Phycology. 25. doi: 10.1007/s10811-012-9951-9.

Tajvidi, E., Nahavandizadeh, N., Pournaderi, M., Pourrashid, A. Z., Bossaghzadeh, F. and Khoshnood, Z. (2021). Study the antioxidant effects of blue-green algae Spirulina extract on ROS and MDA production in human lung cancer cells. Biochemistry and biophysics reports. 28:101139. https://doi.org/10.1016/j.bbrep.2021.101139

Turan, G., and Cýrýk, S. (2018). Sea vegetables. InTech. doi: 10.5772/intechopen.75014.

Ulagalanthaperumal, E.P. and R, Sundararaj. (2020). Algae: A Potential Source to Prevent and Cure the Novel Coronavirus -A review. Int J Emerg Technol 11:479–483.

Wang, X., Pei, L., Liu, H., Qv, K., Xian, W., Liu, J. and Zhang, G. (2016). Fucoidan attenuates atherosclerosis in LDLR-/-mice through inhibition of inflammation and oxidative stress. Int J Clin Exp Pathol. 9(7):6896-6904

Widyaningrum, D. and Prianto, AD. (2021). IOP Conf. Ser.: Earth Environ. Sci. 794: 012148.

Xu, X., Gu, X., Wang, Z., Shatner, W. and Wang, Z., (2019). Progress, challenges and solutions of research on photosynthetic carbon sequestration efficiency of microalgae. Renew and Sustain Energy Rev. 110: 65–82.

Plant Science Research 46 (1&2) : 30-39, 2024

Plant Science Research

ISSN 0972-8546

Enhancing Protein Recovery: A Comparative Analysis of Extraction and Quantification Methods for *Glycine max* Seeds

Gyanajeet Parida, Bishnuprabha Behera and Nihar Ranjan Singh[Ψ]
Department of Botany, Ravenshaw University, Cuttack-753003

ARTICLE INFO

Article history:
Received : 25 August 2024
Revised : 28 September 2024
Accepted : 18 October 2024

Keywords:

Glycine max
Protein extraction
Proteomics
Protein quantification
SDS-PAGE

ABSTRACT

It is quite difficult to choose the most effective protein extraction technique from plant samples that can remove the greatest number of limiting steps. The search for effective plant protein extraction methods faces many trials and tribulations and is still under lots of scrutiny. The challenges that lie in the plant protein extraction methods are in having low protein and high proteinase content, secondary metabolites, and cellulosic cell wall encapsulation. Here, five different extraction methods—Phenol-based, TCA-Acetone-based, Phenol-TCA-Acetone-based, HEPES buffer-based, and TRIzol-based methods were used to extract *Glycine max* seed proteins to evaluate the efficiency of these extraction techniques. Further, the extracted proteins were quantified using Lowry and Bradford protein assay tests. To determine the suitable extraction and quantification method for soybean protein extraction from soybean seeds, two approaches were taken. In one approach, equal volumes and in another approach equal quantity of proteins were made into separation steps using the Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) process. In the present experiment, the HEPES-buffer-based extraction takes the least amount of time, recovers more soluble form, and yields nearly identical values when quantified using both approaches. Proteins separated by gel electrophoresis from the HEPES-buffer method also show very conspicuous and distinct bands.

1. Introduction:

The majority of organisms that are alive, primarily obtain their energy from plants that can generate food by utilizing the sunlight through the process of photosynthesis.Similarly, nitrogen fixation is a process by which microbes make atmospheric nitrogen available to plants which work as building blocks of proteins and nucleic acid synthesis (Soumare *et al.*, 2020). As green plants take place at the primary trophic level in all types of ecosystems, inorganic and organic molecules are transferred directly or indirectly to all living organisms (Nisha *et al.*, 2023). Living organisms pass on traits from generation to generation by converting genetic information into proteins that manifest phenotypic and genotypic characteristics within the organism. Proteins like structural proteins maintain the organisation and shape whereas functional proteins are involved in the chemical and developmental process of the organisms (Yu & Fukagawa, 2020). Different proteins within the plant participate in the growth and developmental activities such as embryo development, seed germination, flowering, and fruit setting. While some proteins are expressed during specific developmental phases, others, such as RUBISCO, expressed throughout the life of the plant (Kosova *et al.*, 2021). Referring to the Lindeman's 10% rule of energy flow, only 10% of the total energy is transferred from one trophic level to another, and, since living beings at higher trophic levels will receive less energy than organisms at lower trophic levels that rely directly on plant products (Libralato *et al.*, 2014). Foods from plants is the major source of nutrition for higher trophic level organisms and thus it is essential to accommodate plant-based nutrition into the human diet. For healthy and disease-free human life, a diet comprised of plant-based proteins,

[Ψ] *Corresponding author; Email:* nihar.singh@gmail.com

fibre, vitamins, and the recommended quantity of carbohydrates and lipids are needed. On the other hand, a diet rich in animal proteins and carbohydrates potentially increases the risk of obesity, type 2 diabetes, cardiovascular diseases, and other metabolism-related health diseases (Vahedifar & Wu, 2022). The growing demand for high-quality proteins prompts researchers and dietitians to seek out environmentally friendly and sustainable protein sources.To meet the increasing needs and mitigate the adverse effects of animal proteins, cost-effective extraction methods combined with integration technologies are needed to improve the nutritional supplements of plant protein-based meals (Kumar *et al.,* 2021). Proteins from plants can be extracted using different methods and their functional properties can be influenced by factors like water content, presence of primary and secondary metabolites, and also physical factors such as temperature, pH, and ionic strength along with plant part, tissue, age, and developmental stages (Isaacson *et al.,* 2006). Protein extraction from various plant tissues is a difficult process since plant tissues often contain low amounts of proteins with high proteases, inhibiting tissue disintegration and subsequent isoelectric focusing. In the 1980s, a lot of effort went into establishing sample preparation techniques for two-dimensional electrophoresis (2-DE) in plants. The initial 2-DE sample preparation consisted of a single phase of denaturing extraction in a lysis buffer. However, this approach is only applicable to "clean" samples and is ineffective for crude plant material. As a result, most plant methods included a precipitation phase to concentrate proteins and eliminate interfering contaminants (Carpentier *et al.,* 2005). Plants produce a huge amount of secondary metabolites, which also impedes protein extraction. Despite this, plant cells are enclosed by cellulose cell walls, which adds another challenge to protein extraction by shattering the cells. For these reasons developing a standard technique for protein extraction from plant tissues has yielded modest results (Singh *et al.,* 2015). Apart from the 2-DE protein extraction approach, other plant protein extraction methods like alkaline extraction, salting in and out, ultrafiltration, microwave-assisted or fractionation methods are also employed for extracting plant proteins. It is worth understanding that all such extraction methods are not free from limitations of poor yield, low stability or uniformity, and uneven coloring (Hewage *et al.,* 2022). In this analysis, five protein extraction methods were employed to extract the protein content of plant samples and to evaluate the suitability of such methods for better extraction of plant proteins.

Soybean is a well-known protein-rich seed plant and is the key source of protein and oil for humans and animals. Cultivated soybeans (*Glycine max* [L.] Merr.) are domesticated from wild soybeans (*Glycine soja* Sieb. & Zucc.) in China some about 5000 years ago and belong to the family of fabaceae. Soybean proteins (SPs) made out from soybean seeds are high in nutritional content and have a variety of functional qualities, including emulsification, foamability, gelation, and the ability to hold water and oil. It has numerous applications in the food industry in the form of dietary supplements, emulsifiers, meat and pharmaceutical goods, and newborn formula. Our everyday diet also incorporates a variety of SP-derived products, including soybean milk, tofu, and fermented soybean products (Miso, Tempeh, and Soybean sauce) (Liu *et al.,* 2023; Huang *et al.,* 2020). Since effective protein extraction is the first stage of proteomics analysis, so in this work attempt has been taken to establish an experimental relationship between four different protein extraction methods and two quantification techniques and also to determine the best protocol for both protein extraction and the correspondence quantification method for soybean seeds.

2. Materials and Methodology:

Soybean (*Glycine max*) seed samples were collected from the commercial market of the Malgodown area of the Cuttack district of Odisha and authenticated at the Department of Botany, Ravenshaw University. The seeds were soaked in luke warm water for 10 hours before being subjected to five different extraction methods.

2.1 Phenol-based extraction method

Extraction of plant proteins through the phenol extraction method was done in accordance with the method proposed by (Faurobert *et al.,* 2007 and Isaacson *et al.,* 2006) with little modification (Faurobert *et al.,* 2007; Isaacson *et al.,* 2006). Briefly, 1g of soaked soybean seeds were grounded with 3 ml extraction buffer with the composition of 0.5M Tris-HCl (pH=8.7) + 0.9M Sucrose + 0.05M EDTA + 0.1M KCl + 1mM PMSF + 2% β- mercaptoethanol, keeping the final pH in the range between 7.9-8.5. The mixture was then transferred into a centrifuge tube and mixed with an equal volume of tris-phenol buffer (1:1 ratio of 50mM tris-HCL: phenol with a pH of 6.6-7.9) and vortexed thoroughly at least for 10-15 minutes. The mixture was then cold centrifuged for 10 minutes at 5500g. The upper phenolic layer was then collected and transferred to another centrifuge tube and mixed again with 3ml of extraction buffer followed by cold centrifugation for 10 minutes at 5500g after a gentle vortexing. Again, the top phenolic layer was collected and mixed with 5 volumes of precipitation buffer with the composition of 0.1M ammonium acetate with pre-chilled methanol + 1% β-mercaptoethanol. The solution was then

mixed thoroughly and incubated at -20°C for at least 4-5 hours and then under went a cold centrifuge for 10 minutes with 5500g. The pellet was then collected and washed with cold precipitation buffer for one time and then with cold acetone for at least three times, cold centrifuging at 5500g for 5 minutes at the end of each washing step and then the final collected pellet was air dried and stored at -20°C for further experimental analysis.

2.2 TCA-Acetone-based extraction method

The protein extraction using the TCA-Acetone method followed the protocol designed by (Fei *et al.,* 2021 and Mechin *et al.,* 2007), with minor modifications (Fei *et al.,* 2021; Mechin *et al.,* 2007). Using a pre-chilled mortar and pestle, 1g of soaked seeds were taken and completely homogenised. The homogenate was then poured into a centrifuge tube and mixed with 6ml of reagent 1 having the composition of 10% TCA-Acetone with 5 mM DTT, and 0.07% 2- mercaptoethanol (2-ME). The mixture was then cold centrifuged at 12,000g for 5 minutes after thorough vortexing. The pellet was collected and combined again with reagent 1 and cold centrifuged at 12,000g for 5 minutes. This pellet was collected and washed with a solution containing 80% acetone and 5 mM DTT by cold centrifuging at 5500g for 5 min. The final pellet was collected and stored at -20°C for further analysis.

2.3 Phenol-TCA-Acetone-based extraction method

The third extraction method, which is a hybrid methodology of phenol and TCA-Acetone extraction method as proposed by Wu *et al* with minor modifications to extract more pure protein from the sample (Wu *et al.,* 2014). Using a pre-cooled mortar and pestle, 1g of soaked seeds were thoroughly homogenised and then transferred to a centrifuge tube mixed with 6ml of reagent 1 having the composition of 10% TCA-Acetone with 5 mM DTT, and 0.07% 2-mercaptoethanol (2-ME). Then cold centrifuged at 12,000g for 5 minutes.The pellet was washed twice with a solution containing 80% acetone with 5mM DTT by cold centrifuging at 5500g for 5 min and the final pellet was then suspended in 5 mL of tris-phenol buffer. The mixture was then again cold centrifuged for 5 minutes with 12,000g. The phenol phase was collected in a tube and mixed with a 5-volume of precipitation solution. After fully mixing, it was allowed to precipitate for at least four to five hours and then vortexed and cold centrifuged at 5500g for 10 minutes. The pellet was collected and cleaned at least once with a cold precipitation solution followed by three times washing with cold acetone centrifuging at 5500g for 5 minutes at the end of each washing step. Then the final collected pellets were air-dried and stored at -20°C for further experimental analysis.

2.4 TRIzol solution-based extraction method

Extraction using TRIzol was followed as per the method of Chan et al with slight modifications (Chan *et al.,* 2018). Using this method, 1g of wet seed samples were ground in a pre-cooled mortar and pestle. This paste was then mixed with 20ml of TRIzol solution and thoroughly vortexed for 5 minutes. Following that, 4mL of chloroform was added to the mixture and vortexed for another 15-20 seconds. This mixture was then cold centrifuged at 14,000g for 5 minutes. The upper layer was discarded, and the lower phenolic layer was mixed with 6 ml of ethanol. The mixture was then allowed for cold centrifuge at 2000g for 5 min. After cold centrifugation, the supernatant was then transferred to a fresh tube and mixed with 10ml of isopropanol that precipitated the proteins. The precipitated solution was then cold centrifuged at 14,000g for 15 minutes and the pellet was collected. This protein pellet was then air-dried and cleaned with 95% ethanol and stored at-20°C before further experimental analysis.

2.5 HEPES buffer-based extraction method

Extraction of protein using HEPES buffer was followed using the protocol of Xia et al with slight modifications (Xia *et al.,* 2021). Approximately, 1g of seed samples were pulverised in a pre-chilled mortar & pestle with 12 ml of 4-(2-hydroxyethyl) -1- piperazine ethane sulfonic acid (HEPES) extraction buffer which contains 10mM HEPES with 0.1% protease inhibitor, and 0.7% β-mercaptoethanol, pH=7.5 (adjusted with Diluted NaOH). The mixture was then incubated on ice for 20 minutes, with occasional mixing. The sample was then cold centrifuged at 16,000g for 20 minutes and finally the supernatant was collected and stored at -20°C for further study.

3. Quantification of extracted proteins

The proteins collected using these five extraction methods either in the form of pellet or supernatant are dissolved in a sample buffer which comprises of 0.5M Tris-HCl and 10% sodium dodecyl sulphate (SDS). After mixing the proteins in the sample buffer, the protein solutions were measured by both Lowry's and Bradford's assay of protein quantification (Bradford, 1976; Lowry *et al.,* 1951). The mg of protein present per gram of seed samples was calculated using the standard bovine serum albumin (BSA).

4. Separation of proteins

Proteins were separated based on their molecular weight using the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) method with minor modifications (Postu *et al.,* 2019). The concentration of the

resolving gel was kept at 12%, while that of the stacking gel was made at4%. To ensure the accuracy of protein separation, two approaches were selected. In the first approach, an equal volume of samples was loaded and separated using electrophoresis. Here, the protein samples were loaded just after the extraction process without any protein quantification keeping the volume constant. In the second approach, an equal amount of samples that were measured using the two quantification methods were used. In this approach, an equal amount of proteins was calculated after quantification and loaded and separated using the electrophoresis method. A general schematic representation of the method used in this study is represented in figure 1.

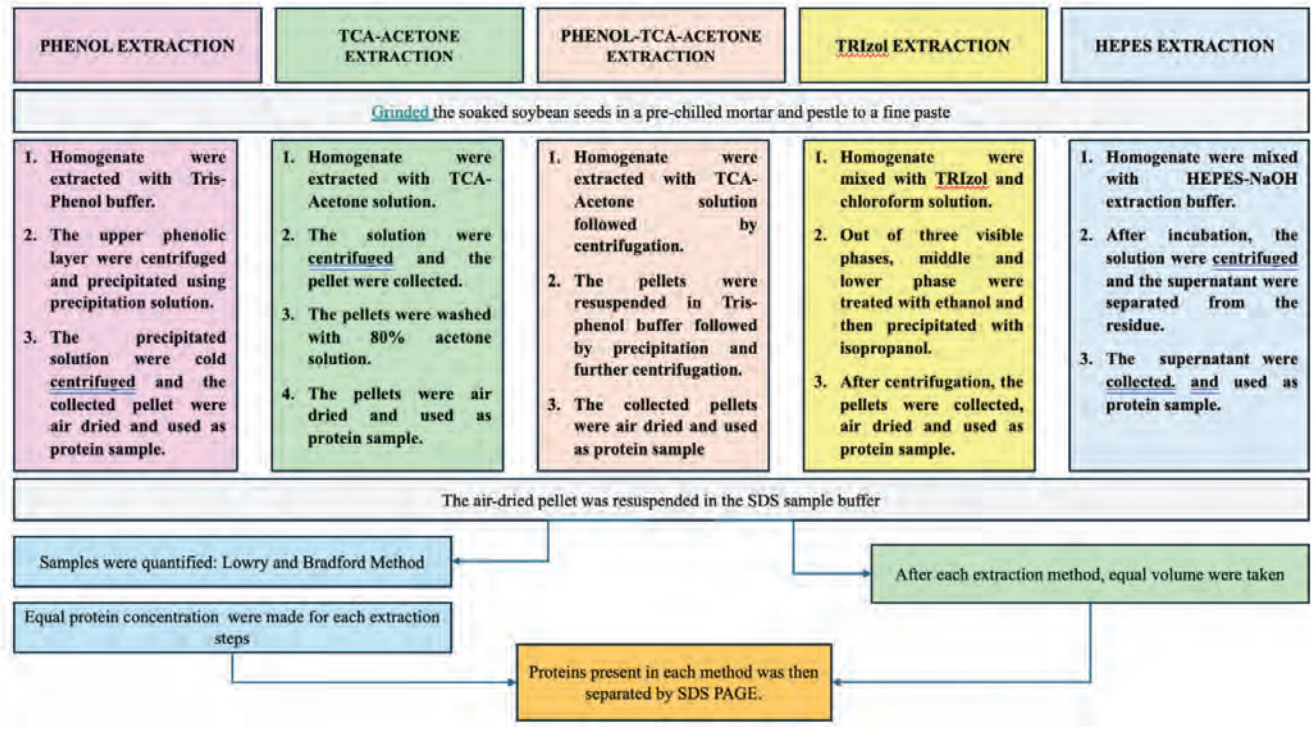


Figure 1: A general schematic representation of methods used in this study.

5. Results:

5.1 Extraction of protein samples:

All five extraction methods used in this experiment show the presence of proteins in the soybean seed samples in a variable range. On visual observation, each of the five different extraction methods as seen in figure 2 shows the variability of protein content in the test tubes after completing the extraction steps. The results of this experiment are depicted in table 1.The results show that the proteins extracted using the TRIzol, HEPES buffer, and Phenol-TCA-Acetone extraction methods were more readily soluble in the sample buffer. This indicates that soluble proteins increase the suitability for protein quantification and separation assays.

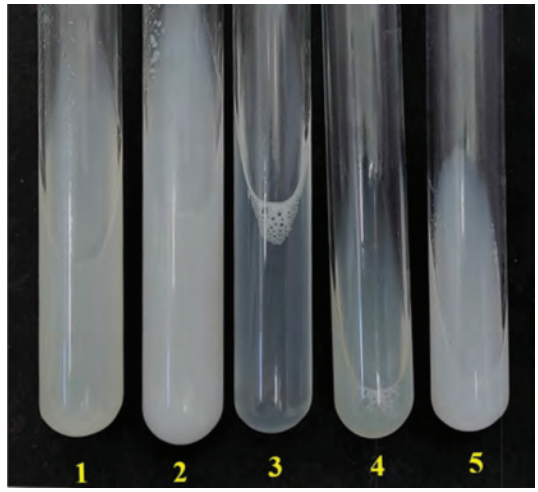


Figure 2: The test tube 1: protein sample extracted from the phenol extraction method, test tube 2: protein sample extracted from the TCA-Acetone extraction method, test tube 3: protein sample extracted from the Phenol-TCA-Acetone extraction method, test tube 4: Protein sample extracted from the HEPES Buffer extraction method, test tube 5: protein samples extracted from TRIzol method of extraction.

Table 1:

The table shows the mechanism used in the five different extraction processes and the subsequent observations in the results.

Methods	Mechanism	Result
Phenol-based extraction method	Diligently dissolve and purify proteins from the liquid extract after protein extraction.	The pellet was not completely soluble in the sample buffer an opaque solution was obtained.
TCA-Acetone extraction method	Secondary metabolites were eliminated by a TCA-Acetone wash before protein extraction.	The pellets were immiscible in the sample buffer as a result a hazy mixture appeared.
Phenol-TCA-Acetone-based extraction method	TCA-Acetone eliminates secondary metabolites first, and phenol is used to separate the proteins from the mixture.	The pellets were completely soluble in the sample buffer, so a completely transparent solution appeared.
TRIzol solution-based extraction method	Proteins precipitated from the TRIzol solution after a range of interfering substances, including pigments, nucleic acids, non-protein components, and salts, were eliminated from the sample.	The pellets were almost soluble in the sample buffer, so a milkish white colour solution was developed.
HEPES buffer-based extraction method	At a pH of 7.5, HEPES NaOH dissociates proteins from the cell and solubilises them in a solution that minimises protein breakdown.	Here proteins were extracted in the aqueous phase directly, no pellet formation occurred as a result we can get a completely soluble protein sample.

5.2 Protein Quantification:

Proteins extracted by five different extraction methods were quantified by both the Lowry's and Bradford's protocol using standard equations ($y = 0.0009x + 0.0134$; $R^2 = 0.9951$) and ($y = 0.0095x + 0.0575$; $R^2 = 0.989$) respectively. The measured values are shown in table 2.

Table 2:

Amount of protein extracted by five different extraction methods determined by two different quantification methods are expressed as mean ± SD from a minimum of three independent experiments. Amounts of proteins are expressed as mg/g of seed samples.

Protein extraction methods	Amount of protein in (mg/g of seed samples) quantified by Lowry method	Amount of protein in (mg/g of seed samples) quantified by Bradford method
Phenol-based extraction method	3.20 ± 0.05	4.48 ± 0.03
TCA-Acetone-based extraction method	3.34 ± 0.03	1.05 ± 0.03
Phenol-TCA-Acetone-based extraction method	0.51 ± 0.03	6.12 ± 0.06
TRIzol solution-based extraction method	27.58 ± 0.06	3.79 ± 0.05
HEPES buffer-based extraction method	5.12 ± 0.06	4.85 ± 0.06

5.3 Protein separation through electrophoresis:

To evaluate the efficacy of the five protein extraction methods and their corresponding protein quantification techniques, extracted proteins were separated based on their molecular weight using one-dimensional gel electrophoresis. The results of protein separation are shown in figure 3.

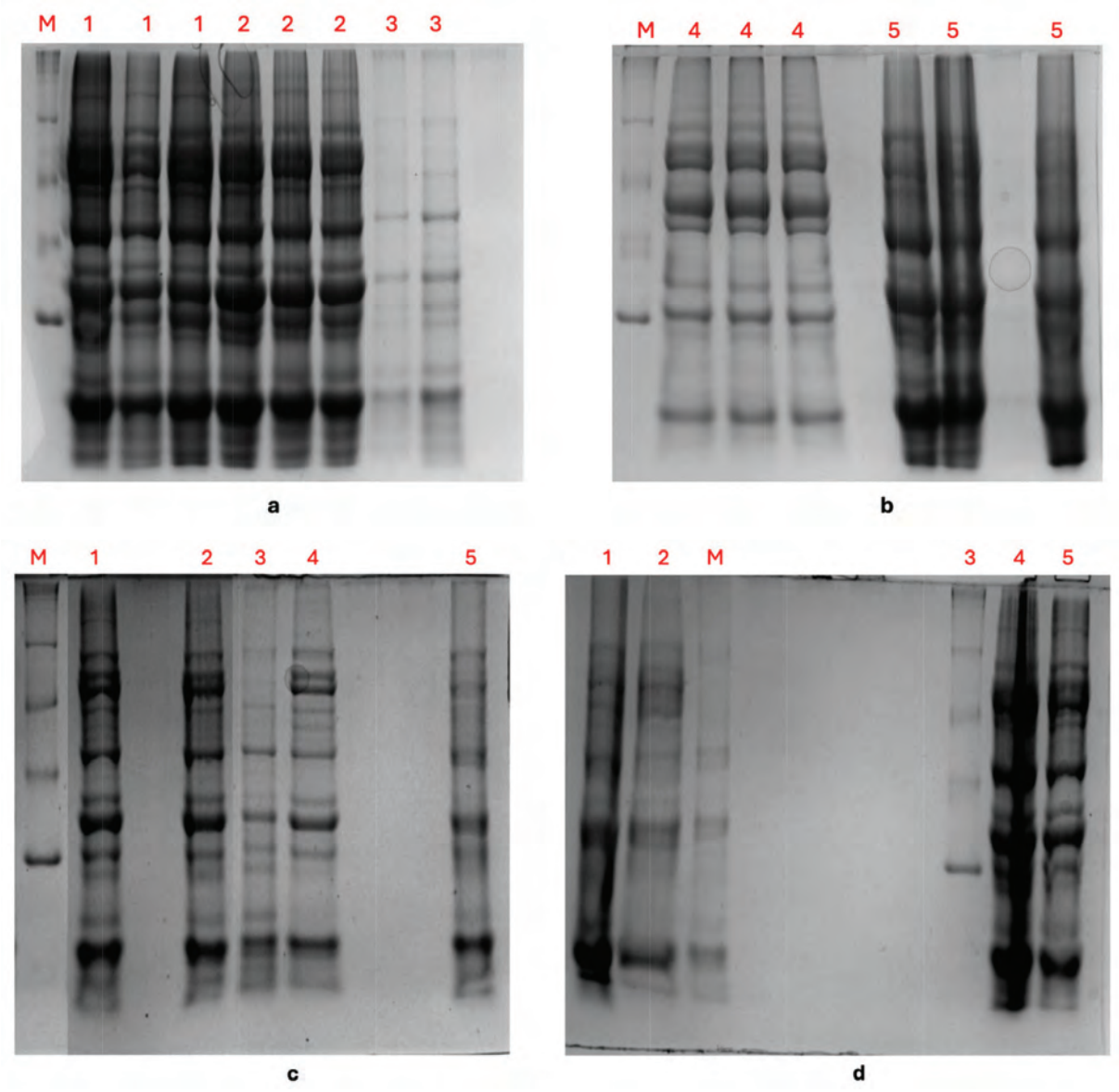


Figure 3: In all four gel images (a, b, c, d) M represents theprotein marker, well 1: Proteins extracted by the Phenol extraction method, well 2: Proteins extracted by the TCA-Acetone method, well 3: Proteins extracted by the Phenol-TCA-Acetone method, well 4: Proteins extracted by HEPES buffer, well 5: Proteins extracted by TRIzol solution method. In gel "a" and "b" equal volumes of samples were loaded in each well and extracted by different extraction methods. In gel "c" an equal amount of proteins was loaded in each well based on the Lowry method of quantification and in gel "d" an equal amount of proteins was loaded based on the Bradford method of quantification.

6. Discussion:

Protein content of any biological sample depends on the chemical composition of proteins of that species, the extent of protein expression and degradation, and the protein regulatory mechanism within the cellular system (Bludau & Aebersold, 2020). In order to show differences in the plant protein extraction process on the yield of the soybean seed proteins, different extraction protocols were examined and evaluated.

Phenol extraction methods were first pioneered on pea epicotyls by Schuster and Davies in 1983 (Schuster & Davies, 1983). Phenol is the basic aromatic alcohol that has an aromatic ring with an attached polar hydroxyl ([OH]) group. It is poisonous and corrosive, with weak acidic qualities. Even though phenol and water are somewhat miscible, in a saturated solution, there is roughly 7% phenol in the aqueous layer and 28% water in the organic layer. Hydrogen bonding is the main mechanism of interacting with proteins, causing denaturation and solubility in the

organic phase. The phenol phase contains proteins, not the interface. Proteins are initially separated in an aqueous buffer before being combined with buffered phenol at pH 7.8-8.0. Proteins denature and move into the phenol phase as a result of this interaction, whilst other water-soluble molecules including nucleic acids, carbohydrates, and salts remain in the aqueous layer. Methanol precipitation separates the co-extracted phenolic chemicals from the proteins. The phenol extraction method is largely utilised for recalcitrant plant tissues and organs, such as wood, rapeseed, potato seedlings, apple, olive, potato, and banana leaves, and also for avocado, banana, and tomato fruits. This method successfully eliminates nucleic acids, which can interact with proteins and cause low resolution and significant background in two-dimensional electrophoresis (2-DE). The drawback of this method is that it is a highly time-consuming process and also the phenol and methanol used in this technique are harmful to the human body (Faurobert *et al.*, 2007).

The TCA-Acetone precipitation technique developed by Damervalin 1986 was a popular and efficient method that works with a variety of plant tissues. Using 2-mercaptoethanol (2-ME) and trichloroacetic acid (TCA) in cold acetone, this method directly precipitates total proteins from homogenised tissues or cells, leading to protein extraction (Wu *et al.*, 2014). Apart from phenolic extraction, seed proteins can also be effectively isolated with a TCA-Acetone solution. Technically, the TCA-Acetone procedure is simpler and relies on the direct precipitation of proteins from plant tissues. (Smolikova *et al.*, 2020). The drawback of this method is in precipitating lignin, an aromatic polymer along with proteins. These aromatic polymers might attach to the colouring agent during the protein quantification procedure, leading to a false-positive result (Sebastiana *et al.*, 2013). In this experiments, it is observed that the phenol extraction method yields a more consistent result than the TCA-Acetone method during the quantification process when measured using both the Bradford and Lowry methods of quantification. On the other hand, the TCA-Acetone approach and the phenol extraction method exhibit nearly identical kinds of banding patterns when we look at the gel electrophoresis profile.

The third extraction method which is the hybrid Phenol-TCA-Acetone extraction method to extract proteins, it may be possible to remove contaminants that impede the protein estimation process, such as polyphenols and aromatic polymers. This allows us to determine the precise levels of protein in a sample (Abdullah *et al.*, 2017). While working on the proteome analysis of the *Jatropha curcas* L. stem in 2017, Song Yang and his colleagues concluded that a combination of the two methods could yield a good number of proteins in 2-dimensional gel electrophoresis, but neither phenol extraction nor TCA-Acetone alone produces better results (Yang *et al.*, 2017). Studies have shown that the amount of protein extracted and protein profiling using the gel electrophoresis of the hybrid phenol-TCA-Acetone method were found more efficient than TCA-Acetone and phenol extraction methods (Rastegari *et al.*, 2011). However, in our studies for soybean seed protein extraction, the individual extraction process provides better results than the combined approach.

TRIzol reagent, a mixture of phenol and guanidine isothiocyanate is a monophasic solution that can extract Protein, RNA, and DNA from a biological sample in a single step (Hummon *et al.*, 2007) Formerly, TRIzol reagent was used for the extraction of RNA but it has also become more well-liked for two-dimensional electrophoresis (2-DE) protein extraction. The efficacy of this technique in removing impurities from protein samples has drawn attention, improving the caliber of 2-DE results. The TRIzol-based extraction method was noteworthy for its effective use in 2006 in halophilic protein samples (Chan *et al.*, 2018). This approach has several benefits over conventional methods, including the ability to extract protein pellets quickly within hours and successfully remove a variety of interfering compounds, such as pigments, nucleic acids, non-protein components, and salts. Furthermore, proteins in the Trizol reagent are safeguarded against protease degradation, eliminating the need for protease inhibitors. During the IEF step, the extracted protein samples can be loaded into the gel for rehydration, producing high-quality 2-DE profiles (Lee & Lo, 2008). However, in contrast to previous approaches, our experiments exhibit significant fluctuation in the quantification result, and the band patterns in the gel electrophoresis profile are not as noticeable and distinct compared to other methods.

HEPES buffer functions as a kosmotrophic reagent due to its positive viscosity index and pH range of 6-8, which aids in the stability of protein structures in solution. This solution is also useful in dissociating proteins from cells at increasing concentrations (Incocciati *et al.*, 2022). Additionally, the amount of protein extracted from the animal cells can be increased if HEPES-NaOH buffer is combined with RapiGest (a mild protein denaturant) or ionic reagents like sodium dodecyl sulphate (Kaleja *et al.*, 2020). Extracting complete protein using HEPES buffer was also uncommon in plant proteome studies. Xia *et al.* in the year 2016 used this technique for determining the seed protein carbonyl content, earlier to this some other scientists also used this technique (Xia *et al.*, 2016). In our experimental

setup,the HEPES buffer extraction method produces a more even result in protein isolation than the other methods. Another positive outcome was that the result observed was similar to the hybrid phenol-TCA-Acetone approach and demonstrated very clear and noticeable bands in electrophoresis suggesting it is one of the suitable approaches for higher protein recovery and with less contamination.

7. Conclusion:

The first and most important stage in proteomic analysis is to extract total proteins in pure form, devoid of impurities. The difficulty lies in breaking down the cellulose cell wall and extracting purified proteins from cells rich in secondary metabolites. In the present study, five different protein extraction methods were evaluated, followed by quantification and separation by gel electrophoresis. Proteins extracted using the phenol and TCA-Acetone methods yielded good quantification findings and a large number of distinct bands in the electrophoretic process, but the pellets were not completely soluble in the sample buffer, which could complicate the exact yield results. To obtain a higher resolution in the process, the combined Phenol and the TCA-Acetone method were used although the protein pellet was completely soluble in the sample buffer, the quantification process produced very uneven results, and the banding pattern was very faint and less prominent than the individual extraction methods.

The TRIzol approach effectively removes contaminants and nucleic acids from proteins, but in our electrophoresis data, it does not achieve the same level of success as we reviewed. Despite the protein pellets being perfectly solubilised in the sample buffer, the findings vary significantly between the two quantification techniques. Finally, the HEPES buffer method of extraction was found to be the quickest and the simplest. The observed extracted proteins were soluble and yielded comparable types of outcomes in both quantification methods. Also, the gel-electrophoresis separation of proteins reveals that the bands are quite clear, with very few contaminants identified between the protein bands.

Acknowledgements

The authors are thankful to the Department of S & T, Odisha for financial support in the form of a grant vide letter no.27552800232014/202820, ST-16/17.05.2015. NRS is also thankful to the Department of Higher Education, Government of Odisha for financial assistance under OHEPEE (Grant No.: HE-PTC-WB-02017) as seed research funding.

References:

Abdullah, F. I., Chua, L. S., & Rahmat, Z. (2017). Comparison of protein extraction methods for the leaves of *Ficus deltoidea*. *Journal of Fundamental and Applied Sciences*, *9*(2), 908-924.

Bludau, I., & Aebersold, R. (2020). Proteomic and interactomic insights into the molecular basis of cell functional diversity. *Nature Reviews Molecular Cell Biology*, *21*(6), 327-340. https://doi.org/10.1038/s41580-020-0231-2

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254.

Carpentier, S. C., Witters, E., Laukens, K., Deckers, P., Swennen, R., & Panis, B. (2005). Preparation of protein extracts from recalcitrant plant tissues: An evaluation of different methods for two-dimensional gel electrophoresis analysis. *PROTEOMICS*, *5*(10), 2497-2507.

Chan, K.K., Kwok, C. S.N., Sze, E. T.P., & Lee, F. W.F. (2018). Evaluation of the Use of TRIzol-Based Protein Extraction Approach for Gel-Based Proteomic Analysis of Dried Seafood Products and Chinese Tonic Foods. *International Journal of Molecular Sciences*, *19*(7), 1998. https://www.mdpi.com/1422-0067/19/7/1998

Faurobert, M., Pelpoir, E., & Chalb, J. (2007). Phenol Extraction of Proteins for Proteomic Studies of Recalcitrant Plant Tissues. In H. Thiellement, M. Zivy, C. Damerval, & V. Mechin (Eds.), *Plant Proteomics: Methods and Protocols* :9-14. Humana Press. https://doi.org/10.1385/1-59745-227-0:9

Fei, J., Wang, Y.-S., Cheng, H., & Su, Y.-B. (2021). An efficient protein extraction method applied to mangrove plant Kandelia obovata leaves for proteomic analysis. *Plant Methods*, *17*(1), 100. https://doi.org/10.1186/s13007-021-00800-y

Hewage, A., Olatunde, O. O., Nimalaratne, C., Malalgoda, M., Aluko, R. E., & Bandara, N. (2022). Novel Extraction technologies for developing plant protein ingredients with improved functionality. *Trends in Food Science & Technology*, *129*, 492-511.

Huang, Z., Qu, Y., Hua, X., Wang, F., Jia, X., & Yin, L. (2023). Recent advances in soybean protein processing technologies: A review of preparation, alterations in the conformational and functional properties. *International Journal of Biological Macromolecules*, *248*, 125862. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2023.125862

Hummon, A. B., Lim, S. R., Difilippantonio, M. J., & Ried, T. (2007). Isolation and Solubilization of Proteins After TRIzol® Extraction of RNA and DNA from Patient Material Following Prolonged Storage. *BioTechniques*, *42*(4), 467-472. https://doi.org/10.2144/000112401

Incocciati, A., Bertuccini, L., Boffi, A., Macone, A., & Bonamore, A. (2022). Unlocking the Treasure Box: The Role of HEPES Buffer in Disassembling an Uncommon Ferritin Nanoparticle. *Separations*, *9*(8), 222. https://www.mdpi.com/2297-8739/9/8/222

Isaacson, T., Damasceno, C. M. B., Saravanan, R. S., He, Y., Catalá, C., Saladié, M., & Rose, J. K. C. (2006). Sample extraction techniques for enhanced proteomic analysis of plant tissues. *Nature Protocols*, *1*(2), 769-774.

Kaleja, P., Emmert, H., Gerstel, U., Weidinger, S., & Tholey, A. (2020). Evaluation and improvement of protein extraction methods for analysis of skin proteome by noninvasive tape stripping. *Journal of Proteomics*, *217*, 103678. https://doi.org/https://doi.org/10.1016/j.jprot.2020.103678

Kosova, K., Vítámvás, P., Prášil, I. T., Klíma, M., & Renaut, J. (2021). Plant Proteoforms Under Environmental Stress: Functional Proteins Arising From a Single Gene [Review]. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.793113

Kumar, M., Tomar, M., Potkule, J., Verma, R., Punia, S., Mahapatra, A., Belwal, T., Dahuja, A., Joshi, S., Berwal, M. K., Satankar, V., Bhoite, A. G., Amarowicz, R., Kaur, C., & Kennedy, J. F. (2021). Advances in the plant protein extraction: Mechanism and recommendations. *Food Hydrocolloids*, *115*, 106595. https://doi.org/https://doi.org/10.1016/j.foodhyd.2021.106595

Lee, F. W.-F., & Lo, S. C.-L. (2008). The use of Trizol reagent (phenol/guanidine isothiocyanate) for producing high quality two-dimensional gel electrophoretograms (2-DE) of dinoflagellates. *Journal of microbiological methods*, *73*(1), 26-32.

Libralato, S., Pranovi, F., Stergiou, K. I., & Link, J. S. (2014). Trophodynamics in marine ecology: 70 years after Lindeman. *Marine Ecology Progress Series*, *512*, 1-7.

Liu, S., Zhang, M., Feng, F., & Tian, Z. (2020). "Toward aGreen Revolution" for Soybean. *Molecular Plant*, *13*(5), 688-697.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J biol Chem*, *193*(1), 265-275.

Mechin, V., Damerval, C., & Zivy, M. (2007). Total Protein Extraction with TCA-Acetone. In H. Thiellement, M. Zivy, C. Damerval, & V. Méchin (Eds.), *Plant Proteomics: Methods and Protocols* (pp. 1-8). Humana Press. https://doi.org/10.1385/1-59745-227-0:1

Nisha, P., John, N., Rubeena, K. A., & Thangavel, M. (2023). Biomass from Terrestrial Environments. In S. Thomas, M. Hosur, D. Pasquini, & C. Jose Chirayil (Eds.), *Handbook of Biomass* (pp. 1-27). Springer Nature Singapore. https://doi.org/10.1007/978-981-19-6772-6_3-1

Postu, P. A., Ion, L., Drochioiu, G., Petre, B. A., & Glocker, M. O. (2019). Mass spectrometric characterization of the zein protein composition in maize flour extracts upon protein separation by SDS-PAGE and 2D gel electrophoresis. *ELECTROPHORESIS*, *40*(20), 2747-2758. https://doi.org/https://doi.org/10.1002/elps.201900108

Rastegari, E., Ahmad, Z., Spencer, D. F., & Ismai, M. (2011). Two-dimensional profiling of proteins from Curculigo latifolia fruit by three different extraction protocols. *J Med Plant Res*, *5*(16), 3719-3724.

Schuster, A. M., & Davies, E. (1983). Ribonucleic Acid and Protein Metabolism in Pea Epicotyls 1: II. Response to Wounding in Aged Tissue. *Plant Physiology*, *73*(3), 817-821.

Sebastiana, M., Figueiredo, A., Monteiro, F., Martins, J., Franco, C., Coelho, A. V., Vaz, F., Simões, T., Penque, D., Pais, M. S., & Ferreira, S. (2013). A possible approach for gel-based proteomic studies in recalcitrant woody plants. *Springer Plus*, *2*(1), 210. https://doi.org/10.1186/2193-1801-2-210

Singh, N., Jain, N., Kumar, R., Jain, A., Singh, N. K., & Rai, V. (2015). A comparative method for protein extraction and 2-D gel electrophoresis from different tissues of *Cajanus cajan*. *Frontiers in plant science*, *6*, 606.

Smolikova, G., Gorbach, D., Lukasheva, E., Mavropolo-Stolyarenko, G., Bilova, T., Soboleva, A., Tsarev, A., Romanovskaya, E., Podolskaya, E., Zhukov, V., Tikhonovich, I., Medvedev, S., Hoehenwarter, W., & Frolov, A. (2020). Bringing New Methods to the Seed Proteomics Platform: Challenges and Perspectives. *International Journal of Molecular Sciences*, *21*(23), 9162. https://www.mdpi.com/1422-0067/21/23/9162

Soumare, A., Diedhiou, A. G., Thuita, M., Hafidi, M., Ouhdouch, Y., Gopalakrishnan, S., & Kouisni, L. (2020). Exploiting Biological Nitrogen Fixation: A Route Towards a Sustainable Agriculture. *Plants*, *9*(8), 1011. https://www.mdpi.com/2223-7747/9/8/1011

Vahedifar, A., & Wu, J. (2022). Chapter Two - Extraction, nutrition, functionality and commercial applications of canola proteins as an underutilized plant protein source for human nutrition. In J. Wu (Ed.), *Advances in Food*

and Nutrition Research (101): 17-69. Academic Press. https://doi.org/https://doi.org/10.1016/bs.afnr.2022.04.001

Wu, X., Gong, F., & Wang, W. (2014). Protein extraction from plant tissues for 2DE and its application in proteomic analysis. *PROTEOMICS*, *14*(6), 645-658. https://doi.org/https://doi.org/10.1002/pmic.201300239

Wu, X., Xiong, E., Wang, W., Scali, M., & Cresti, M. (2014). Universal sample preparation method integrating trichloroacetic acid/acetone precipitation with phenol extraction for crop proteomic analysis. *Nature Protocols*, *9*(2), 362-374. https://doi.org/10.1038/nprot.2014.022

Xia, Q., El-Maarouf-Bouteau, H., Bailly, C., & Meimoun, P. (2016). Determination of protein carbonylation and proteasome activity in seeds. *Plant proteostasis: methods and protocols*, 205-212.

Xia, Z., Zhai, H., Wu, H., Xu, K., Watanabe, S., & Harada, K. (2021). The synchronized efforts to decipher the molecular basis for soybean maturity loci E1, E2, and E3 that regulate flowering and maturity. *Frontiers in Plant Science*, *12*, 632754.

Yang, S., Ding, M.-M., Chen, F., & Xu, Y. (2017). Proteomic analysis of latex from *Jatropha curcas* L. stems and comparison of two classic proteomic sample isolation methods: the phenol extraction and the TCA-Acetone extraction. *Electronic Journal of Biotechnology*, *27*, 14-24.

Yu, Y.-M., & Fukagawa, N. K. (2020). Chapter 2 - Protein and amino acids. In B. P. Marriott, D. F. Birt, V. A. Stallings, & A. A. Yates (Eds.), *Present Knowledge in Nutrition (Eleventh Edition)* :15-35. Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-323-66162-1.00002-0

Plant Science Research 46 (1&2) : 40-42, 2024

Plant Science Research

ISSN 0972-8546

Aphyllorchis montana Rchb. f. (Orchidaceae): A new report for Goa, India

Shreyas Betageri[1Ψ], K. Kotresha[1] and Mayuresh Kulkarni[2]

[1] Taxonomy and Floristic Laboratory, Department of UG, PG and Research in Botany, Karnatak University's, Karnataka Science College, Dharwad-580001, India.

[2] Institute of Natural History, Education and Research, Pune-411038, Maharashtra, India

ARTICLE INFO

Article history:
Received : 27 August 2024
Revised : 30 September 2024
Accepted : 19 October 2024

Keywords:

Mycoheterotrophicorchid
Mainpai Waterfalls
Netravali Wildlife Sanctuary
New record

ABSTRACT

A floristic survey of Mainpai waterfalls, Goa resulted in collection of an interesting leafless saprophytic orchid. It is identified as *Aphyllorchismontana* Rchb. f.,it is reported for the first time in Netravali wildlife sanctuary, Goa and forms a new distribution record for the state. The present paper deals with the nomenclature, botanical description, ecology, distribution and taxonomic status of the species. Herbarium specimens are preserved for further study.

Introduction

Orchidaceae is one of the largest and most diverse family of flowering plants comprising of over 28,000 species in the world (Christenhusz & Byng, 2016). The genus Aphyllorchis Blume has 20 species, which are distributed from tropical and subtropical Asia to North Queensland (POWO, 2024). Aphyllorchis is derived from the Greek word 'aphyllos' meaning leafless and 'orchis'is an orchid i.e., leafless orchid. Singh et al. (2019) reported four species of Aphyllorchisviz. Aphyllorchisalpina King & Pantl., A. montana Rchb. f., A. vaginata Hook. f. and A. gollanii Duthie from India. Jalal (2019) worked on the diversity and distribution of orchids of Goa and reported the occurrence of 68 species of orchids belonging to 28 genera but not Aphyllorchismontana Rchb. f. from the state.

During a field survey of Netravalli Wild life Sanctuary, Goa, we collected a leafless orchid from Mainpai waterfalls area, which after critical study and literature consultation was identified as Aphyllorchismontana Rchb. f. This interesting orchid species has not yet been reported from the Goa stateand hence is considereda new plant record for the state. In India, the species is reported to occur in the states of West Bengal, Sikkim, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Nagaland, Andhra Pradesh, Karnataka, Kerala and Tamil Nadu (Aravindhan *et al.*, 2013; Rao & Kumar, 2015). The nomenclature, botanical description, ecology and distribution of the species are provided below along with photographs of the plant and herbarium specimens.

Taxonomic treatment:

Aphyllorchismontana Rchb. f. Linnaea 41: 57.1876; Hook. f., Fl. Brit. India 6: 116.1890; King & Pantl. in Ann. Roy. Bot. Gard. (Calcutta) 8: t. 349. 1898; Pearce & Cribb. Orch. of Bt. 3(3): 37. 2002; Fischer in Gamble, Fl. Madras 3: 1019. 1957 (repr.ed.).

Saprophytes, 50-60 cm tall, rhizome short, creeping. Stem with many membranous transparent sheaths; proximal sheaths tubular, 0.5-2 cm; Bracts 1cm. Inflorescence with a few to 10 or more losely arranged flowers; floral bracts reflexed, linear-lanceolate, 8×2-2.5 mm, shorter than pedicel and ovary, cream color with nerves. Flowers yellow, spreading, usually becoming pendulous, 3×1 cm; dorsal sepal cymbiform, oblong or obovate, obtuse apex, 9×3 mm,

Ψ *Corresponding author; Email:* shreyasbetgari@gmail.com

3-veined; lateral sepals slightly shorter, not cymbiform; petals sub-oblong, shorter 7 mm long; lip 9×5 mm, ovate, acute, 3-lobed, side lobes obliquely ovate-obtuse; midlobe ovate, acute with 2 triangular or triangular-lanceolate wings; column slightly arcuate, 7-10 mm, apex dilated. Ovary glabrous or sometimes slightly puberulent. Capsule 2.5×1 cm across, purplish tinge.

Habitat:Terrestrial Mycoheterotrophic orchid found in evergreen and semi-evergreen forest floors in leaf litter commonly associated with Peristylusaristatus and Crepidiumverscicolor in sandy soil.

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

Specimen Examined: INDIA, Goa, Netravali Wildlife Sanctuary, Netravali, Mainpai Waterfalls trek trail, 15°03'43.4"N 74°15'17.5"E, 31-08-2024, by Shreyas B. & K. Kotresha, (0351) 20374, HKSCD (Herbarium of Karnatak Science College, Dharwad)

Distribution: India (West Bengal, Sikkim, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Nagaland, Karnataka, Kerala and Tamil Nadu), Cambodia, China, Bhutan, Indonesia, Japan, Malaysia, Philippines, Sri Lanka, Thailand, Vietnam.

Figure 1: *Aphyllorchismontana* Rchb. f. A. Habit and Habitat; B. Rhizome; C. Inflorescence; D. Fruit. (Photography by Shreyas Betageri)

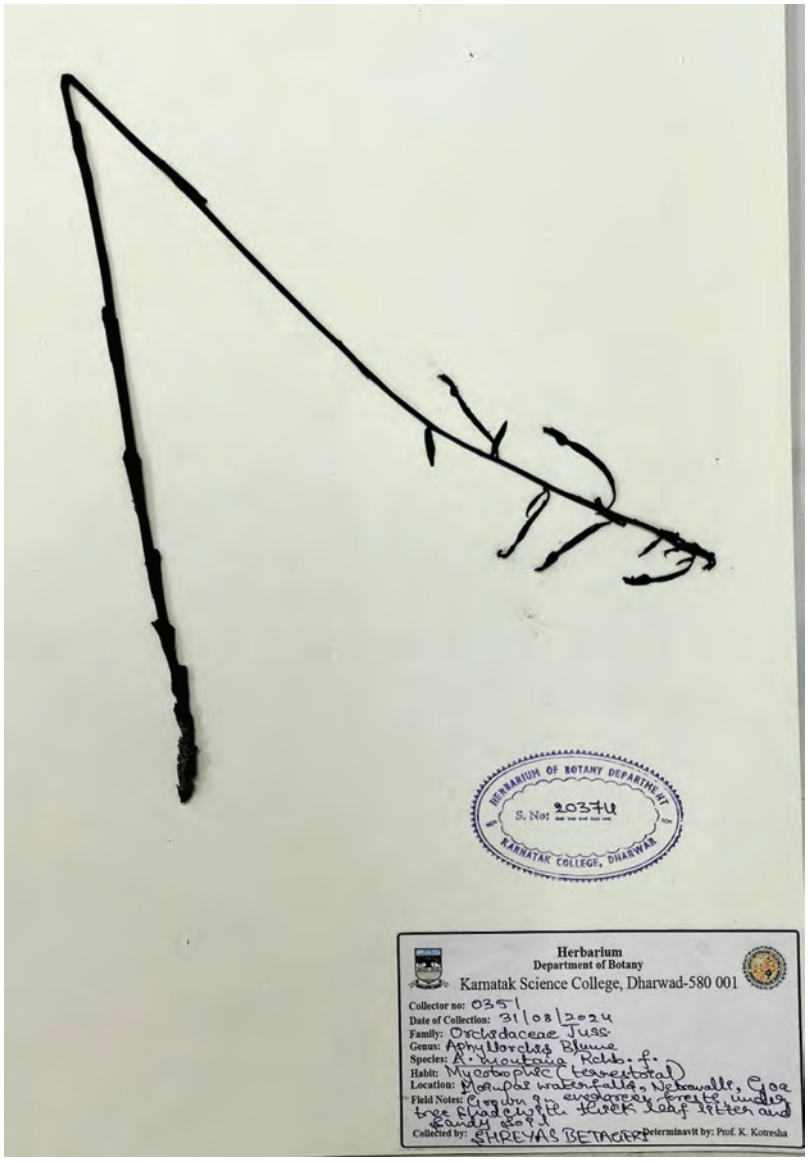


Figure 2: Herbarium of *Aphyllorchismontana* Rchb. f. from Netravali Wildlife Sanctuary submitted to Herbarium of Karnatak Science College, Dharwad

Acknowledgment

I thank the Karnatak University, Dharwad for providing University Research fellowship for my research work. Authors are thankful to Dheeraj Halali, Gajanan Shetye and Pranay Sawant from Goa, who helped during the survey.

References

Aravindhan, V.; Mahendran, G.; Rajendran, A. & Narmatha, B.V. (2013). *Aphyllorchis montana* Rchb. f. (Orchidaceae), a new distributional record for Peninsular India.Ann. Pl. Sci. 2(5): 153 - 155.

Christenhusz M. J.M. and Byng, J.W. (2016). The number of known plants species in the world and its annual increase. Phytotaxa261 (3): 201-217.

Jalal, J. S. (2019). Diversity and distribution of orchids of Goa, Western Ghats, India.Journal of Threatened Taxa11 (15): 15015-15042.

POWO(2024) "Plants of the World Online". Facilitated by the Royal BotanicGardens, Kew. Published on the internet; https://powo.science.kew.org//. (accessed: 28 August 2024)

Rao, A. N. and Kumar, V. (2015). Record of holomycotrophic *Aphyllorchis montana* Reichenbach H.G. (Orchidaceae) from Manipur, India. Pleione9(2): 527 - 530.

Singh, S. K., Agrawala, D. K., Jalal, J. S., Dash, S. S., Mao, A. A. and Singh, P. (2019). Orchids of India: A pictorial guide. Botanical Survey of India, Kolkata, pp. 521.

Plant Science Research

ISSN 0972-8546

Precision farming in rice to improve crop productivity

Laba Hembram, Kusha Hembram, Abahani Mohapatra and Aparna Das[Ψ]
P.G. Department of Botany, Udayanath Autonomous College of Science & Technology, Adaspur, Cuttack

ARTICLE INFO

Article history:
Received : 30 August 2024
Revised : 1 October 2024
Accepted : 20 October 2024

Keywords:

Organic matter
Fertilizers
Soil health
Environmentalfriendly Technique
Precision farming
Rice
Nutritious crops

ABSTRACT

Globally, agriculture faces challenges due to climate change and declining soil health. At the same time, the continuously growing human population makes these challenges even more difficult. Organic matter (OM) is a crucial component in maintaining soil fertility and productivity while providing essential ecosystem services. In the current situation, cultivated land area has been severely decreased due to human settlement and rapid urbanization. However use of good crop variety is important to increase production of rice, wheat, millet, vegetables and fruits, etc. Over dependence on chemical fertilizers and unbalanced nutrient management practices have reduced soil health and ultimate crop production in several countries. Soil organic matter is declining from time to time, which is the most precarious threat to agriculture. Integration of all natural, organic, and inorganic sources of nutrients is an efficient and environmental friendly technique of crop production. Precision farming helps manage fertilizers, pesticides, herbicides, and water in the field based on sources of data i.e. soil reports, temperature, nutrients and other agroclimatic factors. These technologies are utilized in various stages such as field preparation, weed management, irrigated water management, fertilizer application, insect-pest control and harvesting. The present study is to focus on managing and reducing the cost of rice cultivation, and simultaneously emphasizing the production of rice. In order to study the effect of NPK fertilizers (Inorganic) and biofertilizers (organic) on various parameters such as height, tillering, chlorophyll, protein & carbohydrate content, the treatment in different proportions (90% NPK + 10% organic, 80% NPK + 20% organic, 70% NPK + 30% organic, 60% NPK + 40% organic, 50% NPK + 50% organic) were applied to 5 different plots, keeping the control with 100% NPK only of all the combinations, 60% NPK with 40% biofertilizer was proved to enhance the growth and yield significantly.

Introduction

Precision farming is an effective technique that manages fertilizer, pesticides, and herbicides in the agricultural field. Precision agriculture also be defined, as "a management system that is information and technology-based, site-specific and uses more of the sources of data i.e. soil, crops, nutrients, moisture, pests and yield, for optimum profitability, sustainability of the environment (McLoud, 2007)." The concept was given by Dr Pierre Roberts in the 1980s during the problem of nutrient variability in large farms in the USA. In countries like United States and Australia, precision farming was one of the mostly adopted technique beneficial on large scale, but in the Asia- Pacific region, due to obvious reasons the benefits are not so significant. In India 22 Precision Farming Development Centres (PFDCs) have been developed to test new technologies and modify them according to local needs. As India faces challenges from climate change, water scarcity and soil degradation, precision farming offers a sustainable path forward. Optimizing production, saving on input costs, and minimizing adverse effects on the environment are the main objectives of precision farming. Presently, this agricultural technology works with modernized and advanced i.e. global position systems (GPS), remote sensing and variable technology, etc.

[Ψ] *Corresponding author; Email:* dasaparnadr@gmail.com

Rice is a major cereal crop belonging to the family Poaceae. More than 50% percent of rice is cultivated in Asian countries. Approximately half of the world consumes rice regularly, whereas most consumers of rice are found in different corners of Asian origin. The Genus *Oryza* contains 24 species, out of which 22 are wild and 2 cultivated (Gouda *et al.*, 2020). The two cultivars of rice are *Oryza sativa* L. and *Oryza glaberrima* Steud. After China, India is the second largest producer of rice. According to the Ministry of Agriculture and Farmers Welfare, 1357.55 lakh tonnes of rice was produced in India in 2022-2023. It is 62.84 Lakh tonnes higher than previous years rice quantity. Moreover, rice is not only universal staple food but also it plays an important role in various religious occasions and societal rituals. Rice is cultivated in almost all states of India though mostly in irrigated or rainfed areas. Globally, rice cultivation is more challenging due to climate change and declining soil rhizosphere. The unbalanced use of chemical fertilizer leads to deterioration of soil health, crop yield, and bio-organic matter which is the most serious threat, now a days.

This study focuses on managing and reducing the cost of rice cultivation and enhancing the nutritional quality & quantity of rice. Precision farming ensures crop and soil to receive the appropriate amount of water, nutrients and other inputs at the right time and place, resulting in increased crop production with minimum use of resources like land, water and labour. This work presents the effect of chemical fertilization and organic fertilization on productivity and soil health. Both approaches provide solutions to ensure improved fertilizer use efficiency in short term and maintain soil fertility in long term. Thus, the objective of this study was to determine the effect of different proportion of biofertilizers and NPK on growth and biochemical content in rice.

Materials and Methods

Soil Collection

The productivity of crop is proportional to the soil health & quality. So, nutrient analysis is mandatory to know about the soil. Using the V-cut method, soil samples were collected from the garden of UNAC and were processed properly and delivered (Fig. 1) to State Quality Control Laboratory, Bhubaneswar.

Preparation of Land

A part of the Garden of P.G. Department of Botany is divided into six equal plots, of 4x4 feet with one foot distance. The plots were dug to a depth of 2 feet and were filled with agricultural soil up to half foot and then with vermicompost, cow dung compost, jaggery (1:1:1) and plots were flooded with water. Legumes and oil seeds were sown in the soil and allowed to grow till the sapling were up to 15cm. All the germinated legumes along with the oil seeds were mulched inside the plots to enhance the nutritional quality of the soil.

Collection of Seedling and Plantation

Sindhu variety of rice seedlings of 15 days old were collected on 28th January, 2024, from Dr. Subhasisa Bal, consultant scientist, 5G farming at Nimapada, Odisha (Fig. 2). After collecting the seedlings, they were manually transplanted in the ground, with 3 transplants together at a distance of 15 cm from each side (Fig. 3). During plantation, each plant measured a height of about 30 cm. The plots were flooded to facilitate the growth of saplings and reduce the growth of weeds and pests. The height and tillering of the plant were measured at every 15 days intervals during the growing season.

After 30 days of plantation, NPK (2:1:1) and organic manures (vermicompost and cow dung manure in 1:1 ratio) were applied in different proportions. The first plot was the control with 100% NPK and without any organic fertilizers. In the remaining five plots, both organic and NPK were used at different proportion i.e. 90% NPK with 10% Organic, 80% NPK with 20% Organic, 70% NPK with 30% Organic, 60% NPK with 40% Organic, 50% NPK with 50% Organic in plots 2, 3, 4, 5, and 6 respectively.

Biochemical Analysis

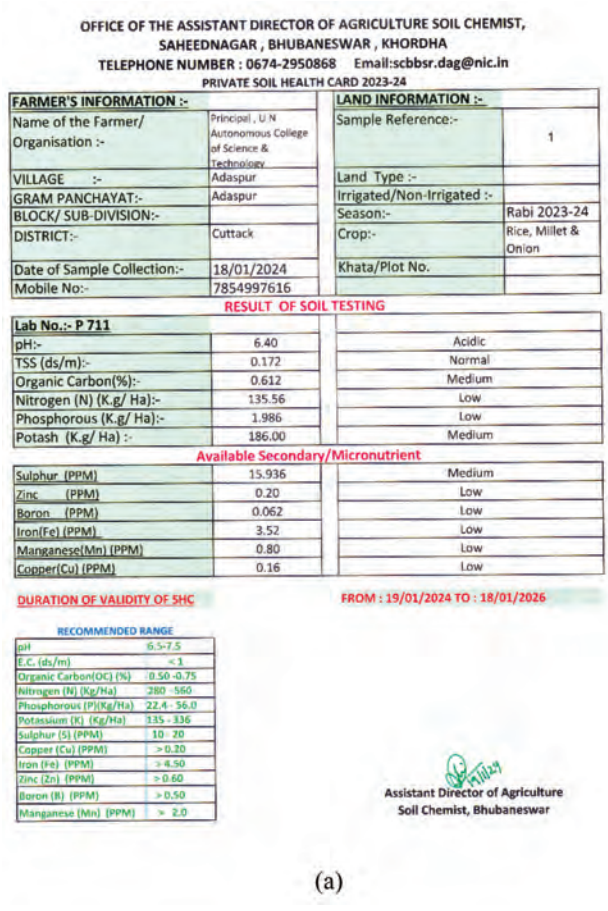
Chlorophyll was extracted in 80% acetone & the absorption at 645nm & 663nm were recorded in UV-Visible dual beam spectrophotometer using the Arnon's formula, the amount of chlorophyll was calculated.

Total carbohydrates were quantitatively analysed using the anthrone method, which is a simple colorimetric technique. This method involves hydrolyzing polysaccharides and dehydrating the resulting monomers through digestion with sulfuric acid and heat treatment. During this process, pentose and hexose sugars are converted into furfural and hydroxymethyl furfural, respectively. When anthrone (an aromatic compound) reacts with these digested products, it produces a coloured compound. The amount of total carbohydrates in the sample was then determined by measuring the absorbance of the resulting solution at 630 nm and comparing it to a glucose standard curve.

Protein was extracted with the help of phosphate buffer pH 7.4. These extracts were measured by the protocol of Lowry method 1951. In this method, the colour developed due to the reduction of phosphomolybdic and

Table 1:

Analysis of soil for various nutrients



OFFICE OF THE ASSISTANT DIRECTOR OF AGRICULTURE SOIL CHEMIST,
SAHEEDNAGAR , BHUBANESWAR , KHORDHA
TELEPHONE NUMBER : 0674-2950868 Email:scbbsr.dag@nic.in
PRIVATE SOIL HEALTH CARD 2023-24

FARMER'S INFORMATION :-		LAND INFORMATION :-	
Name of the Farmer/ Organisation :-	Principal , U N Autonomous College of Science & Technology	Sample Reference:-	1
VILLAGE :-	Adaspur	Land Type :-	
GRAM PANCHAYAT:-	Adaspur	Irrigated/Non-Irrigated :-	
BLOCK/ SUB-DIVISION:-		Season:-	Rabi 2023-24
DISTRICT:-	Cuttack	Crop:-	Rice, Millet & Onion
Date of Sample Collection:-	18/01/2024	Khata/Plot No.	
Mobile No:-	7854997616		

RESULT OF SOIL TESTING

Lab No.:- P 711		
pH:-	6.40	Acidic
TSS (ds/m):-	0.172	Normal
Organic Carbon(%):-	0.612	Medium
Nitrogen (N) (K.g/ Ha):-	135.56	Low
Phosphorous (K.g/ Ha):-	1.986	Low
Potash (K.g/ Ha) :-	186.00	Medium

Available Secondary/Micronutrient

Sulphur (PPM)	15.936	Medium
Zinc (PPM)	0.20	Low
Boron (PPM)	0.062	Low
Iron(Fe) (PPM)	3.52	Low
Manganese(Mn) (PPM)	0.80	Low
Copper(Cu) (PPM)	0.16	Low

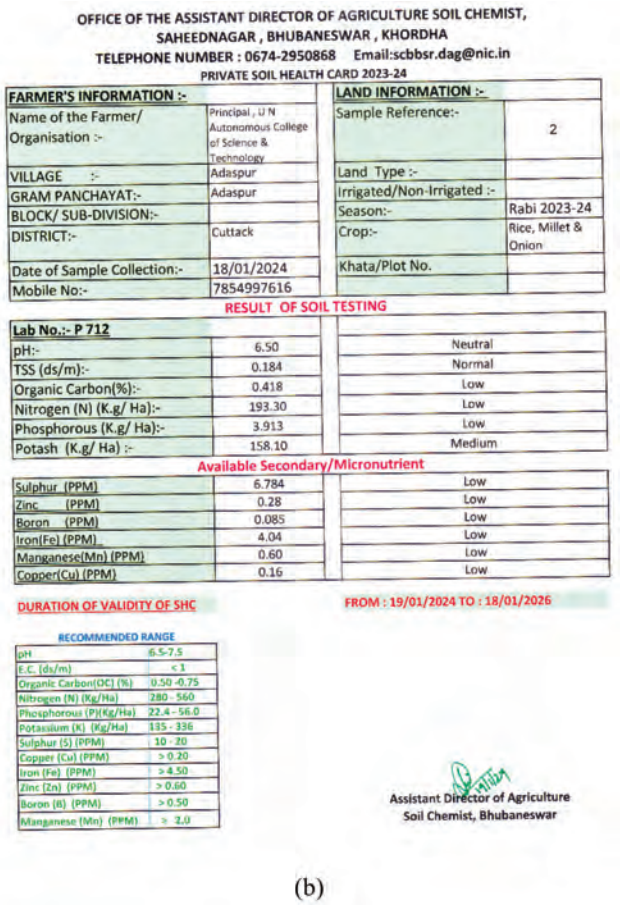
DURATION OF VALIDITY OF SHC FROM : 19/01/2024 TO : 18/01/2026

RECOMMENDED RANGE

pH	6.5-7.5
E.C. (ds/m)	< 1
Organic Carbon(OC) (%)	0.50 -0.75
Nitrogen (N) (Kg/Ha)	280 - 560
Phosphorous (P)(Kg/Ha)	22.4 - 56.0
Potassium (K) (Kg/Ha)	135 - 336
Sulphur (S) (PPM)	10 - 20
Copper (Cu) (PPM)	> 0.20
Iron (Fe) (PPM)	> 4.50
Zinc (Zn) (PPM)	> 0.60
Boron (B) (PPM)	> 0.50
Manganese (Mn) (PPM)	> 2.0

Assistant Director of Agriculture
Soil Chemist, Bhubaneswar

(a)



OFFICE OF THE ASSISTANT DIRECTOR OF AGRICULTURE SOIL CHEMIST,
SAHEEDNAGAR , BHUBANESWAR , KHORDHA
TELEPHONE NUMBER : 0674-2950868 Email:scbbsr.dag@nic.in
PRIVATE SOIL HEALTH CARD 2023-24

FARMER'S INFORMATION :-		LAND INFORMATION :-	
Name of the Farmer/ Organisation :-	Principal , U N Autonomous College of Science & Technology	Sample Reference:-	2
VILLAGE :-	Adaspur	Land Type :-	
GRAM PANCHAYAT:-	Adaspur	Irrigated/Non-Irrigated :-	
BLOCK/ SUB-DIVISION:-		Season:-	Rabi 2023-24
DISTRICT:-	Cuttack	Crop:-	Rice, Millet & Onion
Date of Sample Collection:-	18/01/2024	Khata/Plot No.	
Mobile No:-	7854997616		

RESULT OF SOIL TESTING

Lab No.:- P 712		
pH:-	6.50	Neutral
TSS (ds/m):-	0.184	Normal
Organic Carbon(%):-	0.418	Low
Nitrogen (N) (K.g/ Ha):-	193.30	Low
Phosphorous (K.g/ Ha):-	3.913	Low
Potash (K.g/ Ha) :-	158.10	Medium

Available Secondary/Micronutrient

Sulphur (PPM)	6.784	Low
Zinc (PPM)	0.28	Low
Boron (PPM)	0.085	Low
Iron(Fe) (PPM)	4.04	Low
Manganese(Mn) (PPM)	0.60	Low
Copper(Cu) (PPM)	0.16	Low

DURATION OF VALIDITY OF SHC FROM : 19/01/2024 TO : 18/01/2026

RECOMMENDED RANGE

pH	6.5-7.5
E.C. (ds/m)	< 1
Organic Carbon(OC) (%)	0.50 -0.75
Nitrogen (N) (Kg/Ha)	280 - 560
Phosphorous (P)(Kg/Ha)	22.4 - 56.0
Potassium (K) (Kg/Ha)	135 - 336
Sulphur (S) (PPM)	10 - 20
Copper (Cu) (PPM)	> 0.20
Iron (Fe) (PPM)	> 4.50
Zinc (Zn) (PPM)	> 0.60
Boron (B) (PPM)	> 0.50
Manganese (Mn) (PPM)	> 2.0

Assistant Director of Agriculture
Soil Chemist, Bhubaneswar

(b)

Table 2:

Plant height in centimetre with 15 days interval

PLOT	15th DAP	30th DAP	45th DAP	60th DAP
P1	23.73±0.03	51.26±1.17	64.73±0.17	74.30±0.28
P2	22.91±0.03	49.28±0.28	51.03±1.72	73.39±0.82
P3	23.01±1.60	47.09±0.55	59.52±0.69	80.27±0.94
P4	23.53±0.24	50.35±0.32	61.87±0.38	85.50±0.83
P5	23.78±0.20	51.72±1.70	63.22±0.01	85.72±0.55
P6	23.23±0.65	50.12±1.51	56.03±0.02	72.61±0.61

*DAP= Day after plantation

Table 3:

Number of plant tillering with 15 days interval

PLOT	15th DAP	30th DAP	45th DAP
P1	5.23±0.45	7.35±0.65	7.13±0.02
P2	4.79±0.13	7.65±0.60	7.36±0.45
P3	4.85±0.59	6.38±0.18	7.15±0.42
P4	6.28±0.81	8.14±0.21	8.19±0.05
P5	6.57±0.34	9.12±0.44	8.97±0.07
P6	5.23±0.65	6.64±0.24	6.14±0.36

*DAP= Day after plantation

Table 4:

Total chlorophyll content of *O. sativa* L. with 30 days interval

Different Concentration of Nutrient	30th DAP	60th DAP	90th DAP
100% NPK + 0% Organic (P1)	1.78±0.04	1.90±0.02	1.95±0.08
90% NPK + 10% Organic (P2)	1.68±0.64	1.97±0.21	1.87±0.04
80% NPK + 20% Organic (P3)	1.52±0.49	1.92±0.26	2.21±0.46
70% NPK + 30% Organic (P4)	1.66±0.14	1.97±0.69	2.67±0.09
60% NPK + 40% Organic (P5)	2.16±0.21	2.33±0.59	2.91±0.02
50% NPK + 50% Organic (P6)	1.46±0.24	1.78±0.17	1.95±0.12

* DAP= Day after plantation

Table 5:

Total carbohydrate content of *O. sativa* L. with 30 days interval

Different Concentration of Nutrient	30th DAP Carbohydrate- mg/gdw	60th DAP Carbohydrate- mg/gdw	90th DAP Carbohydrate- mg/gdw
100% NPK + 0% Organic (P1)	38.3 ±3.0	79.9±1.2	78.3 ±0.9
90% NPK + 10% Organic (P2)	38.8 ±0.8	84.6±0.5	79.9 ±1.26
80% NPK + 20% Organic (P3)	37.9 ±5.1	79.9±0.6	78.3 ±0.2
70% NPK + 30% Organic (P4)	42.5 ±1.67	82.7±0.5	79.1 ±1.06
60% NPK + 40% Organic (P5)	44.7 ±1.3	85.0±2.4	82.8 ±2.2
50% NPK + 50% Organic (P6)	37.0 ±0.7	77.2±2.3	74.2 ±3.3

* DAP= Day after plantation

Table 6

Total protein content of *O. sativa* L. with 30 days interval

Different Concentration of Nutrient	30th DAP Protein-mg./gfw	60th DAP Protein-mg./gfw	90th DAP Protein-mg./gfw
100% NPK + 0% Organic (P1)	15.8±0.52	28.1 ±1.03	30.2 ±0.36
90% NPK + 10% Organic (P2)	17.7±0.43	28.9 ±0.96	31.1 ±0.60
80% NPK + 20% Organic (P3)	16.9±0.40	25.7 ±0.03	29.6 ±0.10
70% NPK + 30% Organic (P4)	17.9±0.45	29.4 ±0.65	33.6 ±0.42
60% NPK + 40% Organic (P5)	22.3±0.86	31.8±0.65	36.2±0.75
50% NPK + 50% Organic (P6)	15.7 ±0.66	25.7 ±1.19	28.8 ±0.50

*DAP= Day after plantation

Figure 1: Soil samples for analysis

Figure 2: Collection of seedlings

Figure 3: Plantation of seedlings in P.G. Department Botanical garden Udayanath Autonomous college of Science and Technology

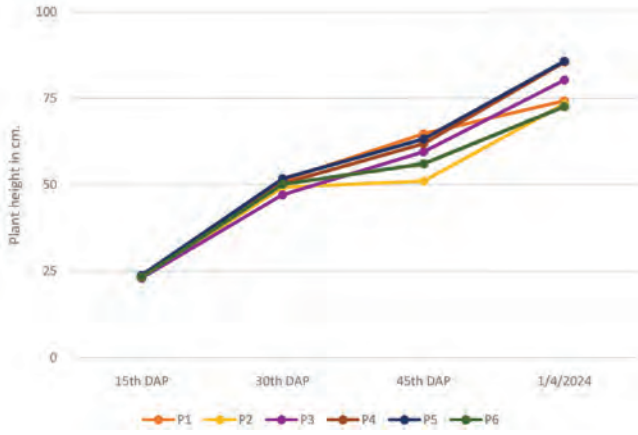


Fig.4: Plant height in centimetre with 15 days interval

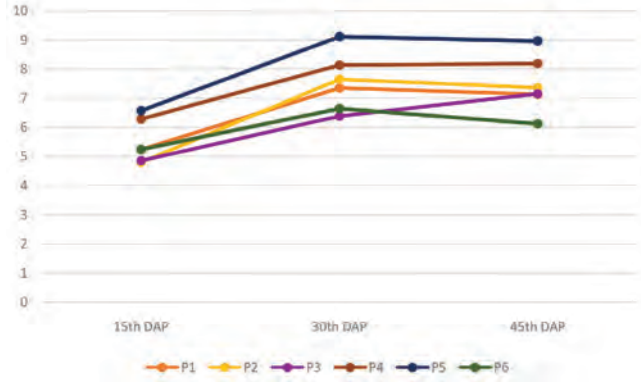


Fig. 5: Plant tillering with 15 days interval

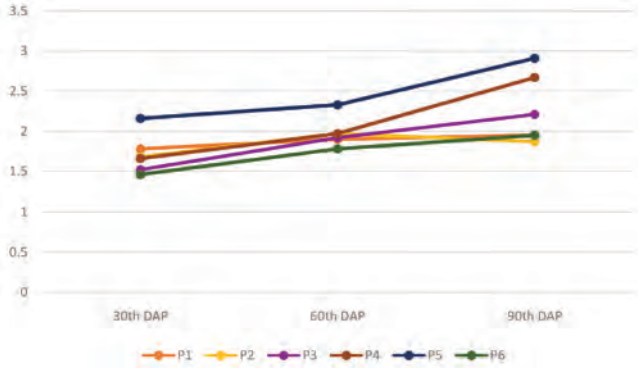


Fig.6: Total chlorophyll content of *O. sativa* L. with 30 days interval

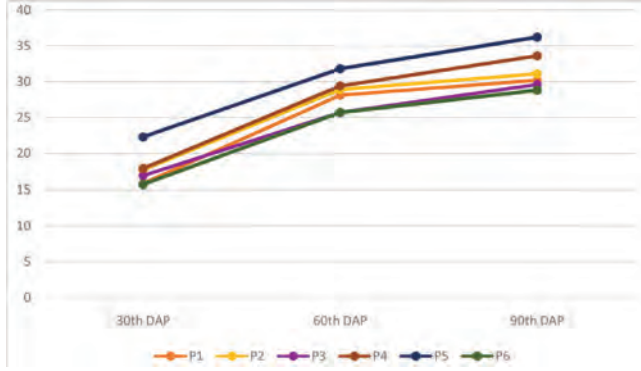


Fig.7: Total carbohydrate content of *O. sativa* L. with 30 days interval

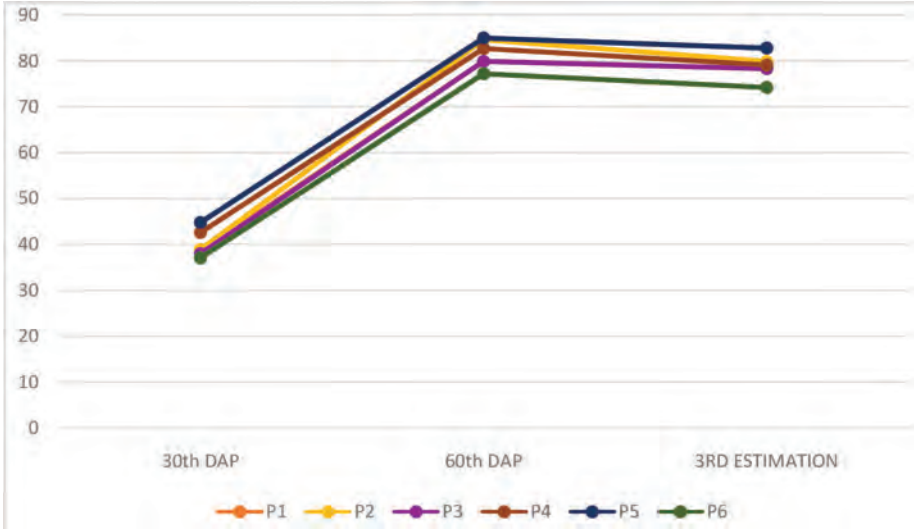


Fig.8: Total protein content of *O. sativa* L. with 30 days interval

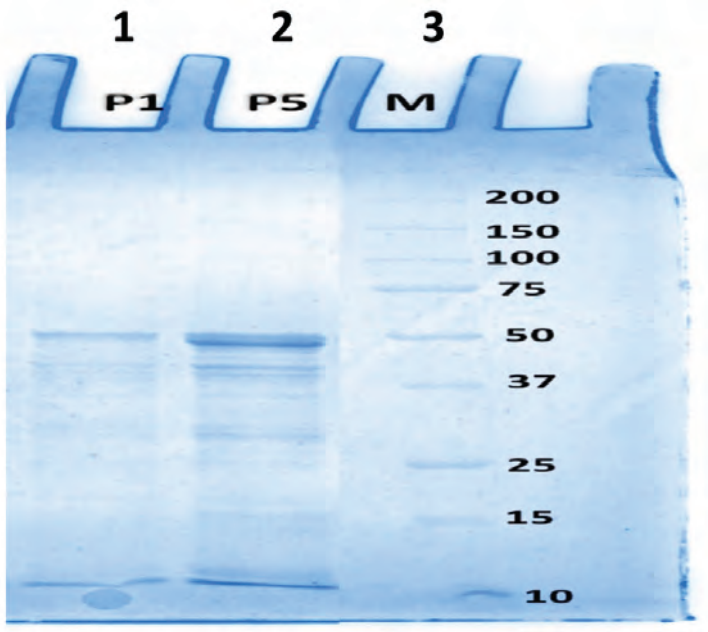


Fig 9: In gel activity by SDS-PAGE in control & supplemented rice

phosphotungstic components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein. Additionally, a colour was developed by the biuret reaction, which occurs when the protein reacts with alkaline cupric tartrate. The intensity of the blue colour produced in reactions was measured at 750nm by UV-Visible dual beam spectro keeping bovine serum albumin as a standard and protein.

The protein was subjected for in gel activities to detect the intensity of bands by SDS-PAGE. The most commonly used technology to obtain high resolution analytical separation of mixtures of proteins is Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The procedure involves initial denaturation of component proteins with an anionic detergent that also binds to them, imparting to all proteins a negative charge proportional to their molecular mass. This step is followed by electrophoresis through a porous acrylamide gel matrix that separates proteins with excellent resolution on the basis of molecular mass.

SDS is a detergent with a strong protein-denaturing effect and binds to the protein backbone at a constant molar ratio. In the presence of SDS and a reducing agent that cleaves disulfide bonds critical for proper folding, proteins unfold into linear chains with negative charge proportional to the polypeptide chain length.

Polymerized acrylamide (polyacrylamide) forms a mesh-like matrix suitable for the separation of proteins of typical size. The strength of the gel allows easy handling. Polyacrylamide gel electrophoresis of SDS treated proteins allows researchers to separate proteins based on their length in an easy, inexpensive, and relatively accurate manner. The polymerization of acrylamide is an example of free radical catalysis and is initiated by the addition of ammonium per sulphate and a catalyst N,N,N'N' tetramethylenediamine (TEMED). TEMED catalyses the decomposition of the persulfate ion to give a free radical

$$S_2 O_8^{2-} + e^- \rightarrow SO_4^{2-} + SO_4^-$$

In this way long chains of acrylamide are built up being crosslinked by introduction of bisacrylamide forming a mesh like structure in which the holes of the mesh represent the pores. Overall protein mobility through polyacrylamide gel is proportional to the pore size which is a function of both the acrylamide concentration (%T) and that of bisacrylamide crosslinker (%C.). In general, the pore size is inversely proportional to %T. Quantified protein were subjected to gel electrophoresis and proteins were separated by loading the samples to stacking gel & running at the current to 10-15 mA, 80V for initial 10-15 minute until the sample travel through the stacking gel. The stacking gel helps concentration of the samples. The run continued at 30mA, 100V until the bromophenol blue reaches the bottom of the gel for 3 hour.

Results and Discussion

Chlorophyll is an important pigment of photosynthesis in plants, which delivers all the photosynthates to be utilised by the plant for enhanced biosynthesis of diversified

metabolites. However, amendment of biofertilizer enhances the nutrient uptake via the microbial mass which ultimately regulates the desired effect on plant growth. N, P, & K fertiliser fulfils the plant's nutrient requirement. At the same time, bio-fertilizer induce plant productivity and quality which is highly essential. The different proportions of organic and inorganic fertilizer exhibited significant enhancement on the productivity of Sindhu rice.

Height as well as number of leaves of the plant was found to be maximum in P5 and P4 with 60% inorganic + 40% organic & 70% inorganic + 30% organic fertilizers respectively although tillering was more in P5. This could be due to the symbiotic activity of micro-organisms in biofertilizers which helped in improving the ultimate growth of rice (Oladele and Awodum, 2014).

The biochemical analysis exhibited increment in chlorophyll, carbohydrate and protein content in P5 followed by P4. The results indicate that increase in biofertilizers has improved the quality of crop by subsequently enhancing photosynthesis, producing bioactive substances causing increase in leaf number and size due to adequate supplementation which in turn possibly had increased nutrient absorption capacity of the plant (Noraida and Hisyamuddin, 2021).

India is successfully able to meet the production targets set by the Government but it was achieved at the cost of degradation of natural resources and with adverse impact on the environment. To meet the projected population of 1.7 billion in 2050, land productivity has to be properly enhanced four times, water productivity 3 times and labour productivity 6 times, (Singh, 2022). Therefore, this emerged technology, so called Precision farming is adopted to increase the agricultural productivity.

The growing demand of rice has to be met by increasing production with less resources i.e., land, water and manpower and by optimizing usage of all agricultural impacts for which precise management of inputs are considered very important. (Tripathi *et.al,* 2021). Precision nutrient management aims to enhance productivity, efficiency and profitability of agricultural products through advanced innovative technologies, while minimizing the cost with reduced environmental foot print. (Goulding *et. al,* 2008).

N, P, K are the macronutrients required for growth of plants. Nitrogen helps in chlorophyll & protein synthesis; Phosphorus helps in synthesis of nucleic acids, coenzymes, nucleotides, phosphoprotein, phospholipid, sugar phosphate and also serve as energy source; Potassium helps in starch formation, serve as cofactor for activating enzymes. Effect of NPK on crop height, number of tillering, leaf area, panicle number per clumps, total grain per panicle, weight of grain was quite significant. Though NPK application result in increase in the growth and yield of rice, it leads to groundwater and air pollution, soil sanitary issues & decline in biodiversity. In case of excessive use, it can accumulate in the soil, causing imbalance of soil pH and fertility. Prolonged exposure to NPK may irritate the respiratory system and other health issues like cancer, diabetes, adverse reproductive outcomes in humans. In case of plants, it affects by blocking the uptake of micronutrients, encourage attack from harmful insects and pests.

In contrast, organic fertilizers improves soil structure and water holding capacity, prevent soil acidification & conserve soil organisms, help plants resistance to pests and diseases. It provides nutrients like magnesium & boron to plants and are non-toxic even at higher concentrations as they have been leached out. Further, organic fertilizers are less expensive, than synthetic ones fertilizers. Those biofertilizers can be applied according to their EMN (estimated mineralizable Nitrogen) that allows the nutrients supplied from organic fertilizers to be synchronised to the nutrient demand of the rice crops, resulting in higher yield (Moe *et al.,* 2019).

Most of the developing countries either over use the fertilizers that causes land degradation and environmental pollution or apply in less amount resulting in yield drop due to lack of spatial variability of nutrient availability. Improper time and rate of application of fertilizers is the major reason behind the low fertilizer use efficiency in rice (Peng *et. al.,* 2010). Variable use of organic manure shelp to reduce the use of chemical fertilizers. Few studies suggested that plants treated with 50% chemical fertilizer in association with 50% compost resulted is higher (Moe *et al.,* 2019).

These fertilizers provide a more balanced mix of nutrients to plant, particularly micro nutrients, which improve rice yield (Moe *et al.,* 2019; Miller, 2007). However, they release nutrients slowly (Myint *et al.,* 2011), thus rice plants treated with these fertilizers may be nutrient deficient early in growth stage. But, no symptoms of nutrient deficiency was observed. In the present study, plants treated with 60% NPK and 40% organic fertilizers exhibited maximum growth, tillering and panicle initiation which could be due to more uptake of available nutrients. This resulted increment in plant height, 15.37%, tillering 25.80% more in P5 (Fig. 4) than P1 as control. In Biochemical analysis, enhanced chlorophyll content was quite significant i.e. 49.23% more in P5 as compared to control. Protein content was also increased up to 19.86% more in P5 along with increment in

6.38% carbohydrate content. Keeping all the above values in view the present data corroborates with the findings of Bassi *et al*. 2018.

Therefore, recommended dose or proper application of NPK fertilizer along with organic fertilizers can increase productivity level up to 2-3 times. Similar results were also obtained from various workers (Peng *et al.*, 2006). How ever, the suitable composition of NPK and organic fertilizers for precision farming varies depending on the soil and type of crop.

Freshly extracted protein samples were subjected to SDS-PAGE to know the quality and intensity of proteins (Fig. 9) P1 as control 8 exhibited protein bands came up in the range of 50-10kD, and the most intense band was approximately 50kD. Simultaneously, in P5 protein content was 19.86% enhanced and the bands were much more intense than P1. They were in the range of 50-10 kD and the most intense band was 50kD, which was also approximately observed in P1 but with very low intensity. So, the results depict the enhanced biosynthesis of proteins when rice was supplemented with 40% organic fertilizers along with 60% NPK.

Conclusion

NPK alone is unable to fulfil a wide range of plant nutrients. However, when both NPK and organic nutrients are used, there is an enhancement in overall plant growth and metabolite content was observed than NPK alone. This was also evident in the SDS-PAGE depicting more conspicuous & intense protein bands. Among the various supplementation P5 with 60% NPK and 40% organic fertilizers exhibited significant growth and overall productivity. This combination of organic and inorganic fertilisers can help farmers in amendment of the soil for maintaining the microbiota and fertility factor.

References

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant physiology, 24(1), 1.

Bassi, D., Menossi, M., & Mattiello, L. (2018). Nitrogen supply influences photosynthesis establishment along the sugarcane leaf. *Scientific reports*, 8(1), 2327.

Belefant-Miller, H. (2007). Poultry litter induces tillering in rice. *Journal of sustainable Agriculture*, 31(1), 151-160.

Dreywood, R. (1946). Qualitative test for carbohydrate material. Industrial & Engineering Chemistry Analytical Edition, 18(8), 499-499.

Gouda, G., Gupta, M. K., Donde, R., Mohapatra, T., Vadde, R., & Behera, L. (2020). Marker-assisted selection for grain number and yield-related traits of rice (*Oryza sativa* L.). *Physiology and molecular biology of plants*, 26, 885-898.

Goulding, K., Jarvis, S., & Whitmore, A. (2008). Optimizing nutrient management for farm systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1491), 667-680.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J biol Chem*, 193(1), 265-275.

McLoud, P. R., Gronwald, R., & Kuykendall, H. (2007). Precision agriculture: NRCS support for emerging technologies. *Agronomy Technical Note* (1).

Moe, K., Htwe, A. Z., Thu, T. T. P., Kajihara, Y., & Yamakawa, T. (2019). Effects on NPK status, growth, dry matter and yield of rice (Oryza sativa) by organic fertilizers applied in field condition. *Agriculture*, 9(5), 109.

Myint, A. K., Yamakawa, T., Zenmyo, T., Thao, H. T. B., & Sarr, P. S. (2011). Effects of organic-manure application on growth, grain yield, and nitrogen, phosphorus, and potassium recoveries of rice variety Manawthuka in paddy soils of differing fertility. *Communications in soil science and plant analysis*, 42(4), 457-474.

Noraida, M. R., & Hisyamuddin, M. R. A. (2021, May). The effect of different rate of biofertilizer on the growth performance and yield of rice. *In IOP Conference Series: Earth and Environmental Science* (Vol. 757, No. 1, p. 012050). IOP Publishing.

Oladele, S., & Awodun, M. (2014). Response of lowland rice to biofertilizesr inoculation and their effects on growth and yield in Southwestern Nigeria. *J. Agric. Environ. Sci*, 3(2), 371-390.

Peng, S., Buresh, R. J., Huang, J., Yang, J., Zou, Y., Zhong, X. & Zhang, F. (2006). Strategies for overcoming low agronomic nitrogen use efficiency in irrigated rice systems in China. *Field Crops Research*, 96(1), 37-47.

Peng, S., Buresh, R. J., Huang, J., Zhong, X., Zou, Y., Yang, J. & Dobermann, A. (2010). Improving nitrogen fertilization in rice by sitespecific N management. A review. *Agronomy for sustainable development*, 30, 649-656.

Singh, A. K. (2022). Precision agriculture in india-opportunities and challenges. *Indian Journal of Fertilisers*, 18(4), 308-331.

Plant Science Research 46 (1&2) : 52-59, 2024

Plant Science Research

ISSN 0972-8546

Isolation and Characterization of Sulphur-Oxidizing Bacteria from Bhitarkanika Mangrove Soil

Sunita Majhi[1], Karisma Dash[1], Prateeksha Mishra[1] and Bhabatarini Panda[1Ψ]

[1]P.G. Department of Botany, Utkal University, Bhubaneswar 751004, Odisha

ARTICLE INFO

Article history:
Received : 25 August 2024
Revised : 27 September 2024
Accepted : 17 October 2024

Keywords:

SOB (Sulphur oxidizing bacteria)
Mangrove
Sulphate
pH
Pyrobaculumarsenaticum

ABSTRACT

The nature of mangrove soils is influenced by a number of variables, including texture, tidal range, redox status, forest type, temperature and rainfall, thus favoring a diverse range of microorganisms to grow in the soils. The major contributors in the carbon, sulphur, nitrogen, and phosphorous cycles in such forests are bacteria. Sulphur is one of the nutrients that are necessary for plant growth and development, also presently regarded as the fourth most important nutrient for plants after N, P, and K. Sulphate (SO_4), is the common form of sulphur that can be absorbed by plant roots. The soil microbial biomass is the key driving force behind all sulphur transformation. These microbes also play significant role in agriculture and also remove toxic H_2S from the environment. In this study, 16 sulphur oxidizing bacteria (SOB) strains were isolated from four soil samples of the Bhitarkanika mangrove forests of Odisha. Based on their pH reduction ability, 8 were chosen for subsequent experiments. The maximum pH reduction up to 5.87 from the initial pH of 9.4 was observed after 12 days. Among the selected strain S5-Bproduced the maximum amount of sulphate ions (403.09 µg/ml) in the thiosulphate medium showing its higher sulphur oxidizing ability. The isolate S5-B was identified as *Pyrobaculum arsenaticum* by 16S rRNA sequencing and further characterized morphologically and biochemically.

1. Introduction

Mangrove ecosystems, characterized by their unique intertidal environments, play a crucial role in coastal protection, carbon sequestration, and biodiversity conservation. Bhitarkannika is a mangrove forest in Odisha, covering an area of 650 square km, located between $86{\cdot}45^0$-$87{\cdot}76^0$ E longitude and 20.17^0- 20.47^0 N latitude in the estuary of Bramhani, Baitarani, Dhamara and Mahanadi (Dash *et al.*, 2019). The mangrove soil is rich in micro and macro nutrients due to sediments, nutrients carried by river water, and the diverse local microbial community thus is considered as a major sink for the bio-geochemical cycle for a sustainable mangrove habitat. Among the diverse organisms that inhabit these complex ecosystems, sulphur-oxidizing bacteria (SOB) are of particular interest due to their pivotal role in biogeochemical cycling, especially in the sulphur cycle. These bacteria can oxidizesulphur compounds, which can influence nutrient availability and the overall health of mangrove ecosystems.

Sulphur (S) plays a crucial role as a component of organic matter and is classified as an essential macronutrient for a variety of life forms, including plants, animals, microorganisms, and humans. In terms of abundance, S ranks after potassium (K), calcium (Ca), and phosphorus (P) in both plants and humans (Scherer, 2009). It is a fundamental part of amino acids, which serve as the building blocks of proteins. Additionally, sulphur is involved into numerous biologically active molecules in their reduced forms (Tandon and Messick, 2002; Kertesz and Mirleau, 2004; Jamal *et al*., 2010). Thus, all living organisms have a requirement for S as an elemental constituent. The uptake of sulphur primarily occurs in the form of sulphate, which is subsequently reduced to sulphide to synthesize various essential compounds. The typical sulphur content in organisms can

Ψ *Corresponding author; Email:* bhabatarini_panda@rediffmail.com

reach up to 0.2% of their dry weight; however, certain organisms utilize S compounds in greater quantities for various functions, including anabolic processes, providing reducing power (as an electron donor), serving as an energy source, or acting as an electron acceptor (Camacho, 2009). The primary source of sulphur in soils is the sulphur-containing minerals present as well as the remains of plants and animals, or through the addition of elemental sulphur from external sources (river flow). The key sulfur-bearing minerals found in rocks and soils include gypsum, anhydrite, epsomite, iron pyrite, sphalerite, chalcopyrite, galena, and arsenopyrite etc. Sulphur is found in both organic and inorganic forms and is cycled between these states through processes such as mineralization, mobilization, immobilization, oxidation, and reduction (Jamal *et al*., 2010).

Sulphur oxidizing bacteria are able to oxidize sulphide into different forms of sulphur which they can use to obtain energy. Most of the known sulphuroxidizing bacteria (SOB) belongs to the genera *Thiobacillus, Thiothrix, Thiomicrospira, Achromatium and Desulfuromona* (Das *et al.*, 1996) whereas sulphur reducing bacteria (SRB) belongs to *Desulfobacter, Desulfobulbus, Desulfomicrobium* (Kushkevych *et al.*, 2021). However, oxidation of sulphur compounds is not restricted to the true sulphur bacteria, this process also occurs in heterotrophic bacteria isolated from soil and marine environment. Most of the heterotrophic bacteria belongs to the genera *Pseudomonas, Xanthobacteria, Escherichia coli* strains are mostly involved in sulphur oxidation (Hart, 1959). The sulphur may originate from the weathering of soil minerals, the atmosphere, and originally bound sulphur by mineralization and biological or chemical oxidation (Fig.1). Transformation of elemental sulphur to sulfate is necessary for sulphur to be available for crop uptake. Sulphur-oxidizing bacteria are crucial for the detoxification of sulphide found in aquatic environments and sediments. Symbiotic sulphur-oxidizers, particularly those associated with the bivalve family Lucinaceae, are frequently located in muddy mangrove ecosystems (Liang *et al*., 2006). Sulphur-reducing bacteria, which thrive in anaerobic conditions, are prevalent in anoxic environments such as mangroves. These microorganisms utilize sulphate as a terminal electron acceptor to degrade organic materials, leading to the generation of sulphide. This sulphide can then be oxidized in toxic environments by chemo lithotrophic sulphur bacteria or in anoxic conditions by phototrophic sulphur bacteria. The growth of sulphur-oxidizing chemolithotrophs primarily occurs in aerobic conditions, where molecular oxygen serves as the terminal electron acceptor. Nevertheless, certain species, including *Beggiatoa* sp., *Thioploca sp., Thiobacillus denitrificans*, and *Thiomicrospira denitrificans*, are capable of oxidizing H_2S while simultaneously reducing nitrate in aerobic conditions (Brock *et al*., 2006). In salt marshes, which serve as the ecological counterpart to mangroves in temperate regions, sulphur oxidation is recognized as the predominant mineralization process. The substantial influx of organic matter facilitates elevated rates of heterotrophic metabolism. Consequently, microbial sulphur transformation is a vital component of the biogeochemical sulphur cycle in marine sediments and is intricately connected to the cycling of other elements. Thus, SOB and SRB play a key role in the biogeochemical cycling of sulphur in soil ecosystem.

This research focuses on the isolation and sulphate ion production ability of sulphur-oxidizing bacteria from the Bhitarkannika mangrove forest, which could provide insight to microbial diversity of these bacteria in mangrove soil as well as their functional roles in nutrient cycling and ecosystem sustainability.

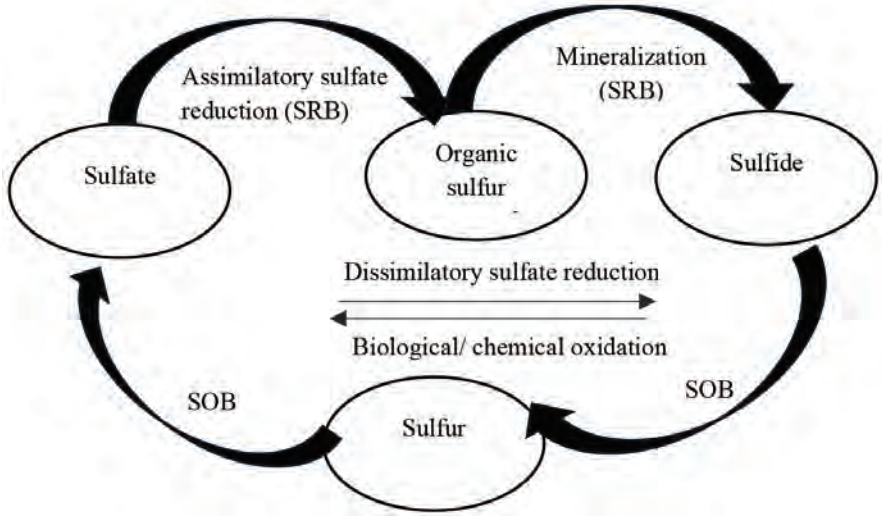


Figure 1. Diagrammatic representation of Sulphur cycle in nature (SRB- Sulphur reducing bacteria, SOB- Sulphur oxidizing bacteria)

2. Material and methods

Soil samples were collected from different locations of the mangrove forests of Bhitarkannika, Odisha and kept in sterile bags, stored in ice box and brought to the laboratory for further analysis. The physicochemical parameters of the soil were estimated to evaluate the soil characteristics (Banerjee, 2018).

2.1. Isolation and purification of sulphur oxidizing bacteria

The spread plate technique, accompanied by serial dilution, was used to isolate sulphur-oxidizing bacteria from the soil samples by using sulphur oxidizing agar plate medium (Visser *et al.,* 1997; Behera *et al.,* 2014). The bacteria colonies observed on this plate were purified by repeated streaking on the sulphur oxidizing agar plates. The purified bacterial isolates from the agar medium were subsequently cultivated on thiosulphate agarand broth (Beijerinck, 1994; Vidyalakshmi and Sridar, 2007) and incubated again at 37°C for 24 hours. The pH of the broth was measured at regular intervals. Bacteria exhibiting the maximum reduction in pH and alteration in broth color were selected for further testing of their ability to determine sulphur ion concentrations.

2.2. Population densitystudy of the SOB isolates

One gram of soil sample was dissolved in ten ml of sterile distilled water. After serial dilution, 0.1 ml of aliquot was plated over sulphur oxidizing agar plate medium and incubated over night for colony formation. The sulphur oxidizing bacteria population in different soil samples were estimated by counting their colony on agar plates and was expressed in colony forming unit (CFU) as follows.

$$\text{Population density}\left(\frac{\text{CFU}}{\text{ml}}\right) = \frac{\text{No of colonies x dilution factor}}{\text{volume of inoculum}}$$

2.3. Morphological and Biochemical characterization of isolates

The bacterial isolates were primarily identified by means of morphological as well as biochemical characterization. The morphological parameters investigated were colony characteristics, shape, size, Gram's reaction, and the biochemical parameters like catalase production, urease production, Voges-Proskauer (V-P) reaction, Indole production test, Methyl red and TSI test were carried out following the standard methods as described in Bergey's Manual of Determinative Bacteriology (Bergey and Holt, 1994).

2.4. Sulphate Ion Production Ability

The production of sulphate ions (SO_4^{2-}) during the growth of sulphur-oxidizing bacteria in thiosulphate medium was quantified using spectrophotometric methods. To measure the sulphate ion concentration, the bacterial culture supernatant was mixed with barium chloride (10% w/v) at 1:1 ratio, and the mixtures were agitated thoroughly. The resulting white precipitate, formed from barium sulphate, was assessed at a wavelength of 450 nm using a Systronics-119 spectrophotometer. The data obtained were then calculated from a sulphate standard curve having potassium sulphate (K_2SO_4) as the standard (Kolmert *et al.,*2005). Standard sulphate solutions were prepared by dissolving K_2SO_4 in deionized water to achieve known concentrations ranging from 20 to 200µg/mL. The degree of turbidity produced is directly proportional to the sulphate ion concentration.

2.5. Phylogenetic characterization of the Isolates

The bacteria exhibiting the highest sulphur-oxidizing activity was subsequently identified through 16S rRNA sequencing. Bacterial DNA was extracted from a loop of overnight cultured cells on nutrient agar using the QIAmp DNA mini kit (Qiagen, Duesseldorf, Germany), following the manufacturer's instructions. PCR amplification was performed using two universal primers, 27f and 907r, in a thermocycler (Bio-Rad T100, USA). The resulting PCR product was analyzed on a 1.2% agarose gel with ethidium bromide staining and visualized using a gel documentation system (Bio-Rad Gel Doc XR, USA). The PCR product was then purified using a DNA purification kit (Illustra GFX PCR DNA and Gel Band purification kit, GE Healthcare, UK) according to the manufacturer's protocol. The purified amplicon was subsequently sequenced by out sourcing service. The DNA sequences obtained were compared with existing sequences using BLAST (http://www.ncbi.nlm.nih.gov/blast). Phylogenetic analysis was performed using the neighbor-joining method in MEGA-X.

3. Result

3.1 Characterization of soil sample

In this study, efforts were made to isolate sulphur oxidizing bacteria from soil samples collected from the Bhitarkannika mangrove ecosystem. Four soil samples denoted as S4, S5, S22, and S23, were collected and used for isolation of sulphur-oxidizing bacteria. A physicochemical analysis of the samples showed that the pH levels ranged from 7.1 to 8.2. The soil was classified as clay type, exhibiting a significant amount of water holding capacity along with notable moisture content (Table 1).

Table 1

Characterization of collected soil samples from Bhitarkannika mangrove forest

Soil samples	Soil type	pH	Water holding capacity (%)	Moisture content(%)
S 4	Clay	7.1	33.23	35.26
S 5	Clay	7.6	46.13	34.22
S 22	Clay	7.3	37.57	36.9
S 23	Clay	8.2	41.29	34.6

3.2 Isolation and characterization of isolates

The bacteria showing positive growth on sulphur oxidizing medium having $Na_2S_2O_3$. $5H_2O$ as the only sulphate source were identified as sulphur-oxidizing bacteria. On the basis of morphological colony characteristics, six distinct colonies were isolated from S4 soil sample, two from S5 sample, six isolates from S22 and, two from S23 soil sample (Table 2). The morphological characteristics of the isolated SOB were examined, focusing on attributes such as colony size, elevation, and margin and color where off-white and yellow coloration of the strains was predominant (Table 2). Further Biochemical tests, including the VP test, urease test, catalase test, indole test, methyl red test, and TSI test, were conducted for each isolates. All isolates tested showed positiveresult for the indole test, while the other tests gave mixed results (Table 3).

3.3 Estimation of population density of the isolated SOB

The population density of SOB from different soil samples were are presented in Table 4. Bacterial count varied from 2.1- 7.6 × 10^6 CFU/ml in different soil samples showing maximum population of S4-A isolate in the S4 soil sample (Table 4).

3.4. pH estimation of sulphur oxidizing bacteria on Thiosulphate media

All isolates of SOB showed a decrease in the pH levels of the culture medium. Four isolates from the S4 soil sample S4-A, S4-B, S4-D, and S4-F showed significant pH reductions of approximately 1.29, 1.32, 1.5, and 1.32 times, respectively, over a 12-day period (Fig. 2a). Similarly, from the S5 sample, S5-B was identified for its remarkable ability to reduce the pH from an initial value of 9.4 to 5.87, achieving of 1.6 fold reduction, which was the highest observed value

Table 2

Morphological characterization of isolated Sulphur oxidizing bacteria

Soil Sample	Isolates	Appearance	Colony Form	Elevation	Margin	Optical Density	Colour
S 4	S4-A	Dull	Irregular	Raised	Weavy	Transparent	Off white
	S4-B	Sticky	Irregular	Raised	Circular	Opaque	Yellow
	S4-C	Sticky	Irregular	Flat	Weavy	Transparent	White
	S4-D	Dull	Irregular	Raised	Weavy	Transparent	Yellowish
	S4-E	sticky	Circular	Flat	Circular	Opaque	Off white
	S4-F	Dull	Circular	Flat	Weavy	Transparent	Light yellow
S 5	S5-A	Dull	Irregular	Raised	Circular	Transparent	White
	S5-B	Sticky	Circular	Flat	Weavy	Opaque	Pink
S 22	S22-A	Dull	Circular	Raised	Circular	Transparent	Off white
	S22-B	Dull	Circular	Flat	Circular	Tranparent	Light yellow
	S22-C	Sticky	Circular	Flat	Circular	Opaque	Yellow
	S22-D	Dull	Circular	Flat	Circular	Transparent	Off white
	S22-E	Dull	Irregular	Raised	Weavy	Transparent	Off white
	S22-F	Sticky	Circular	Raised	Circular	Opaque	Pale yellow
S 23	S23-A	Dull	Circular	Flat	Circular	Transparent	White
	S23-B	Sticky	Circular	Raised	Circular	Opaque	White

Table 3

Biochemical characterization of Sulphur oxidizing isolates

Bacteria Isolates	VP Test	Urease Test	Catalase Test	Indole Test	Methyl Red Test	TSI Test
S4-A	+	-	-	+	+	-
S4-B	-	-	+	+	-	-
S4-D	+	+	+	+	-	-
S4-F	+	+	+	+	+	-
S5-B	+	-	+	+	+	-
S22-C	-	+	+	+	-	+
S22-D	+	+	-	+	+	+
S23-B	+	+	+	+	+	-

Table 4

Estimation of bacterial population density(CFU/ml)

Bacteria isolates	Population density (cfu/ml)	Bacteria isolates	Population density (cfu/ml)
S4-A	7.6×10^6	S22-A	3.2×10^6
S4-B	3×10^6	S22-B	5.5×10^6
S4-C	3.6×10^6	S22-C	2.4×10^6
S4-D	4.8×10^6	S22-D	4.1×10^6
S4-E	2.1×10^6	S22-E	4.9×10^6
S4-F	3.3×10^6	S22-F	5.1×10^6
S5-A	3.9×10^6	S23-A	6.2×10^6
S5-B	3.3×10^6	S23-B	6.1×10^6

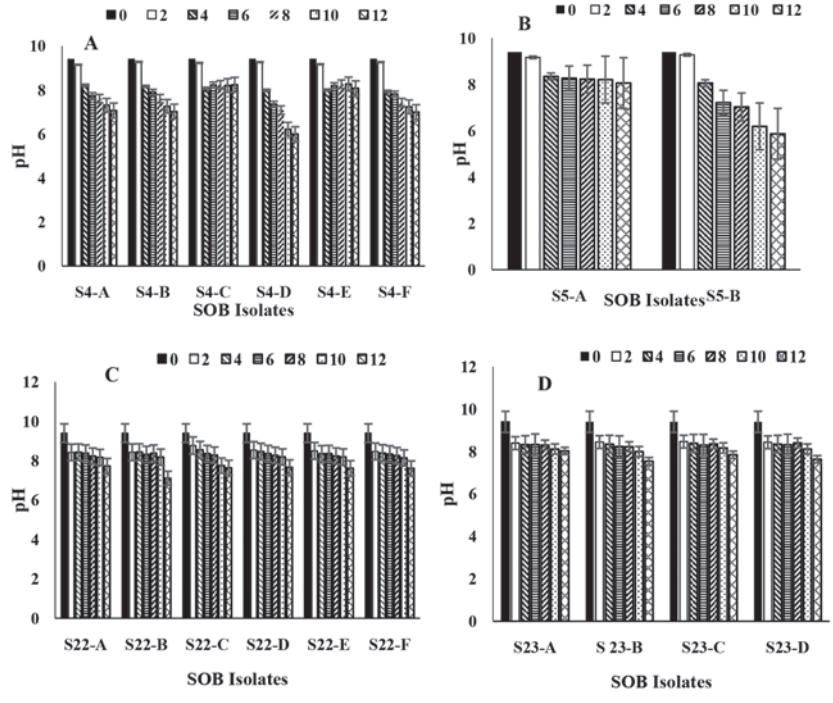


Figure 2. Estimation of pH of Sulphur oxidizing isolates from collected soil samples (A) S4 soil samples (B) S5 soil samples (C) S 22 soil samples (D) S23 soil samples

among other SOB isolate in this study (Fig. 2b). Furthermore, two isolates, S22-C and S22-D from the S22 sample, exhibited a pH reduction of 1.32 and 1.22 times, respectively, while S23-B among the S23 sample showed a 1.24 fold pH reduction (Fig. 2c and 2d). These eight SOB isolates showing significant ($P<0.05$) pH reduction were selected for further study.

3.4. Sulphate ion production ability test

All the eight selected isolates showed a significant amount of sulphate ion production ability. Among the isolates, S5-B showed maximum sulphate ion production (403.09 µg/ml) followed by S4-A isolate (393.72µg/ml) and S4- B isolate (365.07 µg/ml; Fig. 3).

3.5. Phylogenetic identification of the selected strain

The strain showing maximum pH reduction and highest sulphate ion production (S 5-B) was identified at the generic level by 16 SrRNA gene sequencing.The obtained gene sequence of S5B isolate was compared with available data in Gene Bank using BLAST homology search. The isolate was identified as *Pyrobaculumarsenaticum* showing 99% sequence similarity with data available in Gene bank (Fig.4). The sequence is submitted to the NCBI gene bank via accession no PQ699973.

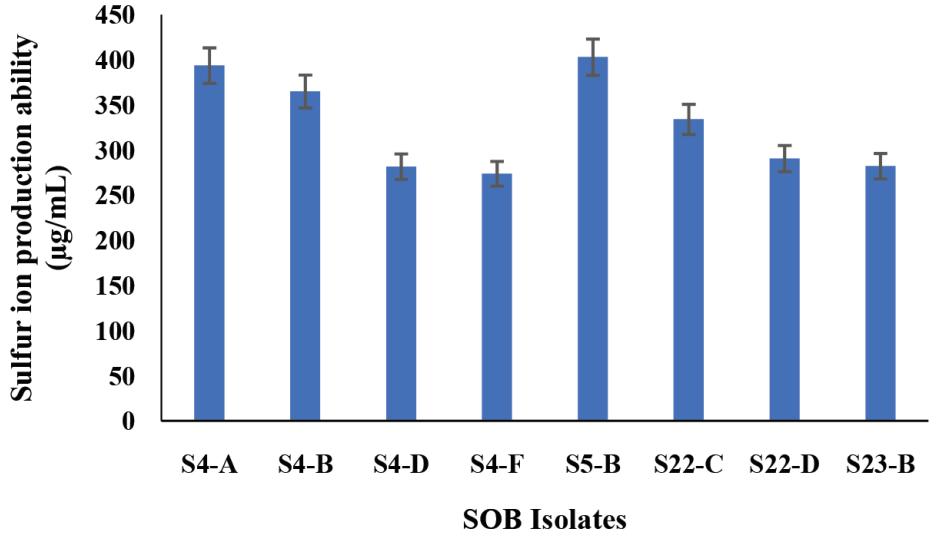


Figure 3. Sulphate ion production ability of isolated SOB

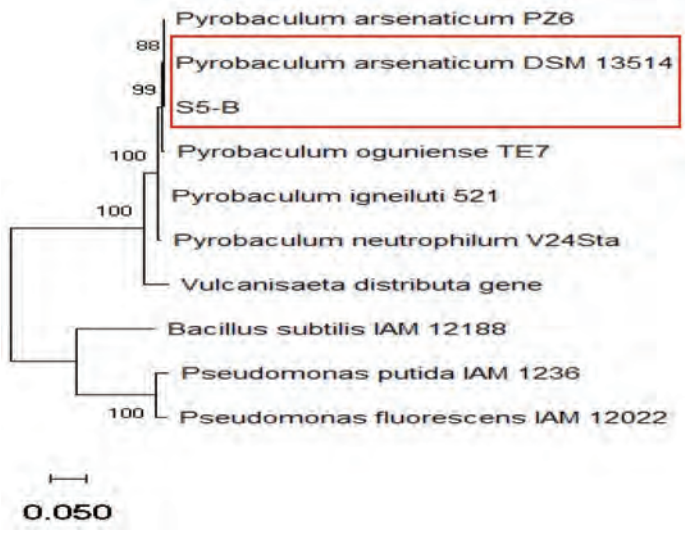


Figure 4. Phylogenetic tree showing the relationships among the isolated bacteria SOB 5- B and between representatives of other related taxa. The tree was constructed by using the software MEGAX.0 and the distance matrix was inferred by the neighbour-joining method using Jukes–Cantor model. The number at the branching points indicate the levels of bootstrap support based on data for 1000 replicates; values greater than 50% are only presented. The scale bar indicates 0.05 substitutions per nucleotide position.

Discussion

The Bhitarkannika mangrove, situated on the east coast, ranks as the second largest tropical mangrove ecosystem in India, hosts a wide variety of microorganisms, thriving in its estuarine environment, which plays a crucial role in nutrient cycling and maintaining ecological balance. The microbial diversity in these mangrove areas varies based on the physicochemical properties of the soil. In this study, the soil samples were identified as clay type, with pH levels ranging from 7.1 to 8.2 (Table 1), whichline up with the reports of Mishra et al. (2012), where a pH variation between 6 and 8 was attributed to seasonal changes. Our results indicate a greater microbial diversity and population density (Table 4).This variation in microbial population size might be influenced by other soil factors, including nutrients, pH, moisture, and salinity (Vikram et al. 2007).

In this study, a total of sixteen SOB isolates were derived from four soil samples labeled S4, S5, S22, and S23. The morphological and biochemical characteristics of these isolates were examined based on their colony traits,Voges-Proskauer (VP) test, Triple Sugar Iron (TSI) test, urease test, catalase test, and methyl red test (Table 2, Table 3). Eight isolates were subsequently selected for their superior ability to reduce pH in the bromophenol blue-containing sulphur-oxidizing medium, as well as in thiosulphate broth significaltly reducing the pH from an initial value of 9.4 to 5.87over a period of 12 days (Fig. 2). This finding aligns with the observations of Behera *et al.* (2014), where sulphur-oxidizing bacterial isolates from mangrove soil of Mhanadi Delta lower the pH in the thiosulphate medium.

Selected bacterial isolates were introduced into the thiosulphate medium to evaluate their capacity for sulphateion production. Among the eight isolates tested, the S5B exhibited the highest sulphate ion production, yielding 403.09 µg/ml (Fig. 3) thus signifying its higher efficiency for sulphur oxidation. Similar results were described by Nakada and Ohta (1999) where sulphate ion production of 11.7 µg/ml was reported from Bacillus sp. In another study, Schook and Berk (1979) reporteda higher sulphate ion production by *Pseudomonas aeruginosa* (130 µg/ml). Ravichandra *et al.* (2007) described the highest production of sulphate ions ranging from 14 to 150 mg/ml by Thiobacillus sp. Simillarly Babana *et al.* (2011) reported two Thiobacillus strains for efficient sulphuric acid production up to 243 mg/l by the oxidation of sulphur and sulphide. These reports indicated S5B higher sulphur oxidizer than *Bacillus* sp. and *Pseudomonas* sp. where as a moderate oxidizer as compared to *Thiobacillus* sp.

Further the bacteria isolated from the mangrove forests of Bhitarkannika S5B was identified as *Pyrobaculum arsenaticum* (Fig. 4) by phylogenetic analysis. Jay *et al.* (2015) previously reported another sp of *Pyrobaculum* (*Pyrobaculum yellostonensis*) isolated from elemental sulphur sediments of Yellostone National park, capable of thriving an extreme temperature, pH, and salinity conditions and playing a major role in biogeochemical cycle of sulphur, carbon and arsenic.

Conclusion

The study isolated and characterized a potent sulphur-oxidizing bacteria from mangrove soil, having maximum sulphur ion production ability of (403.09 µg/ml) showing higher sulphuroxidizing capacity. This bacteria was further identified as *Pyrobaculum arsenaticum* and deposited in the NCBI database. These findings are helpful to understand the biogeochemical cycling of the mangrove environment and provides further scope for bioprospecting biotechnological application and environmental management.

Acknowledgements

Financial support in terms of projects from Start-Up Grant, UGC (F.30-387/2017(BSR)), New Delhi, India, Department of S & T (3365/ST (Bio.)-02/2017), Odishaand the Centre of Excellence in Integrated Omics and Computational Biology (CoE-IOCB) (World Bank) were thankfully acknowledged.

References

Babana, A.H, Samake, F. and Maiga, K. (2011). Characterization of Some Agricultural Soils: Presence and Activity of Tilemsi Rock Phosphate-Solubilizing Thiobacilli," British Microbiology Research Journal, 1(1).1-9.

Banerjee, K., Bal, G. and Mitra, A. (2018). How soil texture affects the organic carbon load in the mangrove ecosystem? A case study from Bhitarkanika, Odisha. In: Singh VP, Yadav, S. & Yadava, R, N. (eds.), Environmental Pollution, Springer, Singapore, pp. 329- 341

Behera, B., Patra, M., Dutta, S., & Thatoi, H. (2014). Isolation and Characterisation of Sulphur Oxidising Bacteria from Mangrove Soil of Mahanadi River Delta and Their Sulphur Oxidising Ability. Journal of Applied & Environmental Microbiology, 2(1), 1-5.

Beijerinck, M. W (1904) "Phenomenes de reduction produits par les microbes (Conference avec demonstrations faite - Delft, le 16 avril 1903)," Archs Neerrl Science Series, 29. 131-157.

Bergey, D.H. and Holt, J.G. (1994) Bergey's Manual of Determinative Bacteriology. 9th Edition, Williams & Wilkins, Baltimore, Maryland.

Brock, T.D., Madigan, M.T. and Martinko, J.M. (2006) Biology of microorganisms upper Saddle River, N,J (U.A) Prentice-Hall

Camacho, A. (2009). Sulphur Bacteria. University of Valencia, Burjassot, Spain. Elsevier Inc. 261-278.

Das, S.K., Mishra, A.K., Tindall, B.J., Rainey, F.A. and Stackerbrandt, E. (1996). Oxidation of thiosulphate by a new bacterium, Bosea thiooxidans (strain BI-42) gen. nov., sp. nov.: Analysis of phylogeny based on chemotaxonomy and 16S Ribosomal DNA sequencing. International Journal of Systematic Bacteriology. 46(4): 981-987

Dash, K.,Mishra, P. and Panda, B. (2019).Cyanobacterial diversity of Bhitarkanika mangrove forests, Odisha. Plant science research. 41 (1&2) : 34-40.

Hart, M.G.R. (1959). Sulphur oxidation in tidal mangrove soils of Sierra Leone. Plant Soil 11, 215-236.

Jamal, A., Moon, Y.S. and Abdin, M.Z. (2010). Sulphur-a general overview and interaction with nitrogen. Australian Journal of Crop Science. 4(7): 523-529

Jay, Z.J., Beam, J.P., Dohnalkova, A., Lohmayer, R., Bodle, B., Planer Friedrich, B., Romine, M. and Inskeep, W.P. (2015). *Pyrobaculum yellowstonensis* Strain WP30 Respires on Elemental Sulphur and/or Arsenate in Circumneutral Sulphidic Geothermal Sediments of Yellowstone National Park. Appl Environ Microbiol 81:

Kabdasli, I., Tünay, O. and Orhon, D. (1995). Sulphate removal from indigo dyeing textile waste waters, Water Science and Technology, 32. 12, 21-27.

Kertesz, M.A. and Mirleau, P. (2004). The Role of Microbes in Plant Sulphur Nutrition. Journal of Experimental Botany. 55: 1939- 1945

Kushkevych, I., Hýžová, B., Vít?zová, M., Rittmann, S.K.R. (2021). Microscopic Methods for Identification of Sulphate-Reducing Bacteria from Various Habitats. Int J Mol Sci 13;22(8):4007.

Liang, J.B., Chen, Y.Q., Lan, C.Y., Tam, F.Y., Zan, Q.J. and Huang, L.N. (2006). Recovery of novel bacterial diversity from mangrove sediment. Marc. Biol. 150:739-747

Mishra, R. R., Swain, M. R., Dangar, T. K. and Thatoi, H. (2012) Diversity and seasonal fluctuation of predominant microbial communities in Bhitarkanika, a tropical mangrove ecosystem in India. Int. J. Trop. Biol 60: 909-924

Nakada Y. and Ohta Y. (1999), Purification and properties of hydrogen sulphide oxidase from Bacillus sp, BN53-1, Journal of Bioscience and Bioengineering's, 87(4), 452-455.

Ravichandra, P., Mugeraya, G., Ganganirao, A., Ramakrishna, M. and Jetty, A. (2007). Isolation of *Thiobacillus* sp from aerobic sludge of distillery and dairy effluent treatment plants and its sulphide oxidation activity at different concentrations", Journal of Environmental Biology, 28(4). 819-823.

Scherer H.W. (2009). Review Article-Sulphur in soils. Journal of Plant Nutrition and Soil Science. 172: 326-335.

Schook L. B. and Berk R.S. (1979). Nutritional studies with *Pseudomonas aeruginosa* grown on inorganic sulphur sources, Journal of Bacteriology, 133, 1377 -1382.

Tandon H.L.S. and Messick D.L. (2002). Practical Sulphur Guide Book. The Sulphur Institute, Washington, Dc

Vidyalakshmi, R. and Sridar (2007). R. Isolation and characterization of sulphur oxidizing bacteria. Journal of Culture Collection. 5. 73- 77.

Vikram, A. and Hamzehzarghani, H. (2008). Effect of phosphate solubilizing bacteria on nodulation and growth parameters of greengram (*Vigna radiata* (L.) Wilczec.). Res. J. Microbiol. 3: 62-72

Visser, J. M., Robertson, L. A., Verseveld, H. W. V. and Kuenen, J. G, (1997). Sulphur Production by Obligately Chemolithoautotrophic *Thiobacillus* Species. Applied and Environmental Microbiology, 63(6). 2300-2305.

Plant Science Research 46 (1&2) : 60-64, 2024

Plant Science Research

ISSN 0972-8546

Anticancer potential of *Terminalia bellirica* Roxb. (Bahera) derived Phytocompounds: A Promising Natural Remedy

Prakash Kumar Senapati and Sujit K Bhutia[Ψ]

Department of Life Science, National Institute of Technology Rourkela, Rourkela-769008, Odisha, India

ARTICLE INFO

Article history:
Received : 5 September 2024
Revised : 20 October 2024
Accepted : 29 October 2024

Keywords:

Terminalia bellerica
Bahera
Antiplatelet
Anticancer

ABSTRACT

Terminalia bellirica (Gaertn.) Roxb. is commonly known as Bahera, belongs to the Combretaceae family, one of the major components of the oldest medicine, 'Triphalachurna'. This belerica myrobalan has a wide native distribution spanning from the Indian subcontinent to china and Malaysia. Tarnabelerica is conventionally used for diabetic diarrhoea, high cholesterol and many other conditions. Skin disease is also controlled by the extract of this plant. Bioactive compounds derived from *Terminalia bellirica* (TB) show their potential for antioxidant, anti-inflammatory, antiviral, antiplatelet, antidiabetic, wound-healing, antidepressant, and anticancer properties. Major components of this plant were chebulagic acid, ellagitannins, galloyl hexahydroxy diphenol and many flavonoid glucosides. This plants habitat is most valleys in India, thats why it is also known as veleric orbastad myrobalan. This plant is a source of vitamin c and minerals like selenium, manganese, potassium, iron and copper. The fruits of *Terminalia bellirica* are astringent (bitter) and acridic (sour) in taste. In the present study, focus is to highlight the anticancer properties of *Terminalia bellirica*-derived bioactive compounds and enlighten the future direction of TB extract as cancer therapeutics.

Introduction

Terminalia bellirica (Gaertn.) Roxb. is one of the ancient medicinal plants foremost in the deciduous regions of the world, such as India, China, Java, Sumatra, Indo–China, Sri Lanka, Nepal, etc. (Deb *et al.,* 2016; Kumari *et al.*, 2017). However, it is dominant in India in Uttar Pradesh, Madhya Pradesh, Punjab, and Maharashtra (Kumari *et al.*, 2017; Umesh Kanna *et al.,* 2024). *Terminalia bellirica* (Gaertn.) Roxb. is readily known for its common names, such as Baheda, Bibhitaki, Belleric Myrobalan, Kalidruma, Bhutavasa and Kaliyugalaya (Deb *et al.,* 2016; Umesh Kanna *et al.,* 2024). As TB fruits have numerous medicinal properties and are used to remove the fear of various diseases, Sanskrit is commonly known as "vibheetaki," which means fearless (Gupta *et al.,* 2020). The genus *Terminalia* is imitative from a Latin term that means terminus or terminal or end (Gupta *et al.,* 2020). The genus name depicts the habit of the plant leaves as the plants come under this genus, having clustered leaves at the terminal part of the branches. *Terminalia bellirica* (Gaertn.) Roxb. mainly belongs to the Combretaceae family of the Myrtales order (Urooj *et al.,* 2023).

Terminalia bellirica (Gaertn.) Roxb. is one of the major constituents of the oldest medicine, 'Triphalachurna' (Baliga *et al.,* 2015; Peterson *et al.,* 2017). Triphalachurna is a combination of fruits of Baheda (*Terminalia bellirica*), Harida (*Terminalia chebula*), and Amla (*Emblica officinalis*) (Peterson *et al.,* 2017; Rana *et al.,* 2018). Several works of literature reported that TB has tremendous therapeutical and pharmacological properties. Various *in vitro*, *in vivo* and clinical reports show its potential for antioxidant, anti-inflammatory, antiviral, antiplatelet, antidiabetic, wound-healing, antidepressant, and anticancer properties. As the mortality rate of cancer now acts as a burden for developing countries, so highlighting the anticancer properties of TB are the main focus in this short communication.

[Ψ] *Corresponding author; Email:* sujitb@nitrkl.ac.in

Table 1:

Phytochemicals present in *T. bellerica*

Compounds	Chemical constituents
Flavone	7-hydroxy 3', 4' (methylenedioxy) flavone [9], luteoline [10]
Steroids	β-sitosterol [11]
Lignans	Termilignan [9], thannilignin [9], anolignan B[9]
Tannins	Gallic acid [10], ellagic acid [10], methyl gallate [10], ethyl gallate (Phenyllemblin) [11], chebulaginine acid [11], chebulagic acid [12], hexahydroxydiphenic acid ester [12]
Glycosides	Fructose, sucrose, galactose, D-glucose, mannose, rhamnose [11]
Terpenoid	Belleric acid [12], chebulagic acid [11], arjungenin [12]
Saponin	Bellericoside and bellericanin [12]
Cardenolide	Cannogenol 3-O-β-galactopyranosyl-(1→4)-O-α-L-rhamopyranoside [13]
Flavonol aglycones	Quercetineand kampferol [10]
Flavonol glycosides	Quercetin-3-O-[6"-a-L-rhamnopyranosyl]-(1→6)-β-D-glucopyranoside (rutin), quercetin-3-O-α-L-rhamnopyranoside, quercetin-3-O-β-D-glucopyranoside and kaempferol-3-O-β-D-glucopyranoside [10]
Fatty acides present in oil	Palmitic acid, linoleic acid, stearic acid, myristic acid and oleic acid [14]
Glycerides of fatty acids	Palmitooleolinolein, stearo-oleolinolein, palmitodiolein, stearodiolein, dioleolinolein and triolein [15]

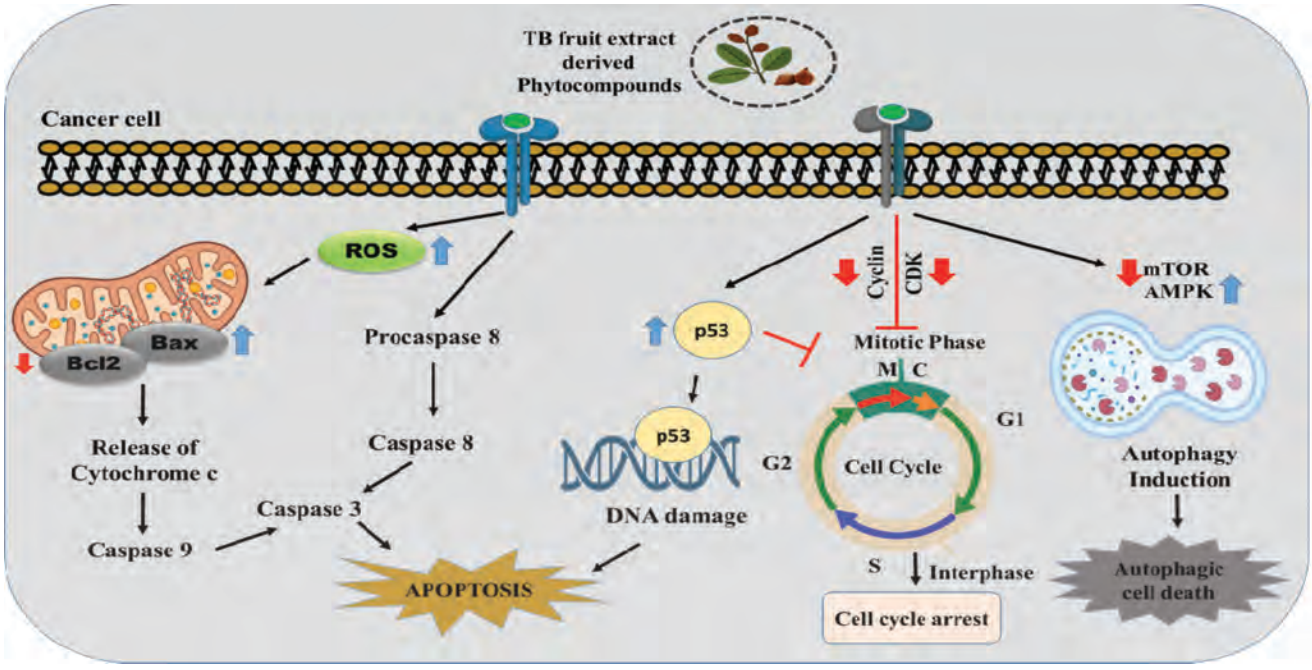


Figure 1. TB fruits-derived phytocompounds show various anticancer activities which are therapeutically important for cancer treatment. Phytocompounds derived from TB fruits increase ROS along with apoptotic proteins to apoptosis in cancer cells. Cell cycle arrest is another anticancer action mediated by TB fruits TB-derived phytocompounds in cancer cells via p53-mediated pathways, along with a decrease in the cell cycle regulator Cyclin and Cdk. Autophagic cell death is one of the anticancer actions governed by TB fruits TB-derived phytocompounds in cancer cells, mainly activated via modulating mTOR/AMPK axis.

Results and Discussion

The anticancer potential of TB is the main topic of discussion as (Figure-1), nowadays, cancer is known to be a leading cause of mortality in the world. Although several therapeutic applications are available for effective and safe cancer treatment, we largely depend on phytocompounds. The phytocompounds derived from TB show tremendous potential for anticancer properties (Kadian *et al.,* 2014). The extract of TB has a wide variety of bioactive compounds, such as tannins, flavonoids, phenolic compounds, terpenes, and glycoside derivatives (Gupta *et al.,* 2020). Studies reported that on a dry-weight basis, approximately 22.3% of polyphenolic compounds (including Gallic acid and its esters-19.1%, Chebulic ellagitannins-0.81%, non-chebulic ellagitannins-1.5%, Ellagic acid and its derivatives-0.64%, and Ellagic glycosides-0.23%) are found in TB fruit extracts (Gupta *et al.,* 2020). Similarly, the seed of TB contains 12.28% oils, out of which we found oleic acid (43%), linoleic acid (29%), palmitic acid (12%), and stearic acid (16%) (Gupta *et al.,* 2020).The most common phytocompounds derived from TB are Chebulagic acid, Gallic acid, Ellagic acid, Bellericaside B, Chebulinic acid, Chebulic acid, Tannic acid, Quinic acid, and many more (Gupta *et al.,* 2020). Interestingly, the major phytocompounds derived from TB have shown the potential for anticancer properties, creating a therapeutic formulation for cancer treatment.

Out of the major phytocompounds derived from TB, Gallic acid has been shown to have anticancer activities in various cancers. The study reported that the methanolic extract containing octyl gallate and gallic acid shows significant inhibition of the growth of breast cancer cells (Sales *et al.,* 2018; Vijayalakshmi *et al.,* 2023). Both gallic acid and octyl gallate show inhibitory action of cell cycle progression via modulating the cell cycle regulators like CDK and cyclin, along with elevating antioxidant enzyme levels (Dinesh *et al.,* 2014; Vijayalakshmi *et al.,* 2023). Patra et al. findings support that the extract of TB shows anticancer properties via modulation of cytoprotective autophagy and apoptosis in oral cancer cell lines, including FaDu, Cal33, SCC-4, and SCC-25 (Patra *et al.,* 2020). Not only in the case of oral cancer, anticancer and anti-proliferative effects have been reported in several other cancers, like prostate cancer PC–3, leukemia HK–63, and many more (Dinesh *et al.,* 2014). TB extracts show anticancer activities in the case of Cholangiocarcinoma, which has also been reported (Chekdaengphanao *et al.,* 2022). Ethyl acetate extract from TB fruits shows anticancer activities via modulating apoptotic pathways, which have been reported by in vitro studies (Chen *et al.,* 2015). In hepatocellular carcinoma, the growth is inhibited by tannins or their derivative phytocompounds of TB fruit extract via modulating EGFR signaling (Chang *et al.,* 2022). Interestingly, TB seed extracts elevate ROS production, apoptosis activation, and inhibition of protective autophagy to show anticancer activities (Padhi, 2014). TB-derived bioactive compounds show anticancer properties via upregulation of p53 to arrest the cell cycle and the proapoptotic protein Bax (Ghate *et al.,* 2014). Ellagic acid, Chebulagic acid, and Chebulinic acids have potential anticancer properties that induce bioactive compounds in TB fruit extracts. Chebulinic acid, a common bioactive compound found in Triphala, belongs to the genus *Terminalia* and shows antitumor properties in human colorectal cancer (Thoithoisana Devi *et al.,* 2023). In the case of retinoblastoma cells, chebulagic acid shows anticancer activities via inducing apoptosis and G1 stage arrest (N. Kumar *et al.,* 2014). Similarly, chebulagic acid shows anticancer activities in gastric cancerby limiting the AURKA/Wnt pathway (Zhao *et al.,* 2023). Moreover, Ellagic acid also has anticancer, anti-proliferative, and anti-inflammatory properties (Han *et al.,* 2006). Recently, in colon cancer, EA has been shown to have anticancer properties via inducing autophagy and apoptosis through modulating mTOR and AMPK pathways (Ni *et al.,* 2023). Similarly, in the case of bladder cancer, EA induces elevated expression of p53 and arrests G1 stage of the cell cycle (Li *et al.,* 2005). However, extensive work needs to be done on Ellagic acids to evaluate their mechanistic way of understanding the anticancer activities in various cancers.

Conclusion

TB-derived phytocompounds offer promising anticancer potential, positioning this plant as a valuable resource for cancer therapeutics. The majority of the bioactive compounds derived from TB extract have the potential to show anticancer, anti-proliferative, and anti-inflammatory properties in various cancers. The key compounds such as gallic acid, chebulagic acid, and ellagic acid exhibit potent anticancer, anti-proliferative, and anti-inflammatory effects across diverse cancers. However, extensive studies need to be done on multiple bioactive compounds like Bellericaside B, Chebulinic acid, Chebulic acid and Ellagic acid to open a futuristic mechanistic path towards Phytocompounds-based cancer therapeutics with limited side effects.

References

Baliga, M. S., Meera, S., Rai, M. P., Saldanha, E., Pais, S., Jayachander, D., & Palatty, P. L. (2015). Use of the ayurvedic drug triphala in medical conditions afflicting older adults. In *Foods and dietary supplements in the prevention and treatment of disease in older adults* (pp. 135–142). Elsevier.

Chang, Z., Zhang, Q., Hu, Q., Liu, Y., Zhang, L., & Liu, R. (2022). Tannins in *Terminalia bellirica* inhibits hepatocellular carcinoma growth via re-educating tumor-associated macrophages and restoring CD8+ T cell function. *Biomedicine & Pharmacotherapy*, *154*, 113543.

Chekdaengphanao, P., Jaiseri, D., Sriraj, P., Aukkanimart, R., Prathumtet, J., Udonsan, P., & Boonmars, T. (2022). Anticancer activity of *Terminalia chebula, Terminalia bellirica, and Phyllanthus emblica* extracts on cholangiocarcinoma cell proliferation and induction of apoptosis. *Journal of Herbal Medicine*, *35*, 100582.

Chen, Y.-X., Tong, J., Ge, L.-L., Ma, B.-X., He, J.-S., & Wang, Y.-W. (2015). Ethyl acetate fraction of *Terminalia bellirica* fruit inhibits rat hepatic stellate cell proliferation and induces apoptosis. *Industrial Crops and Products*, *76*, 364–373.

Deb, A., Barua, S., & Das, B. (2016). Pharmacological activities of Baheda (*Terminalia bellerica*): A review. *Journal of Pharmacognosy and Phytochemistry*, *5*, 194–197. https://api.semanticscholar.org/CorpusID:44514231

Dinesh, M. G., Subbarayan, R., Rallapalli, S., Kansrajh, C., & Kalaivani, R. (2014). *Terminalia bellerica* leaf extracts induce apoptosis in Hep G2 cells and regulates cell cycle progression by inducing G2/M cell cycle arrest. *Indian Journal of Research in Pharmacy and Biotechnology*, *2*(1), 1044.

Ghate, N. B., Hazra, B., Sarkar, R., Chaudhuri, D., & Mandal, N. (2014). Alteration of Bax/Bcl-2 ratio contributes to *Terminalia belerica*-induced apoptosis in human lung and breast carcinoma. *In Vitro Cellular & Developmental Biology-Animal*, *50*, 527–537.

Gupta, A., Kumar, R., Bhattacharyya, P., Bishayee, A., & Pandey, A. K. (2020). *Terminalia bellirica* (Gaertn.) roxb. (Bahera) in health and disease: A systematic and comprehensive review. *Phytomedicine*, *77*, 153278. https://doi.org/https://doi.org/10.1016/j.phymed.2020.153278

Han, D. H., Lee, M. J., & Kim, J. H. (2006). Antioxidant and apoptosis-inducing activities of ellagic acid. *Anticancer Research*, *26*(5A), 3601–3606.

Kadian, R., Parle, M., & Yadav, M. (2014). Therapeutic potential and phytopharmacology of *Terminalia bellerica*. *World Journal of Pharmacy and Pharmaceutical Sciences*, *3*(10), 804–819.

Kumar, N., Gupta, G., & Karnati, R. (2014). Chebulagic acid from *Terminalia chebula* causes G1 arrest, inhibits NFêB and induces apoptosis in retinoblastoma cells. *BMC Complementary and Alternative Medicine*, *14*, 1–10.

Kumari, S., Jeedigunta, M., Joshi, A., Agarwal, A., Mundkinajeddu, D., & G M, G. (2017). *A pharmacognostic, phytochemical and pharmacological review of Terminalia bellerica*. *6*, 368–376.

Li, T.-M., Chen, G.-W., Su, C.-C., Lin, J.-G., Yeh, C.-C., Cheng, K.-C., & Chung, J.-G. (2005). Ellagic acid induced p53/p21 expression, G1 arrest and apoptosis in human bladder cancer T24 cells. *Anticancer Research*, *25*(2A), 971–979.

Ni, X., Shang, F.-S., Wang, T.-F., Wu, D.-J., Chen, D.-G., & Zhuang, B. (2023). Ellagic acid induces apoptosis and autophagy in colon cancer through the AMPK/mTOR pathway. *Tissue and Cell*, *81*, 102032.

Padhi, P. (2014). *Terminalia bellerica (baheda) inhibits protective autophagy and induces apoptosis in oral cancer cell lines*.

Patra, S., Panda, P. K., Naik, P. P., Panigrahi, D. P., Praharaj, P. P., Bhol, C. S., Mahapatra, K. K., Padhi, P., Jena, M., Patil, S., Patra, S. K., & Bhutia, S. K. (2020). *Terminalia bellirica* extract induces anticancer activity through modulation of apoptosis and autophagy in oral squamous cell carcinoma. *Food and Chemical Toxicology/ : An International Journal Published for the British Industrial Biological Research Association*, *136*, 111073. https://doi.org/10.1016/j.fct.2019.111073

Peterson, C. T., Denniston, K., & Chopra, D. (2017). Therapeutic uses of triphala in ayurvedic medicine. *The Journal of Alternative and Complementary Medicine*, *23*(8), 607–614.

Rana, C. S., Kimothi, G. P., NARAYAN, S. B., & SASTRY, J. L. N. (2018). Triphala churna myth and microscopic characterization. *Ananls of Phytomedicine*, *7*(1), 158–164.

Sales, M. S., Roy, A., Antony, L., Banu, S. K., Jeyaraman, S., & Manikkam, R. (2018). Octyl gallate and gallic acid isolated from *Terminalia bellarica* regulates normal cell cycle in human breast cancer cell lines. *Biomedicine & Pharmacotherapy*, *103*, 1577–1584.

Thoithoisana Devi, S., Devika Chanu, K., Singh, N. B., Chaudhary, S. K., Keithellakpam, O. S., Singh, K. B., Mukherjee, P. K., & Sharma, N. (2023). Chemical profiling and therapeutic evaluation of standardized hydroalcoholic extracts of *Terminalia chebula* fruits collected from different locations in Manipur against colorectal cancer. *Molecules*, *28*(7), 2901.

Umesh Kanna, S., Parthiban, K. T., Senthilraja, K., Venkatesan, S., Udhaya Nandhini, D., Mohan Kumar, S., Dhasarathan, M., Kumaresan, P., Sai, M. J.,

Raveendran, M., & Geethalakshmi, V. (2024). Genetic Diversity and Structure of *Terminalia bellerica* (Gaertn. Roxb.) Population in India as Revealed by Genetic Analysis. In *Plants* (Vol. 13, Issue 4). https://doi.org/10.3390/plants13040470

Urooj, A., Hashir, M., Akram, M., & Zaidi, S. S. (2023). Efficacy of *Terminalia bellirica* (Balela): A review. *Indian Journal of Unani Medicine*, *16*(2), 138.

Vijayalakshmi, P., Indu, S., Ireen, C., Manjunathan, R., & Rajalakshmi, M. (2023). Octyl Gallate and Gallic Acid Isolated from *Terminalia bellirica* Circumvent Breast Cancer Progression by Enhancing the Intrinsic Apoptotic Signaling Pathway and Elevating the Levels of Anti-oxidant Enzymes. *Applied Biochemistry and Biotechnology*, *195*(12), 7214–7235.

Zhao, J., Shi, Y., Ma, Y., Pan, L., Wang, Y., Yuan, L., Dong, J., & Ying, J. (2023). Chebulagic acid suppresses gastric cancer by inhibiting the AURKA/â-catenin/Wnt pathway. *Frontiers in Pharmacology*, *14*, 1143427. https://doi.org/10.3389/fphar.2023.1143427

Plant Science Research 46 (1&2) : 65-71, 2024

Plant Science Research

ISSN 0972-8546

Sunflower (*Helianthus annus* L.) plants supplemented with optimized nutrient formulations under controlled hydroponic conditions express improved agro-physiological traits for floriculture

Dayanidhi Sahoo[1] and Khirod Kumar Sahoo[1Ψ]

[1]Department of Botany, Ravenshaw University, Cuttack-753003, India

ARTICLE INFO

Article history:
Received : 9 September 2024
Revised : 21 October 2024
Accepted : 3 November 2024

Keywords:

Sunflower
Hydroponic Cultivation
Nutrient Formulations
NFT system
Agronomic traits
plant physiology

ABSTRACT

Hydroponics is increasingly recognized as a commercial cultivation technology for enhancing agronomic traits and morphological characteristics of ornamental flowering plants. Sunflower, known for their attractive inflorescence, fragrance and vibrant coloration, have become an ideal choice as ornamental plants. The demand is rising steadily but the production of ornamental sunflowers has been declined under conventional cultivation. This is attributed to challenges such as inadequate nutrient availability, hypoxic conditions, adverse climatic factors, and soil contamination, which adversely affect the plant growth, flower production and survivability. This study investigates the potential for growing the sunflower plants supplementing with three different nutrient formulations (F-1, F-2, F-3) under greenhouse hydroponic condition. The results showed that supplementation of nutrient formulations leads to expression of various effects in agro-physiological traits of plants. The best agro-physiological traits like number of flowers produced per plant (9 ± 0.57), total seed number in each plant (661.3 ± 46.97) shoot length (41.37±0.67 cm), root length (24.67±0.28cm), expanded leaves number (8.00±1.15) and plant physiology like photosynthetic rate (19.17± 0.60 µmol $CO_2 m^{-2}s^{-1}$), transpiration rate (16.95 ± 0.30 mmol H, $Om^{-2}s^{-1}$, stomatal conductance (823.3 ± 14.53 molH, $Om^{-2}s^{-1}$) were observed in plants supplemented with F-2 formulation as compared to other formulations. Looking into the sunflower floriculture perspective, it may be concluded that the F-2 nutrient formulation is the optimum nutrient formulation for expression of improved Agronomic traits under hydroponic condition. This outcome could be due to better uptake of macronutrients and maintenance of healthy physiological status of the plant.

1. Introduction

Ornamental sunflower (*Helianthus annus* L.) is an annual flowering plant having a large-circular attractive yellow inflorescence flower head of Asteraceae family. It is quite popular as fresh cut flowers because of the array of colors and growth habits. The *genus Helianthus* L. comprises more than fifty species of sunflowers, all of which are native to North America, with some species (particularly *Helianthus annus*, the common sunflower) are cultivated in Europe and other parts of the world as food crops and ornamental plants. Wild *Helianthus* species have contributed many useful traits to domesticated sunflower, including disease resistance, drought and salt tolerance, and improved oil quality (Adeleke and Babalola, 2020). It is grown all throughout the world and majority of its products have been commercialised as livestock feed (Yegorov *et al.,* 2019). Among the three primary oilseed crops in the world today namely soybean, rapeseed, and sunflower, sunflower has been recognised as a key source of high-quality edible oil (Vilvert *et al.,* 2018). A considerable number of bioactive compounds are present in sunflower seeds. Edible seeds and sprouts are a good source of antioxidants, such as: flavonoids (apigenin, kaempferol, luteolin), phenolic acids (caffeic acid, chlorogenic acid, gallic acid, coumaric, ferulic acid, and sinapic acids), alkaloids, saponin, tannin, steroids,

Ψ *Corresponding author; Email:* khirod555@gmail.com

trace elements and vitamins which contribute to its pharmaceutical activities (Guo *et al.,* 2017; Bohm and Stuessy, 2001). Phenolic compounds and terpenoids are mainly found in florets and aerial parts (Guo *et al.,* 2017). Sunflower seed has an unusually high supply of polyunsaturated fatty acids (about 31.0%), monounsaturated proteins, tocopherols when compared to other oilseeds and also contain significantly higher amounts of vitamin E (37.8 mg/100g) having antibacterial, anti-diabetic, anti-cholesterol, anti-asthmatic and antioxidant properties (Shahidi and Ambigaipalan, 2015; Bashir *et al.,* 2015; Menzel *et al.,* 2019).

With the rapid urbanization and industrialization not only, the cultivable land is decreasing but also conventional agricultural practices are having vast detrimental effects on the environment. Floricultural production in most parts of the world is extremely difficult due to high rate of infection by soil borne diseases, unavailability of fertile land, conducive weather condition, labour intensive and time-consuming processes (Devi *et al.,* 2011; Dinesh *et al.,* 2014; Xu *et al.,* 1990). Over the past two decades, the production of ornamental sunflowers has been reduced and production only possible due to the breeding of new commercial cultivars (Wien 2014). Hydroponics is the art and science of growing crops in a soilless manner that helps to reduce the problems of traditional crop cultivation related to soil (Murumkar *et al.,* 2012). Diverse group of plants like fruiting, flowering, medicinal and ornamental plants cultivated under soil less hydroponic system (Macwan *et al.,* 2020). By utilizing this advanced technique, the growers can cultivate crop throughout the year regardless of climate and weather condition by ensuring enhanced productivity (Sahoo *et al.,* 2024; Domingues *et al.,* 2012). Various commercial and special crops can be grown using hydroponics including leafy vegetables, tomato, cucumber, pepper, strawberry and many more. In recent years, the Netherlands has successfully adopted hydroponic systems for the production of several cut flowers (Kamenetsky and Okubo, 2012). Notable flowering plants such as Lilium species (Ranjbar Sheykhani, 2024), Gerbera (Kharrazi *et al.,* 2020), Tuberose (Kanani and Nazarideljou, 2017), Gladiolus (Wahome *et al.,* 2010), and Rose (Roosta and Rezaei, 2014) have been cultivated under hydroponic conditions, achieving high flower yields. Flowers such as antedeschia aethiopica (Boldrin *et al.,* 2022) etc. had already been established under hydroponics and cultivated worldwide. Vegetable like spinach is grown better by application of optimum nutrient concentration and an uninterrupted cultivation is carried out a year around under greenhouse hydroponic condition (Acharya *et al.,* 2021). Similarly flower like marigold and chrysanthemum are cultivated in optimum nutrient concentration under NFT system, enhances the quality flower production and restrict soil borne diseases (Sarmah *et al.,* 2020; Viyachaia *et al.,* 2015). The objective of the study is to observe the impact of the different nutrient formulations on the agro-physiological traits and yield of flower and seed of the ornamental Sunflower plants under NFT system greenhouse condition. Here, the experiments were carried out through cultivation of sunflower plants in soil as control and NFT hydroponic system, supplementing with three different nutrient formulations.

2. Materials and Methods

2.1 Collection of seed, pre-treatment and germination

Ornamental sunflower seeds were collected in the month of March, 2022. The seeds were subsequently soaked with fungicide (3% of H_2O_2) solution and put it in germination bed, watering and covered with plastic bag. Then germination bed was kept in growth chamber at 27ºC under dark conditions at 85 ± 2% humidity for seed germination. After 7 days,the seeds are going to germinate, uncovered the germination bed and exposed to LED light (8000 lux). After 15 days of observation the seedlings when reached to 5-6 inch, were transferred to greenhouse NFT hydroponic system.

2.2 Experimental design for Nutrient film technique (NFT), hydroponic culture of sunflower plants under greenhouse

In the first week of March 2022, the seedlings were grown in the three NFT System supplemented with three different nutrient formulations (F-1, F-2, and F-3) under greenhouse condition. The seedlings about 5-inch length were transferred in plastic net pots, placed in the 5.5 cm deep water channels of the NFT system for each treatment. The net pots (5 cm diameter) were fixed, maintaining a minimum water depth of 1.5 cm with a 15.5 cm gap between holes. Dissolved oxygen of nutrient media was maintained by using aerators. An inert medium, Leca ball was used to give mechanical support of the growing seedlings in NFT system. Triplicate plants were cultivated in each three nutrient formulations. The nutrient formulations were replaced every 10-15 days. The plants in triplicate number of seedlings planted in soil were considered as control. The temperature and humidity were maintained at 26ºC and 85% respectively till seed maturation. The pH and EC were regulated during the early vegetative stage and gradually increased during flowering and seed setting stage. The optimum nutrient formulation for plant growth and yield was evaluated.

2.3 Applications of Nutrient formulation

The composition of nutrient formulation, stock and working solution preparation and method of application of F-1, F-2, and F-3 were same as described by Sahoo *et al.*, (2024). Initially, the EC of nutrient solutions F-1, F-2, and F-3 was 200, 250, and 300 µs/cm during seeding plantation, which increased to 600, 650, and 700 µs/cm during vegetative stage. During the flowering and seed setting stage, the EC was kept at 900, 950, and 1000 µs/cm. The pH was maintained at 6.5 throughout the experiment.

2.4 Measurement and analysis of agronomic traits

Agronomic parameters such as shoot length, root length, leaf number, fresh root biomass, flower number in each plant, seed number in each flower, total seed number in each plant and seed biomass in each flower were measured randomly from three replicate plants of each treatment. Root morphology and growth were analyzed using the WinRhizo Root scanning system. Development of flower was observed from the third week of April and onwards.

2.5 Measurement of physiological parameters

Physiological parameters were measured during the day time with consistent sunlight by taking the intact leaves of saffron plant cultivated under greenhouse NFT system compared to soil-grown plants. The parameters such as photosynthetic rate (A), stomatal conductance (Gs), transpiration rate (E), and intercellular CO_2 (Ci) were recorded using an Infra-red Gas Analyzer- IRGA (Model: CIRAS-3). Data were taken from at least three replicate plants and were analyzed. The IRGA leaf chamber, fitted with pads, clamped a 1.74 cm^2 area for the study.

2.6 Statistical analysis

For statistical analysis and representation of data we used GraphPad Prism 8.0.1. All data were represented mean ± SEMs. Statistical analyses were conducted by using one way ANOVA followed by Tuckeys multiple range test (Tukey, 1949). The p value ≤ 0.05 was considered to be statistically significant.

3. Result

3.1 Evaluation of agronomic trait of sunflower plants

Three different nutrient formulations such as F-1, F-2 and F-3 were supplemented to plants in NFT System (Sahoo *et al.*, 2024) and soil growing plants, considered as control. Observations were made over a 45-day period, focusing on various vegetative growth parameters. After 15 days, plants supplemented with F-2 exhibited the most prominent vegetative growth, including increased leaf number and root length, followed by F-1, soil, and F-3 treatments (Fig 1). Bud initiation in Sunflower plants commenced at 30 days of observation (Fig 1), with continued development observed until 45 days. At the 45-day observation, Sunflower plants treated with F-2 under the NFT system demonstrated superior growth in several key agronomic traits, including shoot length (41.37 ± 0.67 cm), leaf number (8.00 ± 1.15), root length (24.67 ± 0.28 cm), root diameter (0.57 ± 0.02 mm), root volume (14.74 ± 0.16 cm^3), and root fresh biomass (466.70 ± 16.67 g), when compared to the control group (Fig 1; Table 1). Triplicate plants were taken for all parameter study. There is no statistical difference of shoot length was observed in between F-1 and F-2 supplemented plants (Table-1).

3.2 Optimization of Nutrient management

EC and pH play a vital role for plant growth and development. Supplementation of F-2 nutrient formulation during seed formation produced high number of seeds as compared to control and nutrient treatments (Fig 2a). Highest flower and seed yielding in each plant were observed in F-2 supplemented plants at pH 6.5 and EC 950 µs/cm (Fig 2b; 2d). Similarly, high EC level (1000 µs/cm) at pH 6.5 could decreased the flower and seed yielding in each plant were observed in F-3 supplemented plants (Fig 2b; 2d). 1.05-fold increased or decreased in nutrient EC level in F-2 supplemented plants had a negative impact in both agronomic and physiologic status of the plants.

3.3 Evaluation of flower and seed production of sunflower plants

The observation of 45 days of cultivation is represented in Fig-1 and the result prominently displayed higher number of flower production in F-2 treated hydroponic plants. The observation continued till 120 days where more flowers were generated and seeds were maturated. After 120 days of observation, the Sunflower plants supplemented with nutrient F-2 under NFT system showed significantly highest flower number per plant (9 ± 0.57), seed number in each flower (83 ±1.73), Total seed number in each plant (661.3 ± 46.97), seed biomass in each flower (0.47 ± 0.03) (Fig 2b; 2c; 2d; 2e). F-2 supplemented plants had a 1.28 fold and 1.52 fold increase in flower number and seed production per plant respectively as compared to control (soil) growing plants. But in case of F-1 and F-3 supplemented plants had 1.31 fold, 1.50 fold and 1.66 fold, 2.44 fold decrease in flower number and seed production per plant respectively as compared to control.

3.4 Evaluation of physiological status of sunflower plants

Physiology plays a crucial role in the lifecycle of the plants. The photosynthetic rate (A), transpiration rate (E),

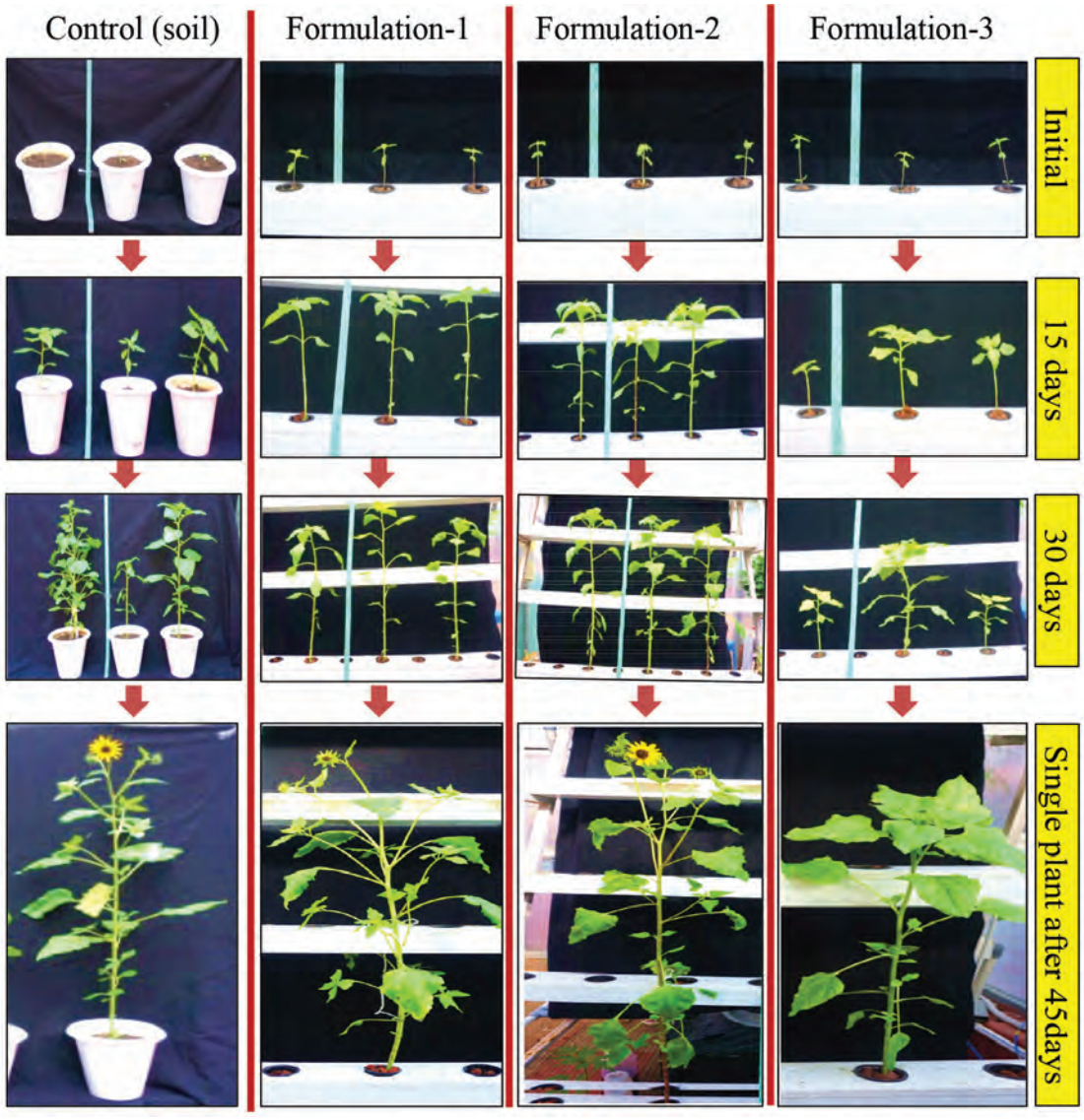


Figure 1: Figure showing the phenotypic analysis of hydroponically cultivated ornamental sunflower plants under NFT system supplemented with three different nutrient formulations (F-1, F-2 and F-3) along with control (Soil). Different developmental stages including initial seedling stage, early vegetative stage (15 days after plantation), late vegetative stage (30 days) and flowering and seed settings (45 days) were shown.

Table 1:

Changes of morphological parameter of different nutrient treated ornamental sunflower plants grown under NFT system

Agronomic traits	Control	F-1	F-2	F-3
Shoot length (cm)	32.83 ± 3.67^{a}	39.60 ± 0.30^{ab}	41.37 ± 0.67^{ab}	20.50 ± 2.84
Leaf Number	26.00 ± 3.46^{abc}	26.00 ± 1.15^{ab}	28.00 ± 1.15^{bc}	15.67 ± 3.18^{a}
Root length (cm)	18.97 ± 0.57^{a}	20.50 ± 0.28^{a}	24.67 ± 0.28	12.63 ± 0.85
Root diameter (mm)	0.39 ± 0.03^{a}	0.50 ± 0.04^{abc}	0.57 ± 0.02^{bc}	0.40 ± 0.03^{ab}
Root volume (cm^3)	10.35 ± 0.17^{ab}	12.35 ± 0.72^{c}	14.74 ± 0.16	9.31 ± 0.44^{a}
Root fresh Biomass (g)	350.00 ± 28.87^{a}	405.00 ± 2.88^{ab}	466.70 ± 16.67^{b}	269.30 ± 5.20

Agronomic traits	Control	F-1	F-2	F-3
Shoot length (cm)	32.83±3.67^{a}	39.60±0.30^{ab}	41.37±0.67^{ab}	20.50±2.84
Leaf Number	26.00±3.46^{abc}	26.00±1.15^{ab}	28.00±1.15^{bc}	15.67±3.18^{a}
Root length (cm)	18.97±0.57^{a}	20.50±0.28^{a}	24.67±0.28	12.63±0.85
Root diameter (mm)	0.39±0.03^{a}	0.50±0.04^{abc}	0.57±0.02^{bc}	0.40±0.03^{ab}
Root volume (cm^3)	10.35±0.17^{ab}	12.35±0.72^{c}	14.74±0.16	9.31±0.44^{a}
Root fresh Biomass (g)	350.00±28.87^{a}	405.00±2.88^{ab}	466.70±16.67^{b}	269.30±5.20
Flower number per plant	7±0.57^{abc}	5.33±0.33^{ab}	9±0.57^{c}	4.66±0.66^{a}
Seed number per flower	65.67±0.88^{c}	45.67±1.45^{b}	83±1.73^{d}	38.67±0.88^{a}
Total seed number per plant	432.7±8.19	260±8.50^{a}	661.3±46.97	1.76.7±8.81^{a}
Seed biomass per flower (gm)	0.29±0.01^{b}	0.22±0.01^{ab}	0.47±0.03	0.18±0.01^{a}

N.B: Means ± SEM followed by the same letter was not significantly different from each other according to the Tuckey's multiple range test at 5% probability level.

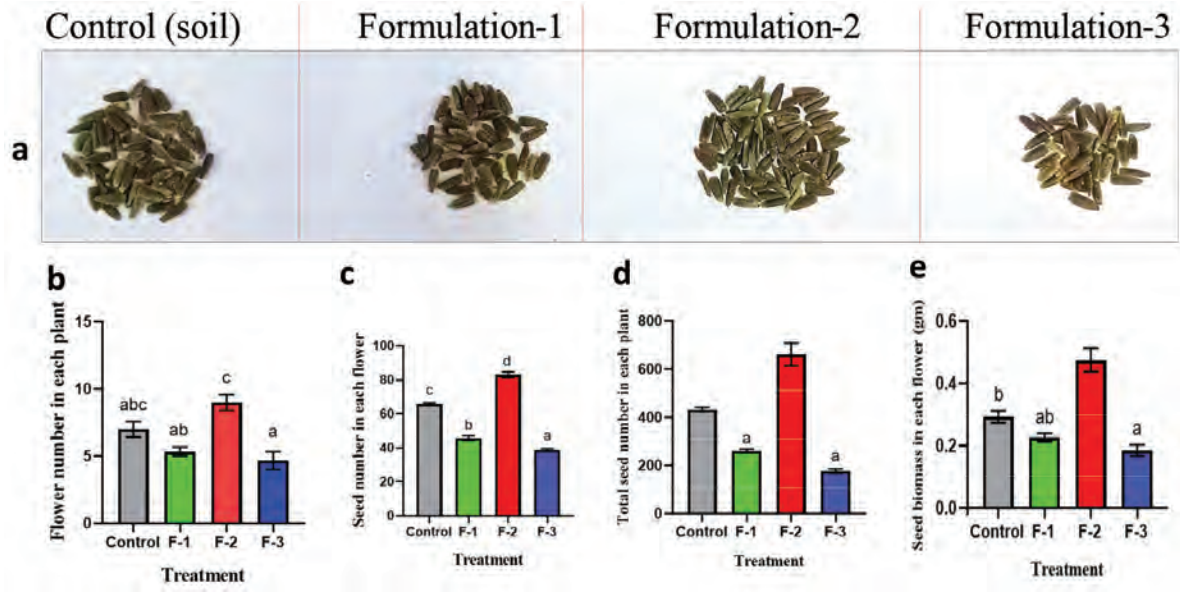


Figure 2: Figure depicting the floral and seed yield related parameters of Sunflower plant supplemented with three different nutrient formulations (F-1, F-2 and F-3) cultivated in NFT system after 120 days of plantation. a: Seed production in control and three different nutrient formulations in each plant. b, c, d, e: Graphs indicate the flower number per plant, seed number per flower, total seed number per plant, seed biomass per flower (in gram) respectively supplemented with three different nutrient formulations as compared with soil control.

stomatal conductance (Gs), and intercellular CO_2 concentrations (Ci) was found to play very essential role in terms of the growth and development of the plants. Plants supplemented with F-2 under NFT system had the highest photosynthetic rate (19.17± 0.60 µmol $CO_2m^{-2}s^{-1}$), transpiration rate (16.95 ± 0.30 mmol $H_2Om^{-2}s^{-1}$), and stomatal conductance (823.3 ± 14.53 mol$H_2Om^{-2}s^{-1}$) with lower intercellular CO_2 concentration (283.3 ± 4.41 µmolCO_2m^{-1}) as compared to control and other formulations (Fig-3). There was 1.36, 1.84-folds increase and 1.18-folds decrease in photosynthetic rate of F-1 F-2 and F-3 supplemented plants respectively as compared to control plants. The lowest intercellular carbon dioxide concentration was observed in F-2 supplemented plants followed by F-1 and control plant groups (Fig 3d).

4. Discussion

Ornamental Sunflower plants supplemented with F-2 nutrient formulation in the NFT hydroponic system expressed better agronomic traits and physiology as compared to control due to incorporation of continuous water flow mechanism through recycle and available of optimized nutrients. According to Chen *et al.*, (2021), optimized balanced nutrient application in plants, help the

plant productivity both qualitatively and quantitatively. The current results aligned with earlier reports that cultivation of different plants such as strawberry (Sahoo *et al.,* 2024), saffron (Nardi *et al.,* 2022), parsley (Razan *et al.,* 2023) under hydroponic condition with optimized nutrient formulation with controlled environmental conditions promote agro-physiological response, increased yield and productivity.

As the photosynthetic rate was increased in F-2 supplemented plants (Fig 3a), it efficiently utilized the intercellular carbon dioxide concentration (Ci) of plants. Thus, less Ci was observed in in F-2 supplemented plant as compared to the plants treated with other nutrient formulations (Fig 3d). In the present study, the significance of nutrient formulation and nutrient pH emerge as one of the most critical factors that influence growth, development, and healthy physiological status of sunflower plant under hydroponic condition which is consistent with the result of Kane *et al.,* (2006). The current result highlighted the optimal EC level for flower and seed productionin sunflower plant is 950 µs/cm. From seedling to flowering stage, the EC of F-2 nutrient formulation was increased at optimum level which was aligned with the result of Santos Junior *et al.,* (2013). More than 1500 µs/cm EC level of nutrient formulation give a negative effect for plant growth and productivity. It was already revealed that exposure to high level of nutrient consequence in decline in overall plant growth in strawberry plant (Sahoo *et al.,* 2024). The outcome of current study result revealed plants supplemented with nutrient formulation 2 (F-2) showed significantly higher agronomic parameters suggesting the better physiological status as compared the other plants treated with other nutrient formulations as well as from the soil grown control plants.

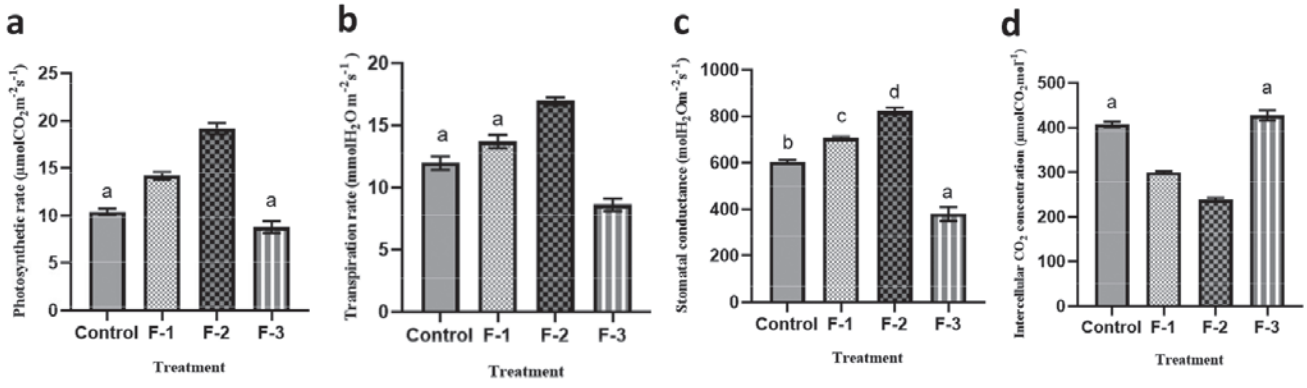


Figure 3: Graphical representation of the physiological parameters evaluated for Sunflower plants supplemented with three different nutrient formulations under NFT hydroponic system along with soil control. a-d: represents the analysis of Photosynthetic rate (A), Transpiration rate (E), Stomatal conductance (Gs) and Intercellular CO_2 concentration (Ci) under above treatments.

5. Conclusion

Sunflower plant growth, flower yield, seed production and physiology were assessed under hydroponic conditions with three nutrient formulations, compared to conventional soil-grown plants. Results from NFT systems indicate that supplement of optimized nutrient formulations 2 (EC 250, 650, 950 µS/cm) improve plant in seedling growth, vegetative growth, flower-seed production respectively. From this study, it was concluded that healthy physiological status play an important role for increasing flower and seed production supplemented with F-2 nutrient formulation. Application of the optimized concentration of nutrient formulation under greenhouse conditions will be helpful for commercial hydroponic sunflower farming and capitalizing on the nutritional and functional benefits of production irrespective of any climatic condition. Continued research and innovation in hydroponic systems, automated nutrient delivery system, sensor networks for real- time monitoring and other advanced technologies can lead to further improvements of sunflower as well as seed productivity.

Acknowledgement

This work was supported by the Department of Science and Technology, Govt. of Odisha (ST-SCST-MISC-0015-2019) and OHEPEE, Ravenshaw University, Cuttack.

Reference

Acharya, S. Kumar, K. Sharma, N. Tiwari, V.K. Chaurasia, O.P. (2021). Yield and quality attributes of lettuce and spinach grown in different hydroponic systems. JSWC. 20 (3): 342-349.

Adeleke, B.S. and Babalola, O.O. (2020). Oilseed crop sunflower (*Helianthus annuus*) as a source of food: Nutritional and health benefits. Food Sci. Nutr. 8 (9): 4666-4684.

Bohm, B.A. and Stuessy, T.F. (2013). Flavonoids of the sunflower family (Asteraceae). SSBM 1th Edn. ISBN-978-3-7091-7245-2. DOI 10.1007/978-3-7091-6181-4.

Chen, Y. Hu, S. Guo, Z. Cui, T. Zhang, L. Lu, C. Yu, Y. Luo, Z. Fu, H.Jin, Y. (2021). Effect of balanced nutrient fertilizer: A case study in Pinggu District, Beijing, China. Sci Total Environ. 754: 142069.

Devi, K. Sharma, M. Singh, M. Singh Ahuja, P. (2011). In vitro cormlet production and growth evaluation under greenhouse conditions in saffron (*Crocus sativus* L.)-a commercially important crop. Eng. Life Sci. 11:189-194.

Dinesh, BM. Ravichandra, NG. Reddy, BMR. Somasekhara, YM. (2014). Interactions between *Radopholussimilis* and *Fusarium oxysporum* f. sp. cubense causing wilt Complex on Banana. Int. J. Adv. Res. 2:976-985.

Guo, S. Ge, Y. & Na Jom, K. (2017). A review of phytochemistry, metabolite changes, and medicinal uses of the common sunflower seed and sprouts (*Helianthus annuus* L.). Chem. Cent. J. 11: 1-10.

Kamenetsky, R. and Okubo, H. (2012). Ornamental geophytes: from basic science to sustainable production. CRC press, Florida. USA.

Kanani, M. and Nazarideljou, M.J. (2017). Morpho-physiological reactions, photosynthetic capacity and quality of cut tuberose (*Polianthes tuberosa* L.) under different soil and soilless cultivation systems. Ejgcst. 7 (28): 61-72.

Kane, C.D. Jasoni, R.L. Peffley, E.P. Thompson, L.D. Green, C.J. Pare, P. & Tissue, D. (2006). Nutrient solution and solution pH influences on onion growth and mineral content. J. Plant Nutr. 29 (2): 375-390.

Kharrazi, M. Sharifi, A. Nejatizadeh, S. Khadem, A. Moradian, M. (2020). Selection of optimal cultivation media for gerbera (*Gerbera jamesonii*) growth in the hydroponic culture system. J. Hortic. Sci. 34 (2): 261-271.

Maboko, M.M. Du Plooy, C.P. and Chiloane, S. (2011). Effect of plant population, fruit and stem pruning on yield and quality of hydroponically grown tomato. Afr. J. Agric. Res. 6 (22): 5144-5148.

Macwan, J. Pandya, D. Pandya, H. & Mankad, A. (2020). Review on soilless method of cultivation: hydroponics. Int. J. Recent Sci. Res. 11: 37122-37127.

Menzel, C. González-Martínez, C. Chiralt, A. & Vilaplana, F. (2019). Antioxidant starch films containing sunflower hull extracts. Carbohydr. Polym. 214: 142-151.

Murumkar, AR. Umesha, B. Palanisamy, D. Bosu, SS. Durairaj, CD. (2012). Evaluation of Soil Less Media for Beet Root under Protected Cultivation. Environ. Ecol. 30 (2): 332-335.

Nardi, L. Metelli, G. Garegnani, M. (2022). Farming for pharming: Novel hydroponic process in contained environment for efficient pharma-grade production of saffron. Mol. 27 (24): 8972.

Ranjbar Sheykhani, R. (2024). Investigating the effect of changing the K: Ca ratio in nutrient solution on the quantitative and qualitative yield of Lilium spp. in hydroponic cultivation. J. Plant Nutr. 1-22.

Razan, K. Aziz, R. Al-Maari, K. Al-biski, F. (2023). Studying the growth and productivity of saffron under different agriculture techniques. Iraqi. J. Agric. Sci. 54 (1): 242-252.

Roosta, H.R. and Rezaei, I. (2014). Effect of nutrient solution pH on the vegetative and reproductive growth and physiological characteristics of rose cv. 'Grand Gala' in hydroponic system. J. Plant Nutr. 37 (13): 2179-2194.

Sahoo, S. Sahoo, D. Sahoo, KK. (2024). Optimization of an efficient hydroponic cultivation method for high yield of strawberry plant. S. Afr. J. Bot. 167:429-440.

Santos Júnior, J.A, Gheyi, H.R, G Filho, D.H, Soares, F.A. & Dias, N.D.S. (2013). Efficiency of water use in sunflower grown in hydroponic system under saline stress. Eng. Agric. 33: 718-729.

Shahidi, F. & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. J. Funct. Foods. 18: 820-897.

Tasneem Bashir, T.B. Zia-ur-Rehman Mashwani, Z.U.R.M. Kulsoom Zahara, K.Z. Shakeela Haider, S.H. Shaista Tabassum, S.T. and Mudrikah, M. (2015). Pharmacology and ethnomedicinal uses of *Helianthus annuus* (sunflower): a review. Chem. 226-235

Tukey, J.W. (1949). Comparing individual means in the analysis of variance. *Biometrics*, 99-114.

Vilvert, E. Lana, M. Zander, P. & Sieber, S. (2018). Multi-model approach for assessing the sunflower food value chain in Tanzania. Agric. Syst. 159: 103-110.

Wahome, P.K. Masarirambi, M.T. Shongwe, V.D. (2010). Evaluating different hydroponics systems for growth, flowering and quality of gladiolus (*Gladiolus grandiflorus*). Int J Agric Biol. 12 (5): 649-654.

Wien, H.C. (2014). Screening ornamental sunflowers in the seedling stage for flowering reaction to photoperiod. Hort. technology. 24 (5): 575-579.

Xu, CX. Ge, QX. (1990). A preliminary study on corm rot in *Crocus sativus* L. *Acta Agric*. Univ. Zhejangensis. 16:241-246

Yegorov, B. Turpurova, T. Sharabaeva, E. and Bondar, Y. (2019). Prospects of using by-products of sunflower oil production in the feed industry. FS&T. 13 (1): 345-678.

Plant Science Research 46 (1&2) : 72-74, 2024

Plant Science Research

ISSN 0972-8546

Sustainable management of anthracnose in *Vigna unguiculata* by *Colletotrichum lindemuthianum* (Sacc. & Magn.)

Manas Ranjan Satpathy[1Ψ] and Debaraj Parida[2]

[1] Department of Botany, Model Degree College, Nayagarh-752079
[2] PG Department of Botany, Dhenkanal (A) College, Dhenkanal-759001

ARTICLE INFO

Article history:
Received : 6 September 2024
Revised : 22 October 2024
Accepted : 2 November 2024

Keywords:

Anthracnose
DAS
RBD
Cowpea
Colletotrichum lindemuthianum

ABSTRACT

Experiments were conducted during kharif season to estimate the role of different botanicals and bio-control agents in Cowpea caused by *Colletotrichum lindemuthianum*. Treatments were done using randomized block design (RBD) and eight treatments were used which were replicated thrice including control treatment. In field experiments, *Trichoderma viride* (29.26) and *Pseudomonas fluorescens* (28.23) @ 2% at 30, 60, 90 DAS, achieved highest reduction of disease intensity. Karanj leaf extract @ 5% in vitro was found most effective inhibiting mycelial growth (99.1%) followed by Trichoderma viride (70.32%). All the treatments significantly reduced the Anthracnose disease under field conditions.

Introduction

The very name Cowpea was evidently coming from United States of America (Small, 2009). Since this crop was mostly used as a good source of fodder and mostly for Cows, the very name Cowpea came into existence (Timko *et al.*, 2007). Cowpea (*Vigna unguiculata*), a native of Central Africa, is an annual herb belonging to family Fabaceae which are known by the common names such as : black-eyed pea, southern pea, yard long bean, catjang and crowder pea. In India, this crop is grown in kharif season across the country for seeds, green pods, animal fodder and organic green manure. It is called as poor man's meat due to high nutritious constitutions with protein 23-24%, carbohydrate 60.3%, minerals, vitamins and it is also rich source of iron and calcium. The Anthracnose disease is a major problem in this crop where blackening of tissue is a characteristic feature affecting the entire plant including leaves, stem, fruit and seed. Apart from India, in all cowpea growing regions across the world, this disease happens to be one of the major fungal diseases which hampers its bumper productivity (Satpathy, 2021). The fungus overwinters in the previous crop debris, and can also be seed-borne (Modi and Tiwari, 2020). The management of plant diseases generally include strategies such as physical and cultural control, resistant cultivars, chemical and biological control. The integration of different management practices has the potential to provide an effective strategy for the control of Anthracnose of Cowpea. Some bio-control agents have also been demonstrated as effective disease control tools.

Materials and Methods

The experiments were carried out during rabi season during 2019-2021 at the farmers field of different locations of Bhubaneswar, Odisha. Before the sowing, the field was made contamination free and thoroughly ploughed making soil particles uniform and weed free. The cultivated land was further divided into 24 sub plots and arrangement of proper irrigation were made. The experiments were conducted in randomized block design (RBD) with seven different treatments and one control. The seeds were sown by line

Ψ *Corresponding author; Email:* manasnitusatpathy@gmail.com

sowing method with a row-to-row space of 30cm and 15 cm from plant-to-plant distance. Healthy seed samples of Cowpea variety Utkal Manika were used for sowing at the rate of 25 kg/ha. Disease intensity and plant height were observed at 30, 60 and 90 DAS. After harvesting yield were also recorded. Different leaf and bulb samples were collected from the nearby area to conduct *in vitro* experiments. All *in-vitro* experiments were conducted by using PDA medium.

Results and Discussion:

The data of 30 DAS shows that the control (40.62) has the highest disease intensity. Minimum disease intensity was recorded in *Trichoderma viride*@ 2% (14.99), followed by *Pseudomonas fluorecsens*@ 2% (16.66), Karanja leaf extract@ 5% (17.26), Neem leaf extract @ 5% (20.48), garlic bulb extract @ 5% (26.01), Citrus leaf extract @ 5% (27.13) and *Calotropis* leaf extract @ 5% (31.17). *Trichoderma viride* shows the best result when it was checked with 30 DAS. The statistical analysis of data showed that all treatments were found significantly effective and significant over control.

These results were also observed under *in vitro* conditions. As it has been clearly revealed from Table 2, that only one botanical exhibits the inhibition of *Colletotrichum lindemuthianum* almost absolutely which was Karanja leaf extract with a radial growth of 03.27 mm with 99.01 percent inhibition followed by *Trichoderma viride* with 70.32 per cent inhibition, *Calotropis* leaf extract 64.22 percent, Neem leaf extract with 63.12 per cent inhibition, *Citrus* leaf extract with 60.13 per cent, *Pseudomonas fluorescens* 61.23 per cent and Garlic bulb extract with least inhibition percent i.e. 57.27 per cent. The statistical analysis of data showed that all treatments were found significantly effective and significant over control.

Disease intensity after 60 DAS had a varied performance with *Trichoderma viride* and *Pseudomonas fluorescens*. The effect of Karanj leaf extract was effective but 28.32% in 60 DAS and 32.11% in 90 DAS, respectively.

Table 1

Effect of disease intensity at different days after sowing in Cowpea.

TRIALS	TREATMENTS	DISEASE INTENSITY (%)		
		30 DAS	60 DAS	90 DAS
T_0	Control	40.62	51.23	64.33
T_1	Neem Leaf extract	20.48	26.33	34.87
T_2	Citrus leaf extract	27.13	33.99	41.19
T_3	Garlic bulb extract	26.01	29.29	40.75
T_4	*Calotropis* leaf extract	31.17	37.30	47.91
T_5	Karanj leaf extract	17.26	28.32	32.11
T_6	*Trichoderma viride*	14.99	23.12	29.26
T_7	*Pseudomonas fluorescens*	16.66	27.82	28.23
F Test		S	S	S
S. Ed. (±)		0.36	1.47	2.36
CD (0.05%)		1.51	3.27	3.92

Table 2

In vitro evaluations of botanicals and bio-agents against *Colletotrichum lindemuthianum*

Trials	Treatments	Radial growth (mm)	% inhibition
T_0	Control	86.7	0.00
T_1	Neem Leaf extract	34.60	63.12
T_2	Citrus leaf extract	35.18	60.13
T_3	Garlic bulb extract	37.31	57.27
T_4	*Calotropis* leaf extract	35.52	64.22

T_5	Karanj leaf extract	03.27	99.01
T_6	*Trichoderma viride*	28.16	70.32
T_7	*Pseudomonas fluorescens*	43.21	61.23
	F Test	S	-
	Sem	0.14	-
	CD (0.05%)	0.91	-

The botanicals and biocontrol agents were utilized to check the growth of the pathogen and percentage of disease incidence. Earlier reports suggested regarding the effectiveness of *Trichoderma viride*, *Pseudomonas fluorescens and Karanj* leaf extract. Studies conducted by Sileshi *et al*., 2014 on common bean observed that *T. viride*, *P. fluorescens* were effective in controlling the incidence of anthracnose. *T. viride* could be able to reduce the incidence by 38.5 against the control of 74.5% in 39 DAS where as in 95 DAS it was 97.4 and 100%, respectively. Very similar results in reducing incidence were also obtained by employing *P. fluorescens*. The results of this work is corroborated with the results of Modi and Tiwari (2020) where both *T. viride*, *P. fluorescens* were found effective in controlling the incidence after 30 DAS and 90 DAS. The efficacy of leaf extract of Karanj was demonstrated by Satpathy and Beura (2021) where it could control the anthracnose by 60.8% and the mean value was 8.1% against the control of 34.5%.

Conclusion

From the experimental results it could be concluded that for the purpose of sustainable control of anthracnose and to increase the productivity of cowpea the farmers can be recommended to use *T.viride*, *P. fluorescens* and the leaf extracts of Karanj as evident from the above experimental analysis.

References

Modi, M. and Tiwari, S. (2020). Eco-friendly management of anthracnose disease of Cowpea (*Vigna unguiculata*) Sacc. & Magn. Int. J. Curr. Mirobiol. Appl. Sc., 9(2): 2720-2725.

Satpathy, M.R. (2021). Chemical control of cowpea anthracnose caused by *Colletotrichum lindemuthianum*. Int.J.Adv.Chem.Res.,3(1):35-37.

Satpathy, M.R. and Beura, S.K. (2021). Evaluation of plant extracts for the management of cowpea anthracnose disease caused by *Colletotrichum lindemuthianum*. J. Curr. Opi. Crop Sc.,2(3):379-383.

Sileshi, F., Mohammed, A., Selvaraj, T. and Negeri, M. (2014). Field management of anthracnose (*Colletotrichum lindemuthianum*) in common bean through foliar spray fungicides and seed treatment bioagents. Sc. Tech. Arts Res. J., 3(2):19-25.

Small, E. (2009).Top 100 food plants. NRC Research Press. p.104.

Timko, M. P., Ehlers, J. D., Roberts, P. A. (2007). "Cowpea". In: Kole, C.Pulses, Sugar and Tuber Crops, Genome Mapping and Molecular Breeding in Plants (PDF). 3. Berlin, Heidelberg: Springer-Verlag. pp. 49–67.

Plant Science Research

ISSN 0972-8546

Rediscovery and extended distribution of *Impatiens goughii* Wight. (Balsaminaceae) in Karnataka

Shreyas Betageri[1], K. Kotresha[1] and Prashant Karadakatti[2Ψ]

[1] Taxonomy and Floristic Laboratory, Department of UG, PG and Research in Botany, Karnatak University's, Karnataka Science College, Dharwad-580001, India.

[2] Department of Studies in Botany, Davangere University, Davangere

ARTICLE INFO

Article history:
Received : 25 August 2024
Revised : 27 October 2024
Accepted : 17 October 2024

Keywords:

Diversity
Impatiens
Western Ghats

ABSTRACT

Impatiens goughii Wight (Balsaminaceae), an endemic species of southern Western Ghats of India and reported from Biligirirangan Hills of Chamarajanagar District, Karnataka by Edward Barnes in the year 1944, is rediscovered from Baba Budangiri and Mullayanagiri hill ranges of Chikkamagaluru district of Karnataka after more than 80 years. The nomenclature, short botanical description, phenology, notes of ecology and conservation status of the species are provided in this paper.

Introduction

The genus *Impatiens* Riv. ex L. (Balsaminaceae) is comprised of about 1120 accepted species across the Old World, North & Central America (POWO, 2024). There are about 242 species of Impatiens, of which 152 species are endemic to the country. The highest species diversity of the genus can be found in the Eastern Himalayas and the Western Ghats (Singh, 2016; Singh & Garg, 2016; Arigela, 2019; Singh *et al.,* 2021). As many as 116 endemic species of Impatiensare known from the Western Ghats alone (Bhaskar, 2012; Arigela *et al.,* 2023).

In Western Ghats, the floristic studies and inventories of the genus *Impatiens* were early reported in floras by Gamble (1915), Fyson (1932) and Matthew (1999). Recently, Bhaskar (2012) provided a comprehensive account of 106 species from Western Ghats, and Dessai and Janarthanam (2011) reported 26 species and 2 varieties from thenorthern and central Western Ghats. Nayar *et al.* (2014) reported the occurrence of 107 species of *Impatiens* from Western Ghat region of India. The taxonomy, distribution and ecology of the genus *Impatiens* in Karnataka state has been studied by a number of workers in the past (Saldanha & Nicolson, 1976; Rao & Razi, 1981; Sharma *et al.,* 1984; Yognarshiman *et al.,* 1990; Bhat, 2014). *Impatiens goughii*, described by R. Wight (1839) based on his collection from Nilgiri (Neelgherry) hills of Tamil Nadu, is endemic to southern Western Ghats covering the states of Karnataka, Kerala and Tamil Nadu). Barnes (1944) recorded *Impatiens goughii* Wight from the eastern ridges of Katari Betta, Biligirirangan Hills of present Chamarajanagar District, Karnataka at an altitude of 5,000 ft. along streams.

Impatiens goughii Wight, now collected from Baba Budangiri and Mullayanagiri hill ranges of Chikkamagaluru district of Karnataka (Fig. 1), is reported here as a new locality from Karnataka state further extending the range of distribution of the species. The nomenclature, botanical description, phenology, notes of ecology and conservation status of the species are described in this paper (Fig. 2).

Ψ *Corresponding author; Email:* prashant.s.k2012@gmail.com

Materials and Method

Study area: Field surveys were conducted in Baba Budangiri and Mullayanagiri hill ranges of Chikkamagaluru district of Karnataka (13.4508N lat, 75.7601E long.), which lie at an altitude of about 1550 m. The region enjoys a cold climate with moderate sunlight. This is one of Karnataka's richest coffee and tea harvesting centres and an area with rich plant diversity area under Western Ghats (Fig 1).

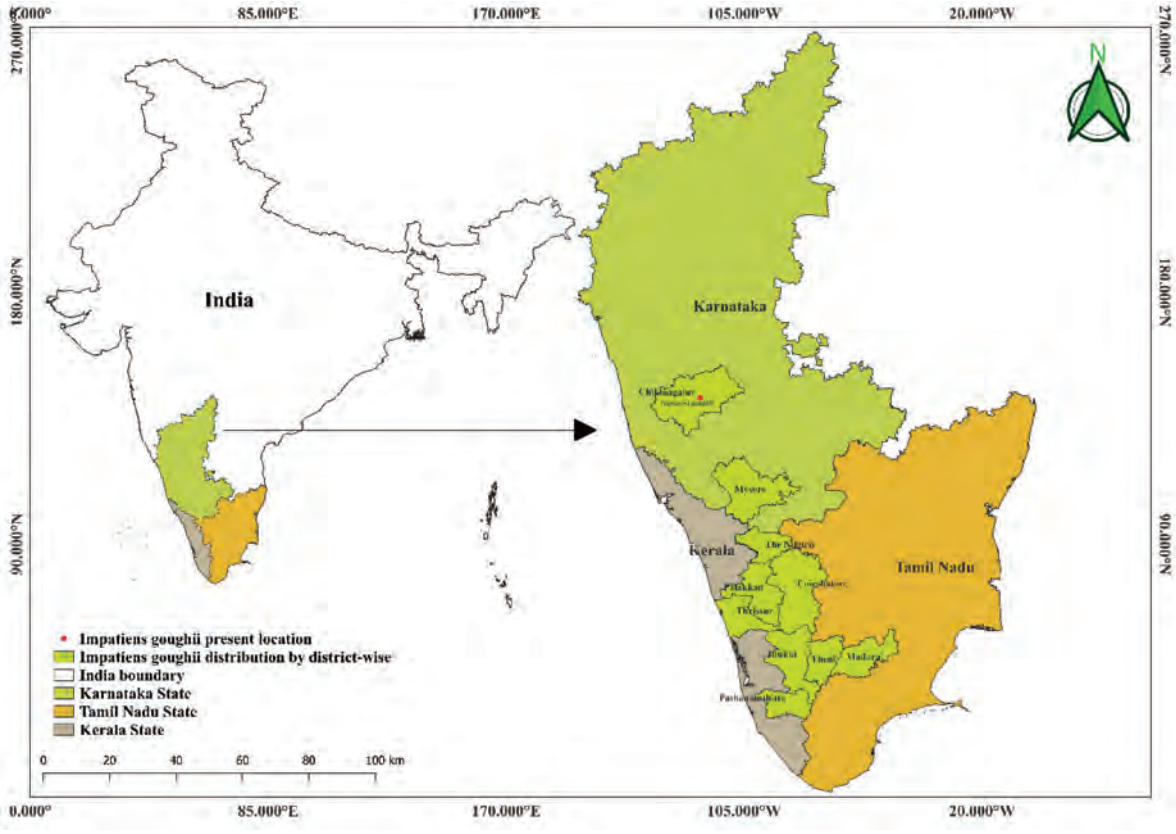


Figure 1. *Impatiens goughii* Wight Distributional Map

Taxonomic treatment:

Impatiens goughii Wight, Ill. Ind. Bot. 1: 160. 1839; Hookf., Fl. Brit. India 1: 452. 1874; Gamble, Fl. Madras: 144. 1915; Vivek. *et al.* in Hajra *et al.*, Fl. India 4: 152. 1997; Bhaskar, Taxon. Monogr. *Impatiens* W. Ghats: 241. 2012. *I. pulniensis* Bedd., in Madras J. Lit. Sci. 19: 176. 1858. *I. anamallayensis* Bedd., in Madras J. Lit. Sci. 20: 68, f. 8. 1859. *I. parvifolia* Bedd., in Madras J. Lit. Sci. 20: 66, f. 1. 1859. *I. omissa* Hook. f., Rec. Bot. Surv. India 4: 43 & 48. 1906. *I. microtheca* Hook. f., Hooker's Icon. Pl. 30: t. 2910. 1910.

Terrestrial herbs, up to 15 cm tall. Stem terete, pink, reddish tinged at nodes. Leaves opposite and decussate, ovate to acute apex, margin serrate with margin tinged red, 3 x 1 cm. Inflorescence long-pedicelled, upto 5-7 cm long, terminal sub-umbels with many flowers, slightly odorous. Flower pink, 1 cm long. Bracts persistent, minute, lanceolate. Petals and sepals 1-nerved; dorsal petals hood-like, with a single thorn at the top. Lower petal bifid with two pink patches. Petal has four teeth like calli. Spur shorter than wings, straight. Capsules ellipsoid, 5-8 mm long; seeds 6-12, almond-shaped, maroon colour (Fig 2).

Flowering & Fruiting: September-October.

Habitat & Ecology: Grows on dripping slopes and permanently wet and damp rocks, associated with Impatiens bababudangiriensis, *Linum* sp., *Utricularia stritula* and *Funaria* sp. The species prefers cold environments in areas located at altitude of 1700 MSL approximately.

Species Examined: India, Karnataka, Chikkamagalur, Bababudangiri hills, Galikere road,13.4508N lat, 75.7601E long., Shreyas B. & K. Kotresha, 0350, 23-08-2024, HKSCD (Herbarium of Karnatak Science College, Dharwad).

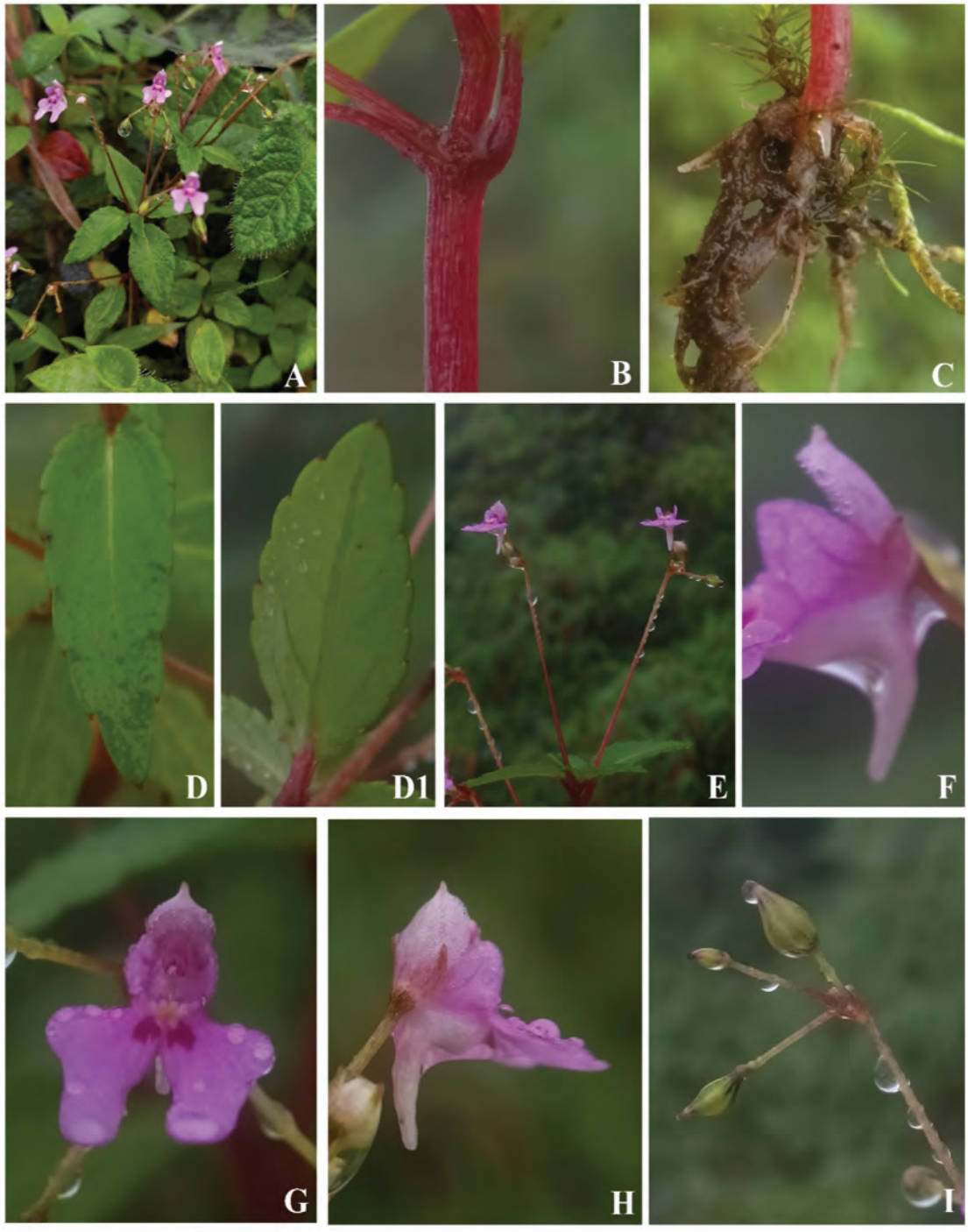


Figure 2. *Impatiens goughii*A. Habit; B. Stem; C. Roots; D. Dorsal leaf; D1. Ventral leaf; E. Inflorescence; F. Spur; G. Front view of flower; H. Side view of flower; I. Fruits.

Conservation Status: The species show a very limited distribution in the state of Karnataka and is considered vulnerable due to habitat loss by invading exotic species and other vigorously growing plant species as competitors. The size of the plant and petal colours vary considerably within and among the populations. The treat status of this interesting taxon needs assessment and accordingly conservation measures to be initiated.

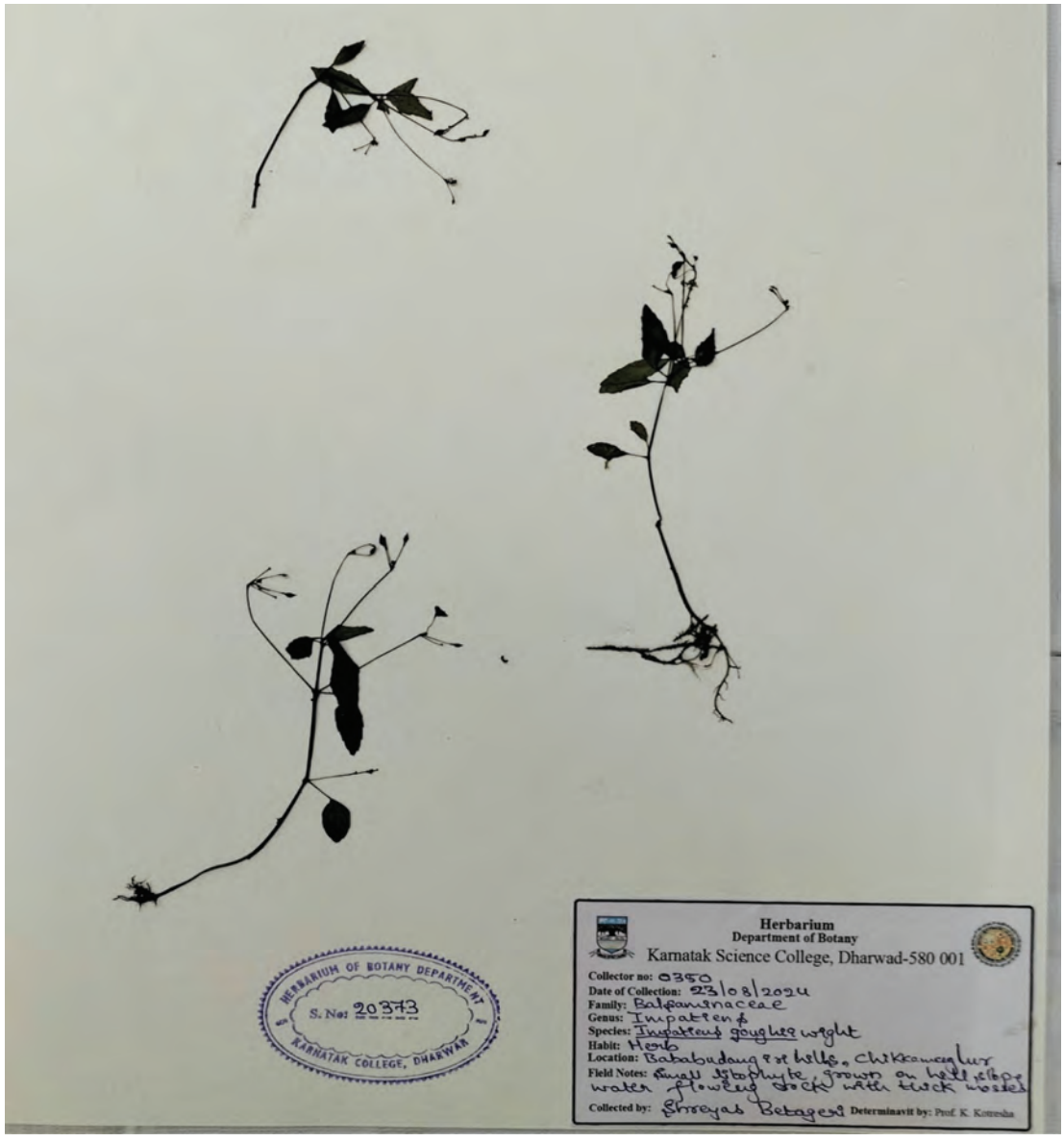


Figure 3: *Impatiens goughii* herbarium specimen

References

Arigela, R.K., Singh, R.K., Kabeer, K.A.A. (2019). *Impatiens tanyae* (Balsaminaceae), a new species from Western Ghats, India. *Kew Bulletin* 74(3): 48.

Arigela, R. K., Singh, R. K. and Rajasekar, C. (2023). Taxonomy and typification of Indian endemic *Impatiens goughii* (Balsaminaceae). *Annales Botanici Fennici* 60(1): 43-59.

Barnes, E. (1944). Notes on flowering plants of Biligirirangan hills. *J. Bombay Nat. Hist. Soc.* 44 (3): 436-459.

Bhaskar, V. (2012). Taxonomic monograph on *Impatiens* L. (Balsaminaceae) of Western Ghats, South India. Centre for Plant Taxonomic Studies, Bangalore.

Bhat, K. G. (2014). *Flora of South Kanara: Dakshina Kannada and Udupi Districts of Karnataka*. Udupi: Taxonomy Research Centre. Chitpady, Udupi, pp. 609-612.

Dessai, J.R.N. and Janarthanam, M.K. (2011). The genus *Impatiens* (Balsaminaceae) in the northern and parts of central Western Ghats. Rheedea 21: 23–80.

Fyson, P.F. (1932). *The Flora of South India Hill Stations*. Vol. 1–2. Govt. Press, Madras.

Gamble, J.S. (1915). *Flora of the Presidency of Madras*. Vol.1, Adlard & Sons, London.

Matthew, K.M. (1999). *Flora of Palni Hills, South India*. Vol. 1. The Rapinat Herbarium, Tiruchirapalli. Nayar, T. S, Rasiya Beegam, A. & Sibi, M. (2014). *Flowering*

Plants of Western Ghats, India. Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Kerala :195-208.

POWO(2024). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the internet; https://powo.science.kew.org//. (accessed: 28 August 2024).

Rao, R. & Razi, D. (1981). A synoptic flora of Mysore district, with an appendix of Unani, Ayurvedic and Trade Names of Drugs. Today & Tomorrow's Printers and Publishers, New Delhi. 485.

Saldanha, C. J. & Nicolson, D. H. (1976). *Flora of Hassan District Karnataka*. Amerind Publishing Co. Pvt. Ltd., New Delhi : 401-404.

Singh, R.K. (2016). Notes on author attribution and typification of two names of *Impatiens* (Balsaminaceae). *J. Jap. Bot.*91(4): 237–241.

Singh, R.K. and Garg, A. (2016). Call for conservation of the critically endangered saffron balsam of Karnataka–*Impatiens raziana* Bhaskar & Razi (Balsaminaceae). *Indian Forester* 142: 803–805.

Sharma, B. D., Singh, N. P., Raghavan, R. S. &Deshapande, U. R. (1984). *Flora of Karnataka: Analysis*. Botanical Survey of India. *Calcutta* : 36-39.

Wight R. (1839). *Illustrations of Indian Botany*, Vol. 1. J.B. Pharoah, Madras.

Yoganarasimhan, S. N., Subramanyam, K. & Razi, B. A. (1981). *Flora of Chikmagalur District*. Karnataka, India. International Book Distributors, Dehra Dun.

Plant Science Research 46 (1&2) : 80-85, 2024

Plant Science Research

ISSN 0972-8546

A comprehensive study of diversity and distribution of subaerial Chroococcales from Similipal Biosphere Reserve, Odisha

S. Behera[1], S. Bhakta[2Ψ], E. Sahu[3] and A.K. Bastia[4]

[1] Depatment of Botany, Maharaja Sri Ram Chandra Bhanj Deo University, Baripada-757003
[2] Botanical Survey of India, Andaman and Nicobar Regional Centre, Port Blair - 744102
[3] Department of Botany, S. R. College, Baliapal, Balasore- 756023
[4] Department of Applied Microbiology, Fakir Mohan University, Balasore-756020

ARTICLE INFO

Article history:
Received : 10 September 2024
Revised : 15 October 2024
Accepted : 7 November 2024

Keywords:

Cyanobacteria
Chroococcales
New record
Similipal Biosphere Reserve
Odisha

ABSTRACT

This study investigates the diversity and distribution of Chroococcales belonging to cyanobacteria were studied within the Similipal Biosphere Reserve, Odisha, which has rich biodiversity and unique ecosystems. The specimens were collected through regular sampling across various habitats, including freshwater streams, subaerial corticolousregions, and moist soils, identified using morphological techniques. A total of 13 species belonging to the order chroococcales were documented under four families and seven genera, with the dominant occurrence of *Gloeocapsa* (5), followed by *Aphanocapsa* (2), *Chrococcidiopsis* (2) and single species each from *Syenechococcus*, *Synechocystis*, *Cyanosarcina* and *Pseudocapsa*. The distribution of the taxa signified five new records to Odisha namely *Synechococcus pevelekii*, *Aphanocapsa grevillei*, *Gloeocapsa violacea*, *Gloeocapsa alpine* and *Pseudocapsa dubia*, which may serve as a baseline for future research and conservation strategies to preserve the unique cyanobacteria of the Biosphere Reserve.

Introduction

Cyanobacteria could grow in any environment where little moisture and sunlight are available. They can also survive the most adverse conditions of deserts (Friedmann and Ocampo-Friedmann, 1984), hot springs (Sompong *et al.*, 2005, Bhakta *et al.*, 2016), and hypersaline ecosystems (Chorus and Bartram, 1999). Chroococcales are characterised by single-celled or colonial forms classified under Cyanoprokaryota. Similipal (21°28”-22°08”N, 86°04”-86°37”E) is the only biosphere reserve of Odisha, situated in the northeastern zones of the state, gets good precipitation from dews, frost, mist etc. Over and above all it receives more rain & thus itharbours, a significant diversity of cyanobacteria occurring in various forms, such as lithic (epilithic and hypolithic), corticolous, benthic, epixylic, epipelic and floating. Though the study of subaerial cyanobacteria is meagre, it’s baseline diversity has been reported from different parts of India for their habitats (Bhuyan *et al.,* 2007, Saha *et al.,* 2007; León–Tejera *et al.,* 2011; Jeyachitra *et al.,* 2013 and Bhuyan *et al.,* 2023). Studies on the taxonomy and diversity of subaerial cyanobacteria are incomplete and limited due to the lack of *exploration*, seasonal collection and where light and air humidity are considered the most influential ecological factors on the growth and development. However, there are also a few reports on these aspects (Tirkey and Adhikary, 2005; Uher, 2007; Neustupa & Skaloud, 2010; Dey and Bastia, 2009, 2010; Farrukh, 2011; Sethi *et al.,* 2012; Halder, 2018, Bhakta *et al.,* 2014 and 2015). Subaerial algae in the form of soil crust received ample attention for their ecological significance in recent years (Rehakova, 2011; Dev *et al.,* 2013; Saminathan, 2013; Kumar *et al.,* 2016, 2019) Similipal Biosphere Reserve is an untapped area for the study of subaerial algae, and the present work is considered as a valuable information of cyanobacterial diversity from this unique ecosystem.

Ψ *Corresponding author; Email:* skmrbhakta@gmail.com

Materials and Methods

Samples in different forms e.g. lithic, soil crust, corticolous, and epixylicwere collected using forceps, anda scalpel. All the samples were collected in sterile specimen tubes (Tarson) of 25 X 50 mm size and brought to the laboratory for further analysis. Each sample was deposited in the Department of Botany, Maharaja Sri Ram Chandra Bhanj Deo University, Baripada, Odisha, in a dark place assigned with a voucher number. The crust and corticolous samples, which were otherwise difficult to identify with natural samples, were first soaked with distilled water in a Petri plate and incubated under white fluorescence light. The growth was observed under a microscope at 24-hour intervals. After a visible growth, each filament or colony or consortia was taken for microscopic photography. Micrographs were taken using Hund Wetzlar Trinocular Research Microscope with Canon-EOS 550D camera attachment. Micrometry was done using ocular and stage micrometers (Erma, Japan) to determine the cell dimensions. The algal species were identified using the monographs Desikachary, 1959 and Komárek & Anagnostidis, 1999.

Results

Systematic enumeration

13 chroococcales cyanobacterial taxa belonging to 34 genera were enumerated from different sampling sites of Similipal Biosphere Reserve (Plate-1). Systematic enumerations of these taxa are given below.

Division - Cyanobacteria/ Cyanoprokaryota

Class - Cyanophyceae

Order - Chroococcales

Family –Synechococcaceae Komárek et Anagnostidis 1995

Genus –*Synechococcus* Nägeli 1849

1. *Synechococcus elongatus* (Nägeli) Nägeli 1849, Komárek and Anagnostidis 1999, P. 123, Fig. 137 (Pl. 1, Fig. 14)

Cells solitary or grouped in irregular clusters, colonies without any envelope, cells long cylindrical with rounded ends, sometimes unusual longer than wide, straight, rarely arcuate or sigmoid, 8-10mm long and 1.5-3mm wide, cell content homogenous, without any granules or aerotopes.

Place of collection - Badamakabadi, Similipal, Occurrence - Bluish green crust in soil, Voucher number– 07.

Distribution – Cosmopolitans

Family: Merismopediaceae

Genus: *Synechocystis* Sauvageau 1892

2. *Synechocystis pevalekii* Ercegoviæ 1925, Act. Bot. Inst. Bot. Univ. Zab. 10: 64-114, P. 77, pl. I: fig. 8; Komárek and Anagnostidis 1999, P. 143, Fig. 163 (Pl. 1, Fig. 13)

Thallus unicellular or colonial, bluish-green in colour, mucilaginous on adverse conditions, mucilage brownish to pale yellow; cells spherical to hemispherical, cell content homogenous; 4.3 µm long, 4.9 µm broad.

Place of collection - Badamakabadi, Similipal, Occurrence - Bluish green crust in soil, Voucher number– 07.

Distribution – First record from Odisha

Genus: *Aphanocapsa* Nägeli 1849

3. *Aphanocapsa grevillei* (Berkeley) Rabenhorst 1865, P. 50; [Basionym: *Palmella grevillei* Berkeley 1832: 16, pl. 5: fig. 1

synonym: *Palmella grevillei* Berkeley, 1832; *Coccochloris grevillei* (Berkeley) Hassall 1845; *Anacystisgrevillei* (Berkeley) Kützing 1849; *Microcystis grevillea* (Berk) Elenkin 1938; *Gloeocystis grevillei* (Berkeley) Drouet & Dailey 1948]; Komárek and Anagnostidis 1999, P. 159, Fig. 194 (Pl.1, Fig. 12)

Thallus bluish green; colonial, colony irregular, clavate shaped, mucilaginous, mucilage slightly lamillated; cells rounded, 5.4µm in diameter.

Place of collection - Barehipani, Similipal, Occurrence - Blackish patch on tree bark surface, Voucher number – 48.

Distribution – First record from Odisha**.**

4. *Aphanocapsa testacea* (Braun ex Kützing) Nägeli 1849, P. 52; [Basionym: *Palmella testacea* A. Braun ex Kützing 1849, P. 211; synonym: *Palmella testacea* Braun ex Kützing 1849; *Microcystis testacea* (Nägeli) Elenkin 1938]; Komárek and Anagnostidis 1999, P. 423, Fig. 553 (Pl.1, Fig. 11)

Colony oval to irregular, mucilaginous, mucilage hyaline, cells arranged loosely, sheath not lamellated, cells bluish green in colour, rounded to oval, 5.3 µm in diameter; cell content vacuolated or homogenous.

Place of collection - Barehipani, Similipal, Occurrence - Blackish patch on tree bark surface, Voucher number - 46.

Family: Microcystaceae

Genus: *Gloeocapsa* Kützing 1843

5. *Gloeocapsa gigas* West et West 1894, J. Lin. Soc. Lon. Bot. 30, P. 276, pl. XIV: figs. 11-13; Komárek and Anagnostidis 1999, P. 259, fig. 340 (Pl.1; Fig.1)

Thallus bluish green, colony macroscopic, gelatinous,

mucilage hyaline; 40-65 µm wide, subcolony indistinct or rare, cells oval or rounded, fine granulated cell content, cells 5-6 µm broad.

Place of Collection – Kairakacha reservoir, Chahala, Similipal, Occurrence - Blue green patch on moist rock, Voucher number -236.

6. *Gloeocapsa gelatinosa* (Meneghini) Kützing 1843, In Phycol. Gen., 2, P. 174; Komárek and Anagnostidis 1999, P. 239, fig. 308 (Pl.1; Fig. 2)

Thallus bluish green; colony microscopic to macroscopic, colony irregular with distinct numerous subcolonies, subcolonies 5.5-7 µm wide, mucilage thick, delimited, colorless or yellowish, hyaline in young colonies or isolated colonies; cells oval, bluish green, finely granulated 1-1.5 µm in diameter.

Place of Collection - Kairakacha reservoir, Chahala, Similipal, Occurrence – epilithic biofilm on the rock surface, Voucher number - 53.

7. *Gloeocapsa violacea* Kützing 1847, In Tab. Phycolog., 1, fasc. 3-5, P. 25, pl.36: fig. IX; [Synonym: *Gloeocapsa lignicola* Rabenhorst, 1865: 41, nom. Illeg.]; Komárek and Anagnostidis, 1999, P. 248, Fig. 320 (Pl.1, Fig. 3)

Thallus blackish, colonial, colony rounded to ellipsoidal, colony contain 8-16 no. of cells, cells mucilaginous, mucilage thick, homogenous, brownish color, cells spherical, 4.5 µm in diameter, cell content homogenous, vacuolated, colony 13,8 µm broad, 17.6 µm long.

Place of collection - Barehipani, Similipal, Occurrence - Blackish patch on tree bark surface, Voucher number – 72.

Distribution – First record from Odisha

8. *Gloeocapsa alpine* Nägeli in Rabenhorst 1865, P. 40; [Heterotypic Synonym: *Gloeocapsa ambigua* Nägel in Kützing, 1849, sp. algarum, pp (i)-vi, 220; Algen. In: Kryptogamen-Flora von Schlesien, 1. Vol. 2, pp. i-iv [vii] 1-284. Breslau: J.U. Kern's Verlag, 1878]; Komárek and Anagnostidis, 1999, P. 248, Fig. 323 (Pl.1, Fig. 4)

Colony rounded to oval, mucilaginous, mucilage thick, lamellated, 7.3µm wide, cells rounded, arranged compactly within the colony, 5.5µm in diameter, cell content granulated.

Place of collection - Chahala, Similipal, Occurrence - Blackish patch on tree bark surface, Voucher number – 64.

Distribution – First record from Odisha.

9. *Gloeocapsa novacekii* Komárek et Anagnostidis 1995, Preslia, Praha 67, P.19; Komárek and Anagnostidis, 1999, P. 252, Fig.328 (Pl.1, Fig. 5)

Thallus colonial, mucilaginous, blackish patch on soil surface, brown to pale bluish green in color after soaking in water; Mucilage lamellated, just copying the cell surface, sheath on each cell as well as on colony, distinct; colony spherical to irregular in shape; cells hemispherical, granulated, 3.54µm long, 5.31µm broad.

Place of collection - Chahala, Similipal, Occurrence - Blackish crust in rocky soil, Voucher number – 64.

Family: Chroococcaceae

Genus: *Cyanosarcina* Kováèik 1988

10. *Cyanosarcina spectabilis* (Geitler) Kovácik1988, Arch. Hydrbiol. Suppl. 50-53, P. 176; [Basionym: *Myxosarcina spectabilis* Geitler, 1933, Arch. Hydrobiol. Suppl. 12: 622-634]; Komárek and Anagnostidis 1999, P. 427 (Pl.1, Fig. 7)

Thallus blackish; colonial, colony rounded to irregular, mucilaginous, mucilage plane, not lamellated; cells, arranged compactly, cells spherical, 4.3µm in diameter; bluish green in colour, cell content homogenous, vacuolated.

Place of collection - Barehipani, Similipal, Occurrence - Blackish patch on tree bark surface, Voucher number - 38.

Genus: *Pseudocapsa* Ercegoviæ 1925

11. *Pseudocapsadubia* Ercegoviæ 1925, Act. Bot. Inst. Bot. Univ. Zab. 10: 64-114, P. 95, pl. I: fig. 4; [Homotypic synonym: *Myxosarcina dubia* (Ercegoviæ) Bourrelly, 1970: 333; expl. pl. 86]; Komárek and Anagnostidis, 1999, P. 318, Fig. 426 (Pl. 1, Fig. 6)

Thallus brownish; colonial, colony rounded to irregular cells, irregular in out line, brownish in colour; 4.7 µm long, 2.82 µm to 3.76 µm broad.

Place of collection - Chahala, Similipal, Occurrence - Blackish crust in rocky soil, Voucher number - 51.

Distribution – First record from Odisha.

Family: Xenococcaceae

Genus: *Chroococcidiopsis* Geitler 1933

12. *Chroococcidiopsis indica* Desikachary 1959, Cyanophyta, pp. [1]-686, P. 167, Pl. 31, Fig. 29 (Pl. 1, Fig. 8)

Colony mucilaginous, mucilage hyaline, cells arranged loosely, 4-16 cells in each colony, cells spherical, 2.3 µm in diameter, cell content homogenous.

Place of collection - Barehipani, Similipal, Occurrence - Blackish patch on tree bark surface, Voucher number – 46.

13. *Chroococcidiopsis kashayi* Friedmann 1961, Öster. Bot. Zeits., 108: 354-367; Komárek and Anagnostidis 1999, P. 423, Fig. 553 (Pl. 1, Fig. 9 & 10)

Colony spherical to oval, margin smooth, mucilaginous; 20-30 µm wide, cell rounded 3-3.5 µm in diameter, cell division transverse, and cell content homogenous.

Place of collection - Barehipani, Similipal, Occurrence - Blackish patch on tree bark surface, Voucher number – 48.

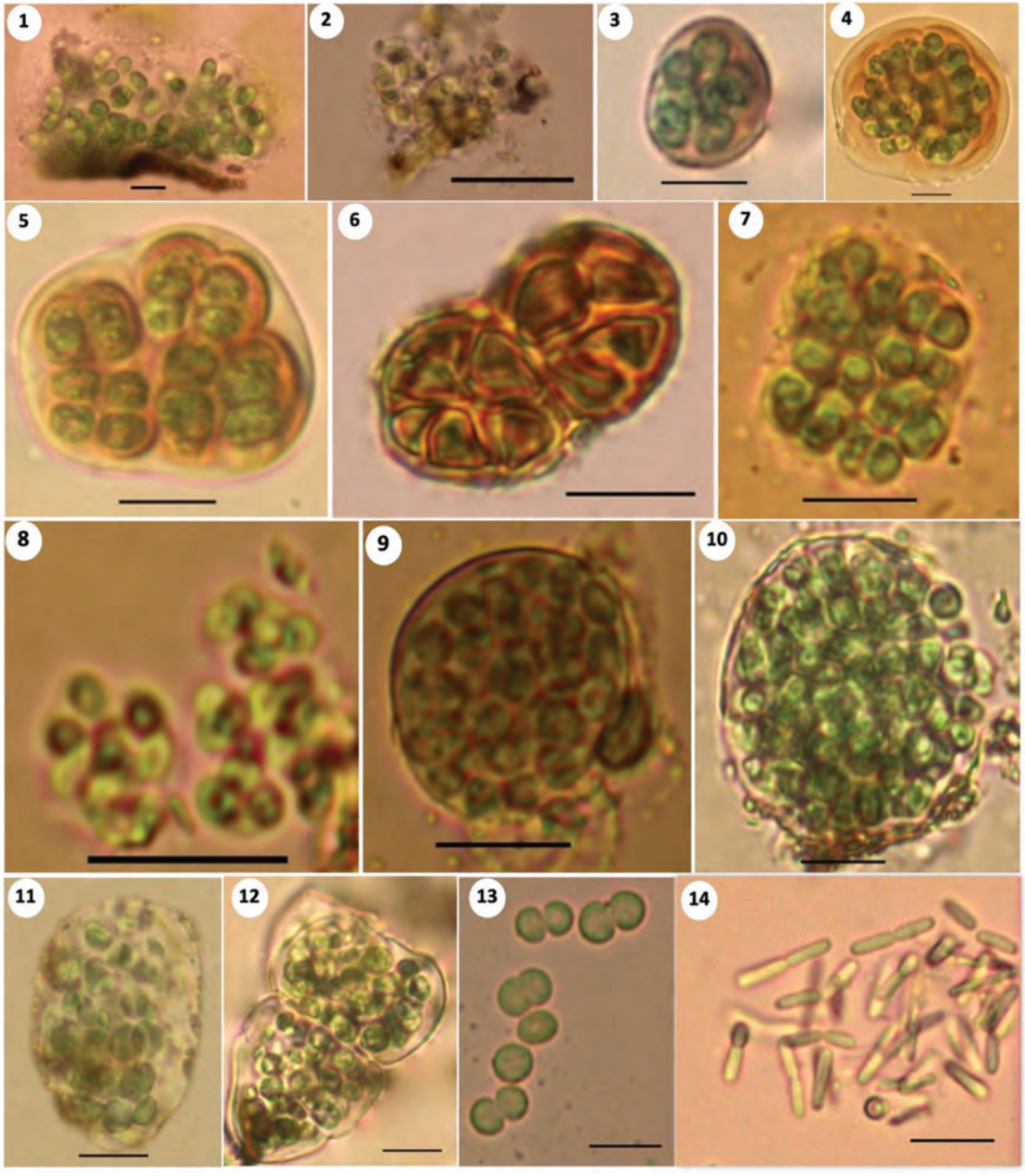


Figure 1. *Gloeocapsa gigas* 2. *Gloeocapsa gelatenosa* 3. *Gloeocapsa violacea* 4. *Gloeocapsa alpine* 5. *Gloeocapsa novacekii* 6. *Pseudocapsa dubia* 7. *Cyanosarcina spectabilis* 8. *Chroococcidiopsis indica* 9 &10. *Chroococcidiopsis kashayi* 11. *Aphanocapsa testacea* 12. *Aphanocapsa grevillei* 13. *Synechocystis pevalekii* 14. *Synechococcus elongates.*

Discussion

The Similipal Biosphere Reserve, located in Odisha, India, is a rich ecological zone known for its diverse flora and fauna. Subaerial algae, which inhabit surfaces exposed to the air such as rocks, soil, and tree trunks, play a vital role in the ecosystem and depict a better threshold for their survival (López-Bautista, 2007). Assessing their diversity provides insights into the health and stability of this

protected area. The results revealed a heterogeneous distribution pattern influenced by environmental factors such as water quality, light availability, and substrate type. Of these 13 taxa, *Gloeocapsa gelatenosa* occurred in the form of lithic biofilm, *Synechococcus elongatus, Synechocystis pevalekii, Gloeocapsa novacekii, Pseudocapsa dubia* from soil crust, *Aphanocapsa grevillei, Aphanocapsa testacea, Gloeocapsa violacea, Gloeocapsa alpina, Cyanosarcina spectabilis, Chroococcidiopsis indica, Chroococcidiopsis kashayi* from the subaerial corticolous substrate and *Gloeocapsa gigas*in the form of biofilm on moist rock (Table-1).

Table 1

Colonization of cyanobacteria in different habitats of Similipal Biosphere Reserve

Sample	Cyanobacterial community
Lithic biofilm	*Gloeocapsa gelatenosa*
Soil crust	*Synechocystis pevalekii, Gloeocapsa novacekii, Pseudocapsadubia*
Corticolous	*Aphanocapsa grevillei, Aphanocapsa testacea, Gloeocapsa violacea, Gloeocapsa alpina, Cyanosarcina spectabilis, Chroococcidiopsis indica, Chroococcidiopsis kashayi*
Moist rock	*Gloeocapsa gigas*

Thedata underscore the ecological significance of Chroococcales in subaerial habitats and their potential as bioindicators for environmental monitoring (Saraphol *et al.*, 2024). The phenotypic variation of chroococcales depends on environmental factors such as moisture levels and substrate type that influence the distribution and abundance of subaerial algae (Chonudomkul *et al.*, 1998; Komarek, 2013). For instance, algae in shaded, humid environments showed higher diversity than those in exposed, arid conditions. The study provides a comprehensive overview of the subaerial algal diversity in the Similipal Biosphere Reserve, highlighting its significance as a repository of unique algal species. The data revealed five new records to Odisha state, namely *Synechococcus pevelekii*, *Aphanocapsa grevillei*, *Gloeocapsa violacea*, *Gloeocapsa alpine* and *Pseudocapsadubia* that reported from different other parts of the country though (Choudhary and Singh, 2013; Gupta, 2023; Singh *et al.*, 2023). The discovery of new distributional records for India emphasizes the reserve's ecological importance and the need for conservation efforts. Understanding algal diversity also aids in monitoring environmental changes and assessing the health of the biosphere reserve. The findings contribute to the broader knowledge of biodiversity in Indian biosphere reserves and support conservation strategies to preserve unique and potentially threatened algal species. Future research should focus on long-term monitoring and the ecological roles of these algae within the Biosphere Reserve.

Acknowledgements

The authors are thankful to the authorities of MSCBD University, Botanical Survey of India, and S. R. College, Baliapal, Balasore and F.M.University for their support in carrying out the entire work.

References

Bhakta, S., Das, S. K., & Adhikary, S. P. (2016). Algal Diversity in Hot Springs of Odisha. *Nelumbo*, 58: 157-173.

Bhakta, S., Pattanaik, L. Dutta, P., Sahu, E. and Bastia, A. K. (2014). Diversity of corticolous algae from Similipal Biosphere Reserve, Mayurbhanj, Odisha. *Phykos*, 44 (1): 9-16.

Bhakta, S., Dutta, P., Sahu, E. and Bastia, A. K. (2015). Soil crust algae of Similipal Biosphere Reserve (SBR), Odisha. *J. Adv. Microbiol*., 2 (1): 54-63.

Bhuyan, S., Dey, H. S. and Bastia, A. K. (2007). Cyanobacterial Diversity of Selected Patches in Similipal Biosphere Reserve, Mayurbhanj, Orissa. Biannual Bulletin in SBR, BRIS, RPRC, Bhubaneswar, 2 (1&2): 30-31.

Bhuyan, P. P., Behera, S. K., Bhakta, S., Pradhan, B., Jena, M., Hansdah, B. and Bastia, A. K. (2023). Subaerial algal flora of Similipal biosphere reserve, Odisha, India. *J. Ind. Bot. Soc.*, 103 (1): 1-12.

Choudhary, K. K. and Singh. R. K. (2013). Cyanobacterial diversity along altitudinal gradient in Eastern Himalayas of India. *J. Algal Biomass Utln.*, 4 (2): 53–58

Chonudomkul, D., Yongmanitchai, W., Sookpreedee, C., Yongmanitchai, P., Arunpairojana, W., Kermanee, P. (1998). Divesity of Blue green algae and green algae in the deciduous dipterocarp forest. *Nat. Sci.,* 32: 339-346.

Chorus, I., and Bartram, J. (1999). Toxic cyanobacteria in water In: A Guide to their public health consequences, monitoring and management, E&FN Spon, London.

Deb, S. Sarma, B. Rout, J. and Sengupta, M. (2013). Algal diversity in soil crusts of Assam University, Silcher campus (North East India). *Phykos*, 43: 56-67.

Dey, H. S. and Bastia, A. K. (2009). The genus Oscillatoria voucher from rice field soils of North Orissa. *Plant Sci. Res.*, 31: 78-82.

Dey, H. S. and Bastia, A. K. (2010). Taxonomical enumeration of Chroococcales from rice field soils of Northern Orissa, India. Ind. *Hydrobiol.*, 13: 46-58.

Farrukh, H., Nizaz, A., Imtiaz, A., and Sara Mubara K.S. (2011). Subaerial algae of Peshawar university campus, Pakistan. *Pakistan J. Pl. Sci.*, 17: 41.

Friedmann, E. I. and Ocampo-Friedmann. (1984). Endolithic microorganisms in extreme dry environ-ments: analysis of a lithobiontic microbial habitat. In: Klug, M.J. & Reddy, C.A. Eds. Microbial Ecology. American Society for Microbiology, *Washington DC* : 177-185

Gupta, P. (2023). First report of diversity of Cyanoprokaryotes and Algae from aero-terrestrial biofilms on Roxburgh building of Acharya Jagadish Chandra Bose Indian Botanic Garden, Howrah, West Bengal, India. Trop. *Plant. Res.*, 10(1): 1-10.

Halder, N. (2018). Observation of Taxonomic Features of A Terrestrial Algae Oedocladium Terrestre Biswas (Chlorophyceae) With Soil Analysis From West Bengal, India. *J. Natl. Hist. Mus.*, 29: 1-8.

Jeyachitra, K., Panneerselvam, H., Rajendran, R, Mahalakshmi, M. and Sundaram, S. K. (2013). Physicochemical and Biological factors in the distribution of Cyanobacteria population in three different sampling sites of South India. African J. *Microbiol. Res.*, 7(25): 3240-3247.

Komárek, J. (2013). Phenotypic and ecological diversity of freshwater coccoid cyanobacteria from maritime Antarctica and islands of NW Weddell Sea. I. *Synechococcales.* Czech Pol. Rep., 3: 130-143.

Komárek, J. and Anagnostidis, K. (1999). Cyanoprokaryota, I. Teil Chroococcales. Band 19/1, Süßwasserflora von Mitteleuropa, Gaustav Fischer Verlag, Jena, 548 pp.

Kumar, D., Kastanek, P. and Adhikary, S. P. (2016). Diversity of Cyanobacteria in biological crust on arid soils in the Eastern region of India and their molecular phylogeny. *Curr. Sci.*, 110(10): 1999- 2004.

Kumar, D., Bhakta, S. and Adhikary, S. P. (2019). Molecular phylogeny and spectral characteristics of selected soil crust cyanobacteria from tea gardens of Darjeeling (India). *Indian J. Biotech.*, 18: 330-336.

León-Tejera, H., Pérez-Estrada, C.J., Montejano, G., & Serviere-Zaragoza, E. (2011). Biodiversity and temporal distribution of Chroococcales (Cyanoprokaryota) of an arid mangrove on the east coast of Baja California Sur, Mexico. *Fottea*, 11(1), 235-244. doi: 10.5507/fot.2011.022

López-Bautista, J.M.; Rindi, F.; Casamatta, D. (2007). The systematics of subaerial algae. In Algae and Cyanobacteria in Extreme Environments; Seckbach, *J., Ed.; Springer: Dordrecht, The Netherlands*:599–617.

Neustupa, J. and Škaloud, P. (2010). Diversity of subaerialalgae and cyanobacteria on tree bark and wood in the lowland tropical forests of Singapour. *Plant Ecol. Evol.*, 143: 51-62.

Rehakova, K. Chlumska, Z. and Dolezal, J. (2011). Soil cyanobacterial and microalgal diversity in dry mountains of Ladakh, NW Himalaya, as related to site, altitude and vegetation. *Microb Ecol.*, 62: 337-346.

Rishi, V., Singh, R. and Awasthi, A. K. (2016). A systematic account of order chroococcales from River Ganga at Kanpur, UP, India. *Ind. J. Fund. Appl. Lif. Sci.*, 6(2): 91-100.

Saha, S.K., Das, R., Bora, K.N. and Uma, L. (2007). Biodiversity of epilithic cyanobacteria from freshwater streams of Kakoijanaresearve forest, Assam, India. *Indian J. Microbiol.* 47: 219-232.

Saminathan, S. Subramaniyan, V. K. and Jeyachandran, S. (2013). Cyanobacteria in biological soil crust of Thanjavur and Thiruvarur district in Tamilnadu, India. *Inter. J. Agr. Sc. Res.*, 3: 59-66.

Saraphol, S., Rindi, F. and Sanevas, N., (2024). Diversity of epiphytic subaerial algal communities in Bangkok, Thailand, and their potential bioindicator with air pollution. Diversity, 16(1), p.55.

Sethi, S. K. Samad, L. K. and Adhikary, S. P. (2012). Cyanobacteria and micro-algae in biological crusts on soil and sub-aerial habitats of eastern and north eastern region of India. *Phykos*, 42:1- 9.

Singh, Y., Singh, A. and Singh, D.P. (2023). An updated checklist of algae from Himachal Pradesh, India. J. Indian bot. Soc., 103 (4): 221-244.

Sompong, U., Hawkins, P. R., Besley, C. and Peerapornpisal, Y. (2005). The distribution of cyanobacteria across physical and chemical gradients in hot springs in Northern Thailand. FEMS. *Microb. Ecol.*, 52: 365–376.

Tirkey, J. and Adhikary, S. P. (2005). Cyanobacteria in biological soil crusts of India. *Curr. Sci.*, 89: 515-521.

Uher, B. (2007). Morphological characterization of three subaerial *Calothrix* species (Nostocales, Cyanobacteria). *Fottea*, 11: 187-201.

Plant Science Research 46 (1&2) : 86-92, 2024

Plant Science Research

ISSN 0972-8546

Phytochemical analysis, antioxidant and antibacterial activity of *Desmodium gangeticum* (L.) DC. root extract against uropathogenic bacteria

Pratikshya Beura[1], Jaga Bemal[1], Shaikh Ameeruddin[1], Jyotirmayee Dash[1] and Sarita Das[1Ψ]

[1] Microbiology Laboratory, Department of Botany, Berhampur University, Bhanja Bihar, Berhampur-760007, Odisha, India

ARTICLE INFO

Article history:
Received : 12 September 2024
Revised : 17 October 2024
Accepted : 10 November 2024

Keywords:

Desmodium gangeticum (L.) DC. Root
Phytochemical analysis
Antioxidant
Antibacterial activity
Uropathogenic bacteria

ABSTRACT

Since antiquity, plants and their products are extensively used by human and animals for their health care as they are easily accessible. The root of *Desmodium gangeticum* (L.) DC. is a significant part of the "Dasamula" which has many medicinal applications. The phytocompounds found in the methanolic extract of *D. gangeticum* root (MDG) were evaluated in this study in terms of their antioxidant potential and antibacterial activity against the uropathogenic bacteria *E. coli*, *S. aureus* and *E. faecalis*. The free radical scavenging capacity of MDG in DPPH assay, is expressed via IC50 value of 56.48 in comparison to the IC50 value of 65.81 for Ascorbic acid. The total phenolics and flavonoids are quantified to be 184±4.91mg/g gallic acid equivalent and 141.47±3.96mg/g rutin equivalent, respectively from the standard calibration curves. By qualitatively testing the potential bioactive molecules, it was determined that MDG contained alkaloids, proteins, steroids, flavonoids, coumarins, and glycosides, all of which might have contributed to the compound's bactericidal action against uropathogens. However, MDG could be further investigated for its possible role in uropathogenesis at a molecular level to validate our results and before then possibly such natural products can be prescribed on regular basis to improve our renal and urinary health.

1. Introduction

Medicinal plants are nature's gift to human beings and animals for disease free healthy life (Sermakkani *et al.*, 2012). There are currently a wide variety of phytochemical medications on the market for the treatment of different illnesses. According to estimates from the World Health Organization (WHO), 80% of people in the majority of developing nations practice traditional medicine (Ruikar *et al.*, 2009). *Desmodium gangeticum* (L.) DC. is an important species of the genus *Desmodium andis* used widely dueto its broad-spectrum therapeutic potentiality and is extensively practiced as traditional medicine in India along with other parts of the Sub-Continent (Krishnasamy *et al.*, 2012). It is distributed wildly in several regions in Asia, Africa and Australia (Fern *et al.*, 2018; Rastogi *et al.*, 2011).

D. gangeticum belonging to the family Fabaceae, popularly known as 'Shalaparni' is a perennial shrub. Fabaceae consists of about 20,000 species and about 170 species are widely distributed as tropical and sub-tropical species growing throughout India. This plant is an erect or ascending under shrub, grows up to 2-4 feet. When the fruit ripens, the joints split into indehiscent segments with one seed each, and the seeds are compressed and reniform. It's flowering and fruiting season is during the month of March to December (Mahajon *et al.*, 2015). Shalparni means leaves like those of Sal tree (Shorearobusta). It is botanically equated to *D. gangeticum*. The roots of *D. gangeticum* are the official drug used as Shalparni (Rastogi *et al.*, 2011). But, aerial parts (chiefly stem) are being used or sold as Shalparni in different herbal drug markets of the country.

In Ayurveda (Indian traditional system of medicine), it has been reported for various therapeutic uses such as in oedema, fever, cough, worm infestation, dyspnoea, diarrhoea, vomiting, pain and inflammation (Prajapati *et al.*, 2010). It is one of the ingredients of 'Dashamula' which is an essential

Ψ *Corresponding author; Email:* saritadas7@yahoo.com; mohap003@gmail.com

herbal formulation of Ayurveda and widely prescribed for many health ailments (Singh *et al.,* 2015). Its roots have been used ethnomedicinally as an expectorant for lower respiratory tract infections and as an antitoxin in snake bite and scorpion sting (Chopra *et al.,* 1956; Nadkarni *et al.,* 1976).

Various pharmacological studies like antimicrobial, antioxidant, antiamnestic, psychopharmacological, anthelmintic, cardioprotective, anticancer, antiulcer, sun protective, antidiabetic, hepatoprotective, renal protective and anti-inflammatory activities revealed the potentiality of *D. gangeticum* on various types of diseases. The roots are one of the maining redients of famous Ayurvedic preparations i.e., Dashmula ristha, Dashmulak wath, Chitrak Haritika, Dashmula Kadha, Brahma Rasayan, Dashmula ark, Dashmula Taila, Dhanvantar Tailum and several other Ayurvedic formulations (Niranjan and Tiwari, 2008).

In recent scenario most deadly diseases are very much frequent. Analysing the reality of health concern urinary tract infection is the second most common disease caused by many common uropathogens like *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* etc. These elements are rapidly detected in urine samples of infected people. The most habituated adaptability of these uropathogens is the antibiotic resistance which is manifested mainly by high mutability rate and horizontal gene transfer ability. Thus, it is high time call for exploring the potential of different medicinal plants, as they are safe, cost effective, mostly available and easy to use. The present study was carried out to investigate the antibacterial activity of the root extract of *D. gangeticum* against standard and clinically isolated uropathogens.

2. Materials and Methods

2.1. Root extract of Desmodium gangeticum

The dried root of *Desmodium gangeticum* was collected from Berhampur University, Berhampur, Odisha, in the month of December, 2022 and authenticated by the botanist of Berhampur University. The oven dried roots were powdered and subjected to exhaustive soxhlet extraction using methanol (300ml) for 72 hours at 60-70^{0}C. The filtered extract was rotary evaporated. The crude extract was stored at 40C in a desiccator for future use and mixed with methanol or lukewarm distilled water to prepare the required working concentration depending on the type of study. The method of methanolic extract of D. gangeticum (MDG) preparation is presented in Fig.1.

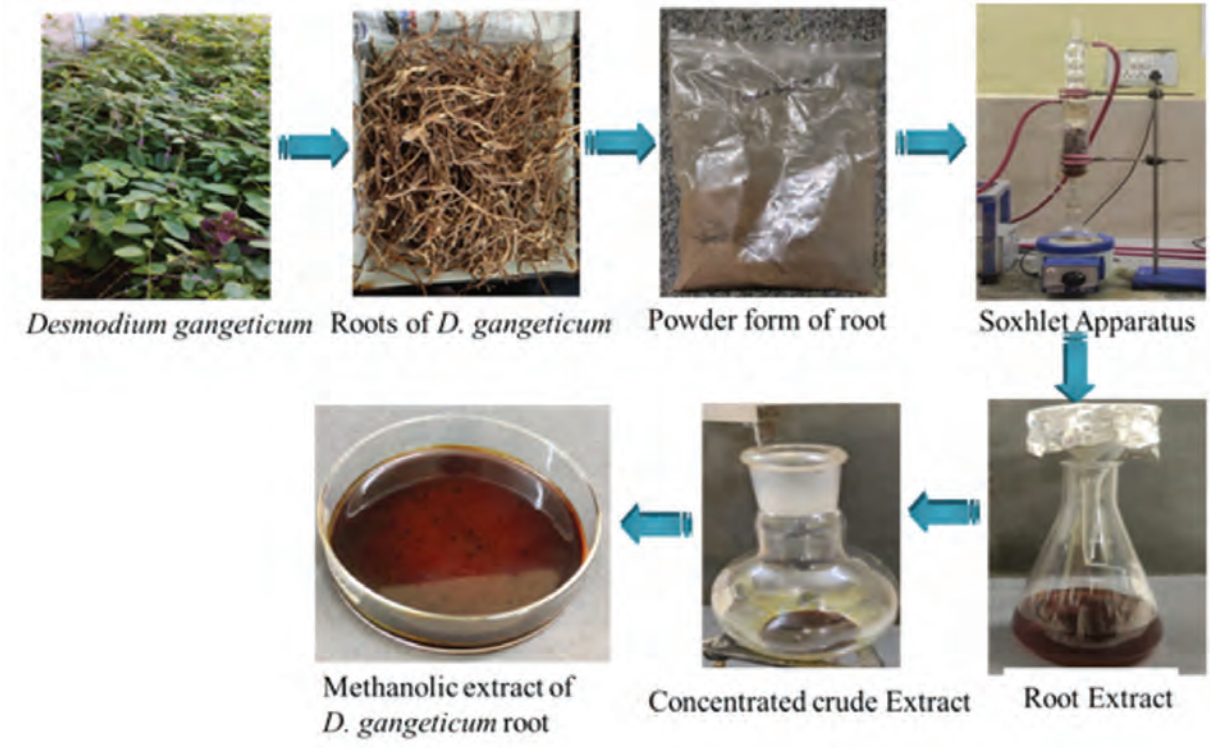


Figure 1: The preparation of methanolic extract of *Desmodium gangeticum* (MDG) roots

2.1. Bacterialstrain,maintenanceandstorage

Clinically isolated *E.coli, S.aureus* and *E. faecalis* were obtained from M.K.C.G Medical College, Berhampur. For routine use, the cultures were maintained on LB agar media plates. For long term storage, glycerol stocks were prepared by inoculating a single colony into LB broth incubated at 37^0C for 16-18 hrs. To 0.8ml of this culture 0.2ml of 50% sterile glycerol was added, mixed thoroughly and stored at 4^0C for 1hr and then stored at -20^0C.

2.2. Phytochemical analysis

2.3.1.Qualitative test

Standard lab procedures were followed to determine the presence of various bioactivc compounds as per the given protocol (Trease and Evans, 1989; Sofowora, 1993; Harborne, 1998).

2.3.2.Quantitative test

2.3.2.1. Total phenolic content (TPC)

The amount of TPC present in MDG was determined by the Folin-ciocalteu (FC) method (Singleton *et al*., 1965). Briefly, 200 µl of crude extract (1mg/ml) was added to 3ml of distilled water followed by 0.2ml of FC- reagent and mixed thoroughly for 8min, then 0.6ml of 10% Na_2CO_3 was added and incubated in dark for 1hr and absorbance was measured at 765nm. Triplicate sets of test and standard were prepared. The TPC was calculated from the gallic acid standard curve and expressed as mg of gallic acid equivalent per g dry weight of MDG.

2.3.2.2. Total flavonoid content (TFC)

The TFC was estimated by spectrophotometric method. Briefly, MDG crude extract was dissolved in methanol (1mg/ml). To 1ml of this solution, 1ml of 2% $AlCl_3$ dissolved in methanol was added and incubated for an hour at room temperature. Test and standard sets were prepared in triplicate. At 415 nm, the absorbance was measured. The rutin standard curve was used to calculate the TFC and the test results were reported as mg of rutin equivalent per g dry weight of MDG.

2.4. Statistical analysis

MS excel 2013 software was used to carryout the statistical analysis and results were expressed as mean ± standard deviation.

2.5. Antibacterial test

The efficacy of methanol extract of *D. gangeticum* root abbreviated as MDG was determined by different antibacterial techniques (disc diffusion, agar well diffusion) (Das *et al*., (2003) against the standard and clinically isolateduro bacteria (*E.coli, E.faecalis* and *S.aureus*).

2.5.1 Disc diffusion method

To prepare discs, Whatmann No. 1 filter paper was used. After adding different concentrations of MDG per disc, they were left to dry. Then LB plates were prepared and swabbed with sterile cotton swabs dipped in bacterial culture. The extract treated discs were placed aseptically on the bacteria swabbed plates. The zone of inhibition (ZOI) was measured after the plates were incubated for the entire night at 37°C.

2.5.2 Agar well diffusion method

An isolated bacterial colony was inoculated into sterile saline/peptone water mixed thoroughly by vortex and activated at 45°C for 15min. This activated bacterial culture was swabbed on LB plates and in cubated at 37°C for 15min. Fourwells were made on it with the help of sterile micropipette tips and MDG extract was added into the wells. The plates were kept in room temperature for 1hr to allow drug diffusion and then incubated overnight at 37°C and the ZOI was measured.

3. Results

3.1. Yield of Extract and its physical properties

MDG crude extract was dark brown coloured, sweet smelling sticky mass, soluble in lukewarm water and it had a mean yield of 18.45%.

3.2. Phytochemicalanalysis

3.2.1. Qualitative test:

The phytochemical screening of MDG revealed the presence of alkaloids, proteins, steroids, flavonoids, coumarins and glycosides and the results were presented in Table 1 and Fig. 2.

3.2 Quantitative test for Totalphenolic and total flavonoid content

The total phenolic content (TPC) of methanolic *D. gangeticum* calculated from the calibration curve (R2= 0.9521) was found to be 184±4.91mg/g gallic acid equivalents. The total flavonoid content (TFC) of the methanolic *D. gangeticum* calculated from the calibration curve (R2=0.9521) was found to be 141.47±3.96mg/g rutin equivalent.

3.3. DPPH Antioxidant assay

The free radicle scavenging capacity of MDG was presented via IC50 value of 56.48 in comparison to the IC50

Table 1:

Phytochemical analysis of methanolic extract of *D. gangeticum* root

PHYTOCHEMICALS	Present/Absent
Alkaloid	+
Terpenoid	-
Phenol&Tannins	-
Reducingsugar	-
Saponins	-
Protein	+
Steroids	+
Flavonoids	+
Anthocyanin	-
Coumarin	+
Leucoanthocyanin	-
Glycosides	+

Note: + indicates presence and – indicates absence

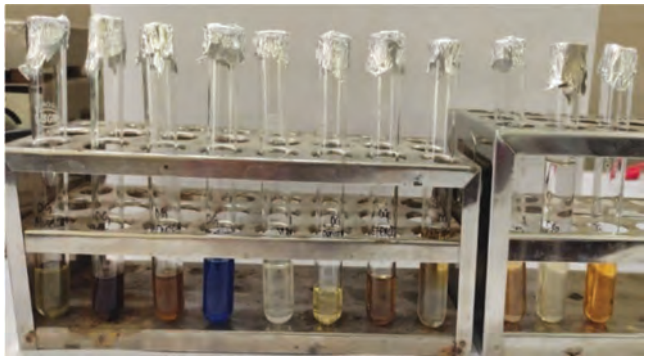


Figure 2: Qualitative phytochemical screening of methanolic extract of *D. gangeticum* root

value of 65.81 for Ascorbic acid, which was determined through the DPPH as say by taking as corbicacid as standard which showed MDG had significant antioxidant properties and high medicinal value against above uropathogenic bacteria. The results were presented in Fig. 3.

3.4. Antibacterial tests

3.4.1.Disc diffusion method

In disc diffusion method 5mg of MDG showed highest zone of inhibition against *E. coli*, *S. aureus* and *E. faecalis*. *S. aureus* and *E. faecalis* were comparatively more sensitive than *E.coli*. The results were presented in Table 2 and Fig.4.

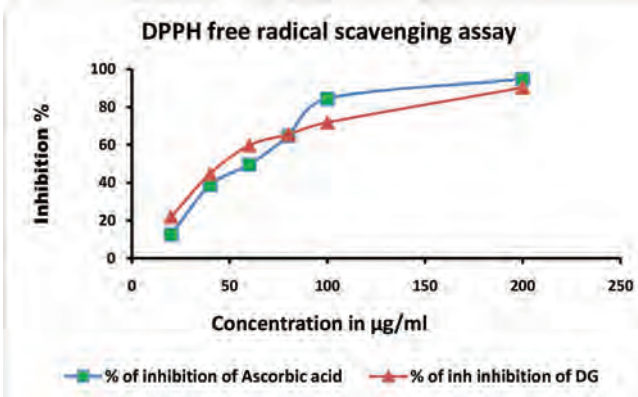


Figure 3: DPPH free radical scavenging assay of methanolic extract of *D. gangeticum* root

Table 2

Antibacterial activityof methanolic extract of *D. gangeticum* root in disc diffusion method

Zone of Inhibition in mm per concentration of MDG in mg/ disc				
Bacterial strain	1.25mg	2.5mg	3.75mg	5mg
S.aureus	6	8	9	10
E.coli	6	7	7	8
E. faecalis	9	10	10	11

3.4.2. Agar well diffusion method

In agarwell diffusion method 20mg of MDG showed highest zone of inhibition against *E. coli*, *S. aureus* and *E. faecalis*. *E. coli* and *E. faecalis* were observed to be more sensitive than *S. aureus.* The results were presented in Table 3 and Fig. 5.

Table 3

Antibacterial activity of methanolic extract of *D. gangeticum* root in agarwell diffusion method

Zone of Inhibition in mm per concentration of MDG in mg/ well				
Test bacteria	5mg	10mg	15mg	20mg
E.coli	0	10	11	13
S.aureus	0	0	9	11
E. faecalis	0	10	12	15

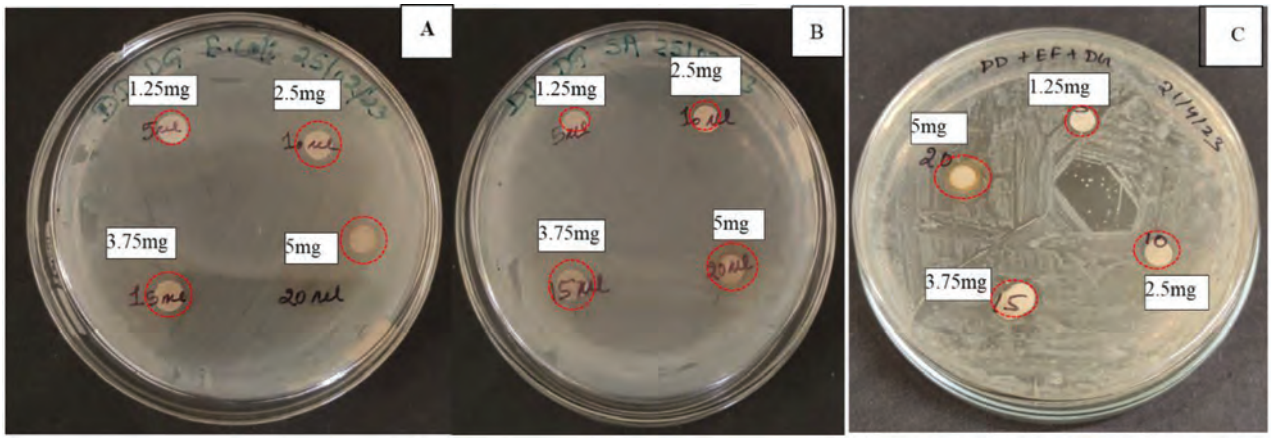


Figure 4: Growth inhibition study of MDG by Discdiffusion method (A) *E.coli* (B) *S.aureus* (C) *E. faecalis*

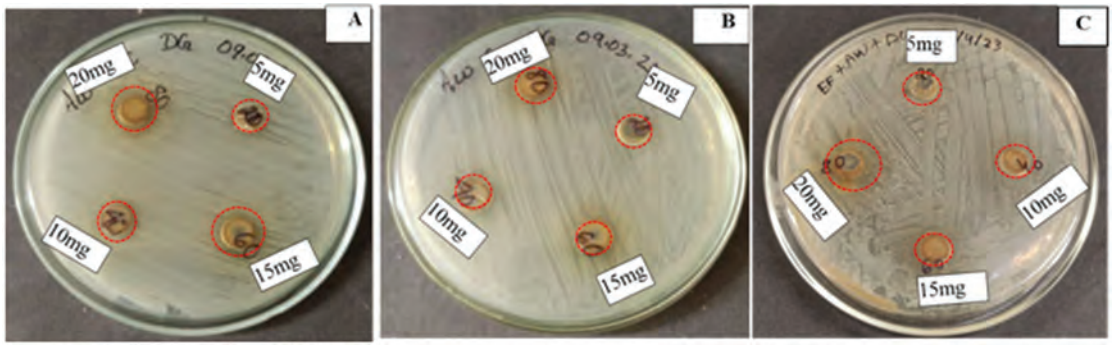


Figure 5: Growth inhibition study of MDG by agarwell diffusion method (A) *E.coli* (B) *S. aureus* (C) *E. faecalis*

Discussion

The well-known medicinal plant *Desmodium gangeticum* (L.) DC. is used extensively in many indigenous medical systems to treat a wide range of illnesses. Numerous phytoconstituents, including kaempferol-3-O-rutinoside, methyl salicylate α-D-glucopyranoside, leonuriside A, syringaresinol-4'-O-α-D-glucopyranoside, (17Z, 20Z)-hexacosa-17, 20-dien-9-one and gangenoid, were already reported in this species. The effectiveness of *D. gangeticum* to exhibit antimicrobial, antioxidant, antiamnestic, psychopharmacological, anthelmintic, cardio-protective, anticancer, antiulcer, sun protective, anti-diabetic, hepatoprotective, renal protective, and anti-inflammatory activities have been demonstrated by a variety of *in vitro* and *in vivo* pharmacological experiments. *D. gangeticum* is a significant candidate species for upcoming drug discoveries due to its wide range of biological activities (Mohan *et al*., 2020).

Phytochemical analysis of *D.gangeticum* root and aerial parts showed the presence of lupeol, lauric acid and mixture of α-sitosterol and stigmasterol. The total phenolic contents were 14.4, 13.8mg/g GAE; antioxidant activity 58.9, 54.8% and reducing power in terms of ascorbic acid equivalent 2.7 and 2.9 in roots and aerial parts of plant, respectively. Free radical scavenging activity by DPPH showed IC_{50}0.31, 0.35mg/ml; EC_{50} 13.48, 15.22 mg/mg DPPH and antiradical power of 7.42, 6.57 in roots and aerial parts, respectively (Niranjan and Tewari, 2008). In this present study, we found that the free radical scavenging capacity of MDG or the IC_{50} value is 56.48 in comparison to the IC_{50} value of 65.81 for Ascorbic acid in DPPH assay. The total phenolics and flavonoids are quantified to be 184±4.91mg/g gallic acid equivalent and 141.47±3.96 mg/g rutin equivalent, respectively.

Antifungal activity of petroleum ether, chloroform, acetone and aqueous leaf extractsof *D. gangeticum*was assessed against six pathogenic fungal strains viz., *Microsporum gypseum* (MTCCNO 4524), *Trichoderma viride* (MTCC NO 793), *Aspergillus niger* (MTCC NO 281), *Curvularia lunata* (NCFT), *Cladosporium oxysporum* (NCFT) and *Candida albicans* by using Poison food technique. Alkaloids, phenols, reducing sugar, saponins, tannins, terpenoids, flavonoids, fixed oils and anthraquinone were found in the preliminary phytochemical screening, but cardiac glycoside was not present in any of the four solvent extracts (Kanoje *et al*., 2020).

The highest level of antimicrobial activity observed in *D. gangeticum* leaf extracts is greater than that of aqueous, chloroform and hexane extracts. When compared to the high concentration of crude (100 mg/ml), the antimicrobial activity of the low concentrations (25 mg/ml and 50 mg/ml) was less potent. Methanol extract at 100 mg/ml concentration exhibited maximum antimicrobial activity against *P. aeruginosa* followed by *B. subtilis* and *E. coli*. The microbial strains *B. subtilis, E. coli, P. aeruginosa, C. albicans* and *S. cerevisiae* were susceptible for all extracts (Lagudu and Owk, 2016).

E. coli and *S. aureus* strains were considerably inhibited by the methanol extract, with a MIC value of 1.25 mg/ml. Conversely, with a MIC of 10 mg/ml, this extract exhibits minimal activity against the *P. vulgaris* strain. All of the tested strains' growth was also inhibited by the ethanol extract, but more strongly than by the methanol extract. The strains of *C. albicans* and *S. oralis* were more sensitive to the extract with 0.625 mg/ml for MIC, while *S. aureus* and *P. vulgaris* were less sensitive (MIC = 5 mg/ml). The residual ethanol extract exerted inhibition on all microorganisms at a concentration of 20 mg/ml, except the strain of *E. faecalis* that was resistant (Toyigbenan *et al*., 2020). In this present study, the phytochemical screening revealed the presence of alkaloid, flavonoids, reducing sugar, protein, steroid, anthocyanin, coumarin and glycosides. However, the presence of anthocyanin, flavonoids, saponins, terpenoids, tannins, mucilage were not detected in MDG.

Toyigbenan *et al*. (2020), reported that methanolic extract inhibit the growth of *E.coli* and *S.aureus*. Inconcordance to this report, MDG showed inhibitory effect against these bacteria in this present study. However, *S. aureus* showed less inhibition in growth as compared to *E. coli* and *E. faecalis*. *E. coli* and *E. faecalis* both are considered as the major uropathogenic bacteria responsible for causing urinary tract infections and they are found to be inhibited by MDG in both disc diffusion and agar well diffusion in this present study.

Conclusion and future prospectives: The root of *D. gangetiucum* shows significant bioactive phytocompounds responsible for its significant antibacterial activity against the urropathogenic bacteria. However, more research is needed to understand its potential role in preventing uropathogenesis and safeguarding against a range of urinary disorders.

Declaration of conflict of interest

The authors declare that there is no conflict of interest with any person or organization.

Acknowledgements

The authors thank all officials of Microbiology Department of Maharaja Krushna Chandra Gajapati Medical College, Berhampur. We also thank the authorities of Berhampur University for providing necessary amenities for the present work.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Chopra, R.N., Nayar, L. and Chopra, I.C. (1956). Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi. https://doi.org/10.4236/ajps. 2017.8201912.

Das S., Prakash R. and Devaraj S.N. (2003). Antidiarrhoeal effects of methanolic root extract of *Hemidesmus indicus* (Indian sarsaparilla) - an *in vitro* & *in vivo* study. Ind J Expt Biol, 41: 363-366.

Fern, K. (2018). Tropical plants database, Ken Fern. tropical. The ferns info. Available from: http://tropical.theferns.info.

Harborne, J.B. (1998). Phytochemical methods guide to modern technique of plant analysis, 3rd Edition, Chapmen all Hall, London

Kanoje, T., Sharma, K. and Verma, J. N. (2020). Antifungal activity and phytochemical screening of *Desmodium gangeticum* (L.) DC: An economic important plant of Narayanpur, Bastar, Chhattisgarh. 6(5): 34-45.

Krishnasamy, K., Gandhi, S.S., Rajendran, S., Chidambaram, C. and Subramaniyan, K. (2012). *In vitro* antibacterial activity of *Desmodium gangeticum* (L.) DC. Asian Pac. J. Trop. Dis. 421-424.

Lagudu. M.N. and Owk A.K. (2016). Antimicrobial activity and phytochemicals constituents of *Desmodium gangeticum* leaves. Int.Res.J. Agricul. Food Sci. 1(1): 44-52.

Mahajon, B., Deepak, M., Rema, Shree, A.B., Remadevi, R. (2015). Comparative phytochemical analysis of different parts of Sthiraa- *Desmodium gangeticum* (L.) DC. World J. Pharmaceut. Res. 4(8): 1821-1828.

Mohan, P.K., Moola, A.K., Kumar T.S. and Kumari, B.D.R. (2020). A comprehensive review of the phytochemical and pharmacological properties of *Desmodium gangeticum* (L.) DC. J. Adv. Sci. Res. 11 (4) Suppl 8: 90-97.

Nadkarni, K.M. (1976). *Hedysarumgegeticum* Linn. In: The Indian Materia Medica. Popular Prakashan Private Limited, Bombay.

Niranjan, A. and Tiwari, S.K. (2008). Phytochemical composition and antioxidant potential of *Desmodium gangeticum* (Linn.) DC. Natural Prod. Rad. 7(1): 35-39.

Prajapati, N.D., Purohit, S.S., Sharma, A.K. and Kumar T. (2010). Medicinal Plants. Agro bios. (India). 194.

Rastogi, S., Pandey, M.M. and Rawat, A.K.S. (2011). An ethnomedicinal, phytochemical and pharmacological profile of *Desmodium gangeticum* (L.) DC. and *Desmodium adscendens* (Sw.) DC. J. Ethnopharmacol. 136: 283-96.

Ruikar, A., Arbale, V., Tambe, A., Puranik, V., and Deshpande, N. (2009). GC-MS study of a steam volatile matter from *Mimusops elengi.* Int. J. Chem. Tech. Res. 1: 158-161.

Sermakkani, M. and Thangapandian, V. (2012). GC-MS analysis of *Cassia italica* leaf methanol extract. Asia. J. Pharm. Clinic. Res. 5: 90- 94.

Singh, S., Parmar, N. and Patel, B. (2015). A review on Shalparni (*Desmodium gangeticum* DC.) and *Desmodium* species (*Desmodium triflorum* DC. and *Desmodium laxiflorum* DC.) Ethnomedicinal Perspective. J Medicinal Plants.3(4): 38-43.

Singleton, V.L. and Rossi J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American J. Enol.Viticult. 16(3): 144-158.

Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd (Pub.), Ibadan.

Toyigbenan, B.F., Durand, D.-N., Haziz, S., Chimene, N., Wassiyath, M., Aklesso, N., Sylvestre, A., Majoie, T., Martial, N., Lehmane, H., Adolphe, A., Aly, S. and Lamine, B.M. (2020). Phytochemical screening and antimicrobial activity of *Desmodium ramosissimum.* American J. Plant Sci. 11: 51-63.

Trease, G.E. and Evans, W.C. (1989). Pharmacognosy, 13th edition, Bailliere Tindall, London : 345-346.

Plant Science Research 46 (1&2) : 93-96, 2024

Plant Science Research

ISSN 0972-8546

Seasonal incidence of brinjal shoot and fruit borer (*Leucinodes orbonalis* Guene) during *Kharif* Season in 2023

Shiv Shankar Kumawat[1], B Gangwar[1] and Pradeep Kumar[1Ψ]

[1] Institute of Agricultural Sciences, Bundelkhand University, U.P.

ARTICLE INFO

Article history:
Received : 12 September 2024
Revised : 18 October 2024
Accepted : 31 October 2024

Keywords:

fruit borer
kharif
Leucinodes orbonalis
meteorological week

ABSTRACT

The field experiment was conducted to observe the Seasonal incidence of Brinjal shoot and fruit borer (*Leucinodes orbonalis* Guene), at Organic Research Farm Kargua ji, Department of Entomology, Institute of Agricultural Sciences, Bundelkhand University, Jhansi (Uttar Pradesh) during kharif season of 2023. The experiment was laid out in Randomized block design. Result showed that the infestation of brinjal shoot and fruit borer (*L. orbonalis*) started in fourth week of July i.e., 30th standard meteorological week with 2.5 per cent shoot infestation. Shoot infestation increased gradually and reached its peak to 31.4 per cent during second week of September coinciding with 39st standard meteorological week. And The infestation of shoot and fruit borer on brinjal crop fruits began in August, increasing to 14.8% in the fourth week and reaching its peak at 41.2% in the second week of October. The infestation began with 1.3% in August and enhanced very fast.

Introduction

Brinjal (*Solanum melongena* L.) is one of the most important vegetables in South Asia which accounts almost fifty percent of the world's area under cultivation and also popular in some part of Africa and Central America (Harish *et al.,* 2011). In 2023, production in India was upto 12.61 million metric tons with a cultivable land of more than 760,000 hectares. However, it is slightly less than the previous year. Our country is the second-biggest producer of brinjal in the world, after China. The average yield of brinjal in India is 20-30 tons per hectare for a particular season and variety. Brinjal being a hardy crop, grows well in dry areas with limited irrigation and during the off-season. It's also a moderate source of vitamins and minerals. (NHB 2023). Unripe fruits are used primarily as vegetable in the country due to its nutritive value, as fruits are consisting of minerals like iron, phosphorous, calcium and vitamins like A, B and C, (Singh *et al.,* 1963). It has been reported as Ayurvedic medicine for curing diabetes. In addition, it is used as a good appetizer, aphrodisiac, cardiotonic, laxative and reliever of inflammation. Brinjal plants are very much susceptible to insect pest attack right from seedling stage to final harvesting. This solanaceous plant is attacked by 53 species of insect pests of which 8 are considered as major causing enormous damage to crop in every season throughout the year (Chakraborti and Sarkar, 2011). In this cultivation, there are several constraints, which are responsible for reduction in yield, out of which insect pests are one of the major factor. It has been reported that this crop is damaged by one hundred forty (140) species ofinsect pests at different stages of growth (Prempong *et al.,* 1977). Among the insect-pests infesting this vegetable, the major ones are shoot and fruit borer, *Leucinodes orbonalis* (Guen.), whitefly, *Bemisia tabaci* (Genn.), leafhopper, *Amrasca biguttula biguttula* (Ishida), *Epilachna beetle*, *Henosepilacha vigintioctopunctata* (Fab.) and red spider mite, *Tetranychus macfurlanei* (Baker and Pritchard) of these, *L. orbonalis* is considered as one of the main constraints as itdamages the

Ψ *Corresponding author; Email:* pradeepentomology@gmail.com

crop throughout the year. It is known to damage shoot and fruit of brinjal in allstages of its growth. The yield loss due to the insect-pests is to the extent of 70-92 per cent (Eswara Reddy and Srinivas, 2004). The shoot and fruit borer, *Leucinodes orbonalis* Guenee is the most destructive pest of brinjal at both vegetative and reproductive stages of almost all regions of India causing significant reduction in the yield by 40-80 per cent. After hatching, the young larvae bore into the petioles/growing shoots/flower buds/fruits and close the bore holes with frass, after entering in them. The larvae feed inside the midrib/flowers and in the pulp of fruit. The damaged shoots and flowers drop down. The large one or more round exit holes are visible on the fruits. Affected fruits get rotten from inside and such fruits lose their market value (Raina and Yadhav, 2018)

Material and Methods

The present field experiment was conducted at Organic Research Farm Kargua ji, Department of Entomology, Institute of Agricultural Sciences, Bundelkhand University, Jhansi (U.P) during kharif season of 2023 It is situated at 78^0 36'47" E Longitude and 25^0 27'2"N Latitude and is about 178.36 m above mean sea level. Thirty days old seedling of the brinjal cultivars were transplanted in the well-prepared field. The details of material used and methodology adopted during the present investigation are given below:

Net plot size	-	1.8 × 2.40 m2
Gross plot size	-	2 × 2.60 m2
Field size	-	19 × 9.2 m2
Spacing	-	60 × 45 cm
Season	-	Kharif (2023)

Method of recording observation

The incidence of shoot and fruit borer was recorded on regular basis to know the ETL. Spray of bio-pesticides was done when the shoot/fruit damage reached/ crossed 5% in any one of treatments. Per cent of shoot and fruit infestation was worked out by using following formula.

$$\text{Per cent shoot Infestation (\%)} = \text{x } 100 \frac{\text{Number of infested shoots}}{\text{Number of total Shoot}}$$

$$\text{Per cent fruit Infestation (\%)} = \text{x } 100 \frac{\text{Number of infested fruits}}{\text{Number of total fruits}}$$

Result and Discussion

Shoot infestation

The results presented in table.1 and figure. 1 revealed that the infestation of brinjal shoot and fruit borer, *L. orbonalis* started in fourth week of July *i.e.,* 30th standard meteorological week with 2.5 per cent shoot infestation. Shoot infestation increased gradually and reached its peak to 31.4 per cent during second week of September coinciding with 39th standard meteorological week. Thereafter, the infestation of shoot and fruit borer on shoots started declining gradually and persisted up to 48th standard meteorological week (6.3%) and disappeared completely during last week of December.

Fruit infestation

The results presented in table. 1 and figure. 1 revealed that the infestation of shoot and fruit borer on fruits of brinjal crop commenced from first week of August *i.e.,* 31st standard meteorological week with 1.3 per cent fruit infestation. Further, the infestation suddenly increased to 14.8 per cent in fourth week of August *i.e.,* 34th standard meteorological week. Thereafter, the infestation increased gradually and reached its peak to 41.2 per cent during second week of October *i.e.,* 41st standard meteorological week. Further there, was gradual decrease in infestation and persisted up to last week of November *i.e.,* 48th standard meteorological week. (Gupta *et al.,* 2021) reported that the infestation of *Leucinodes orbonalis* on developing shoots was first observed during fourth week after transplantation. However, the maximum fruit damage (50.2%) caused by *Leucinodes orbonalis* was observed during fourth week of October (43rd SMW).

Data on correlation coefficient of per cent fruit infestation by shoot and fruit borer with weather parameters are shown in table. 2 and illustrated in Fig. 1.

Table 1

Population dynamics of *Leucinodes orbonalis*in relation to weather parameters during *Kharif* 2023

SMW	Shoot Infestation (%)	Fruit infestation (%)	Max. temp.(C)	Min. temp.(C)	Max. RH(%)	Min. RH(%)	Rainfall (mm)
27	0 ± 0	0± 0	36.7± 1.10	25.8± 0.97	84± 3.51	73± 1.91	33.4± 0.75
28	0± 0	0± 0	35.8± 1.08	26.1± 0.98	86± 2.36	73± 1.91	86.2± 2.3 9
29	0± 0	0± 0	36.3± 1.09	27.1± 0.98	83± 2.42	62± 1.01	4.8± 0.12
30	2.5± 0.27	0± 0	36.6± 1.07	25.9± 0.87	76± 2.0	66± 1.05	4.6± 0.11
31	4.8± 0.30	1.3± 0.11	32.6± 0.99	25.4± 0.85	88± 2.63	80± 2.01	123.4± 3.23
32	7.6± 0.40	7.5± 0.41	33.4± 0.98	24.7± 0.76	85± 2.69	66± 1.07	65± 1.57
33	10.5± 0.51	11.6± 0.53	34.9± 0.96	25.2±0.75	83± 2.57	70± 1.91	3.8± 0.32
34	14.5± 0.62	14.8± 0.63	34.4± 0.97	25.1± 0.71	86± 2.62	68± 1.03	58.8± 1.21
35	16.8± 0.87	19.7± 0.91	35.4± 0.98	24.6± 0.71	84± 2.71	62± 1.02	0± 0
36	19.8± 0.91	24.5± 0.99	34.4± 0.94	24.5± 0.70	87± 2.91	76.1± 1.07	119.9± 3.11
37	23.5± 0.97	28.8± 0.98	33.7± 0.91	24.7± 0.69	90± 3.01	73± 1.51	66± 1.51
38	27.5± 0.91	32.5± 1.10	34.4± 0.90	24.5± 0.68	85± 2.51	66± 1.09	3.4± 0.23
39	31.4± 0.93	35.2± 1.13	35.9± 0.89	23.4± 0.64	87± 2.41	69± 1.08	0± 0
40	28.2± 0.92	38.5± 1.18	35.9± 0.85	20.6± 0.52	80± 2.01	48± 0.99	0± 0
41	24.8± 0.91	41.2± 1.21	37.1± 0.96	21.4± 0.53	77± 2.02	42± 0.94	0± 0
42	21.6± 0.98	35.2± 1.13	33.9± 0.83	18.3± 0.42	87± 2.51	68± 1.01	5.4± 0.33
43	17.2± 0.33	31.5± 1.19	33.2± 0.71	16.7± 0.40	84± 2.49	66± 1.02	0± 0
44	14.8± 0.25	27.5± 0.93	34± 0.75	17.4± 0.41	84± 2.43	57± 0.99	0± 0
45	12.2± 0.34	22.8± 0.81	34.5± 0.77	17.4± 0.42	79± 2.11	48± 0.81	0± 0
46	10.2± 0.31	19.5± 0.72	29.5± 0.90	13.2± 0.35	83± 2.32	49± 0.83	0± 0
47	7.8± 0.24	15.3± 0.74	29.6± 0.91	12.6± 0.29	81± 2.40	47± 0.79	0± 0
48	6.3± 0.40	13± 0.27	25.4± 0.89	15.6± 0.41	85± 2.51	49± 0.71	0± 0

Table 2

Correlation between *L. orbonalis* infestation with weather parameters during *Kharif* 2023

Weather parameters	Correlation coefficient (r)	
	Shoot damage (%)	Fruit damage (%)
Maximum Temperature (°C)	0.173± 0.008	0.054± 0.001
Minimum Temperature (°C)	-0.115± 0.001	-0.385± 0.003
Rainfall (mm)	-0.211± 0.007	-0.362± 0.004
Maximum relative humidity (%)	0.130± 0.005	-0.011± 0.001
Minimum relative humidity (%)	-0.133± 0.010	-0.358± 0.004

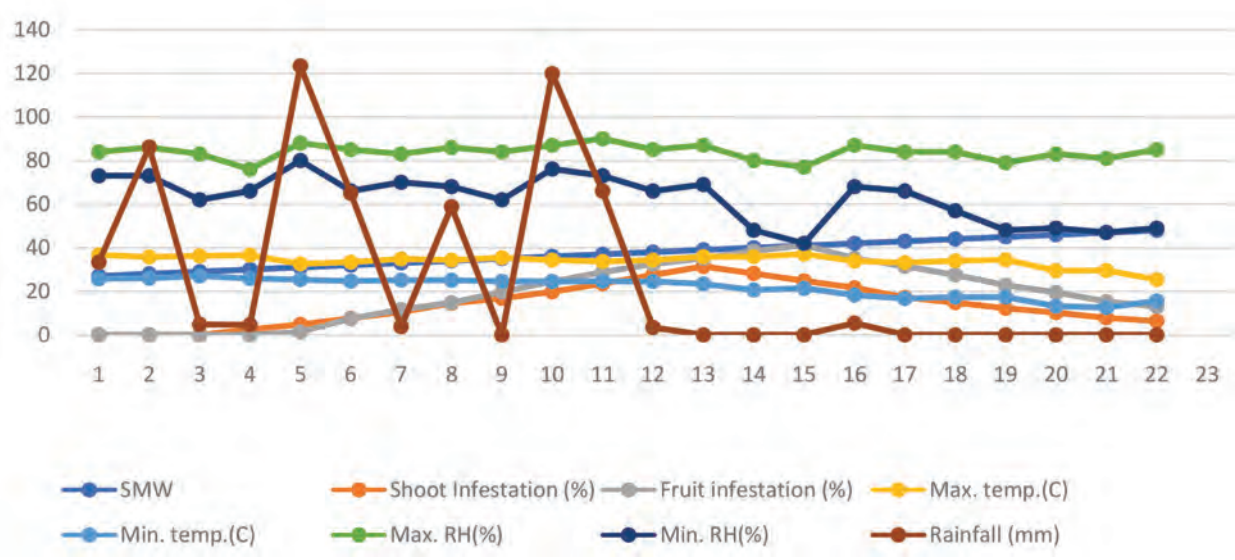


Figure 1. Population dynamics of *L. orbonalis* in relation to weather parameters during *Kharif* 2023

Correlations between weather parameters and shoot incidence

Correlation coefficients worked out between shoot infestation and weather parameters indicated that among the various weather parameters shoot damage was positively significant correlation with maximum temperature (r=0.173), maximum relative humidity (r= 0.130) and rainfall (r= -0.211). Whereas minimum temperature (r= -0.115), minimum relative humidity (r= -0.133) came us with negative, non – significant correlation.

Correlations between weather parameters and fruit incidence

The incidence of brinjal shoot and fruit borer was positive significant correlation with maxim temperature (r=0.054) and negative non–significant correlation with other weather parameters such as minimum temperature, rainfall, maximum and minimum relative humidity (r= -0.385, r= -0.362, r= -0.011 and r= -0.358, respectively). (Maru and Kumar, 2018) reported that BSFB incidence on shoot showed significant positive correlation with maximum temperature. It was negatively correlated with evening relative humidity. BSFB incidence on fruit showed significant positive correlation with maximum temperature.

Acknowledgements

We are privileged in availing this opportunity to express our deep sense of gratitude to Prof. B. Gangwar for undertaking this research work

References

Gupta, A., Chandrakar, G., Mohanan, A., and Sidar, R.(2021) Seasonal occurrence and impact of weather parameters on population dynamics of brinjal shoot and fruit borer (*Leucinodes orbonalis* Guenee) during *Kharif* 2020-21 *The Pharma Innovation Journal* 2021; SP-10(11): 262-265.

Chakraborti, S., Sarkar, P.K. (2011). Management of *Leucinodes orbonalis* Guenee on egg plant during the kharif season in India. J Pl. Protec. Res.; 51(4):325-328.

Reddy SG, Srinivasa (2004). Management of shoot and fruit borer, *Leucinodes orbonalis* (Guen.) in brinjal using botanicals/oils. Pestol; 28:50-52.

Harish DK, Agasimani AK, Imamsaheb SJ, Patil Satish S. (2011). Growthand yield parameters in brinjal as influenced by organic nutrient management and plant protection conditions. Res. J Agri. Sci.; 2(2) 221-225.

Raina, J., Yadav GS. (2018). Brinjal shoot and fruit borer: Bio- ecology and management. *Journal of Pharmacognosy and Phytochemistry*; 7(4):444-449

Maru, N.K., Kumar, A. (2018) Incidence of Shoot and fruit borer, *Leucindodes orbonalis* Guen. On Brinjal in relation to weather parameters in Allahabad region *Journal of Plant Development Sciences Vol. 10 (6) : 323-330. 2018*

Prem Spong E, Buahim. (1977). Studies on the insect pest of egg plant, *Solanum melongena* Linn. in Ghana. Bulletin de institute fundamental de Afrigue Neire seria A; 39(3):627-641.

Singh, S., Krishna, S.K., Katyal, S.L. (1963). Fruit culture in India. Indian Council of Agricultural Research, New Delhi, 412.

Plant Science Research 46 (1&2) : 97-102, 2024

Plant Science Research

ISSN 0972-8546

Plants: An innovative tool for Cancer

Swarna Kumar Dash[1], Bhagyajyoti Baral[2Ψ], Itisam Sarangi[3] and Santilata Sahoo[4]

[1,2,4] P.G. Department of Botany, Udayanath Autonomous College of Sc. & Tech., Adaspur, Cuttack, Odisha-754011

[3] Cancer Metabolism Lab, Bme Department, University of Michigan, Ann Arbor USA - 48109

ARTICLE INFO

Article history:
Received : 15 September 2024
Revised : 27 October 2024
Accepted : 7 November 2024

Keywords:

plant-derived Compounds
anticancer activity
plant-based therapies
synthetic drugs

ABSTRACT

Cancer remains one of the leading causes of mortality worldwide, necessitating novel approaches for effective treatment. In this context, plants have emerged as a promising source of anticancer agents due to their vast repository of bioactive compounds with therapeutic potential. Various plant-derived Compounds, such as alkaloids, flavonoids, phenolics, terpenoids, and glycosides, have demonstrated significant anticancer activity through mechanisms including apoptosis induction, cell cycle arrest, angiogenesis inhibition, and metastasis suppression. Despite significant advancements in the synthesis of chemical pharmaceutical agents, nature remains an unparalleled source of bioactive molecules. The exploration of natural products offers an invaluable pathway for discovering and developing novel therapeutic compounds with distinctive structures and mechanisms of action. While their origins trace back to traditional medicinal practices, plant-derived compounds continue to constitute a substantial portion of modern pharmaceutical agents. Their significance is particularly evident in oncology, where they provide promising alternatives to conventional chemotherapy, addressing its major drawbacks such as severe side effects and the growing challenge of multidrug resistance. Advances in biotechnology, including tissue culture and genetic engineering, have further enhanced the production of these bioactive molecules, ensuring sustainability and scalability of plant-based therapies. Furthermore, plant-based compounds exhibit selective toxicity toward cancer cells, often with reduced side effects compared to synthetic drugs, making them an attractive alternative for cancer management. This review highlights the plants as innovative tools in cancer research and treatment.

Introduction

Cancer stands as one of the foremost causes of morbidity and mortality worldwide. Among all non-communicable diseases, it ranks as the second leading cause of death, following cardiovascular diseases (Mathers & Loncar, 2006). The burden of cancer is immense, accounting for one in every eight deaths globally-a figure that surpasses the combined toll of AIDS, tuberculosis, and malaria. The incidence and mortality of cancer are disproportionately higher in regions such as North America, Australia, New Zealand and Western Europe when compared to other parts of the world (Parkin *et. al.*, 2001). In the United States alone, cancer is responsible for approximately one in four deaths (Jemal *et. al.*, 2007). On a global scale, cancer-related deaths are anticipated to rise significantly, from 7.1 million in 2002 to an estimated 11.5 million by the year 2030. Cancers can grow and spread either by direct invasion of surrounding tissues or through metastasis, a process in which malignant cells travel via the blood or lymphatic system to form new tumors in other parts of the body. The term "cancer" refers to over 100 different diseases that can affect nearly every area of the body, all of which can be life-threatening. The main categories of cancer include carcinoma, sarcoma, melanoma, lymphoma and leukemia. Carcinomas, the most frequently diagnosed type, develop in the skin, lungs, breasts, pancreas and other glands or organs. Lymphomas target lymphocytes, a type of white blood cell, while leukemia is a cancer of the blood that typically does not form solid

Ψ *Corresponding author; Email:* bhagyajyoti91@gmail.com

tumors. Sarcomas, which are less common, originate in connective tissues such as bone, muscle, fat, cartilage, or blood vessels. Melanomas arise in pigment-producing cells of the skin. Nature serves as an abundant and inexhaustible resource for the discovery of novel drugs, chemotypes, and pharmacophores (Veeresham *et. al.*, 2012).

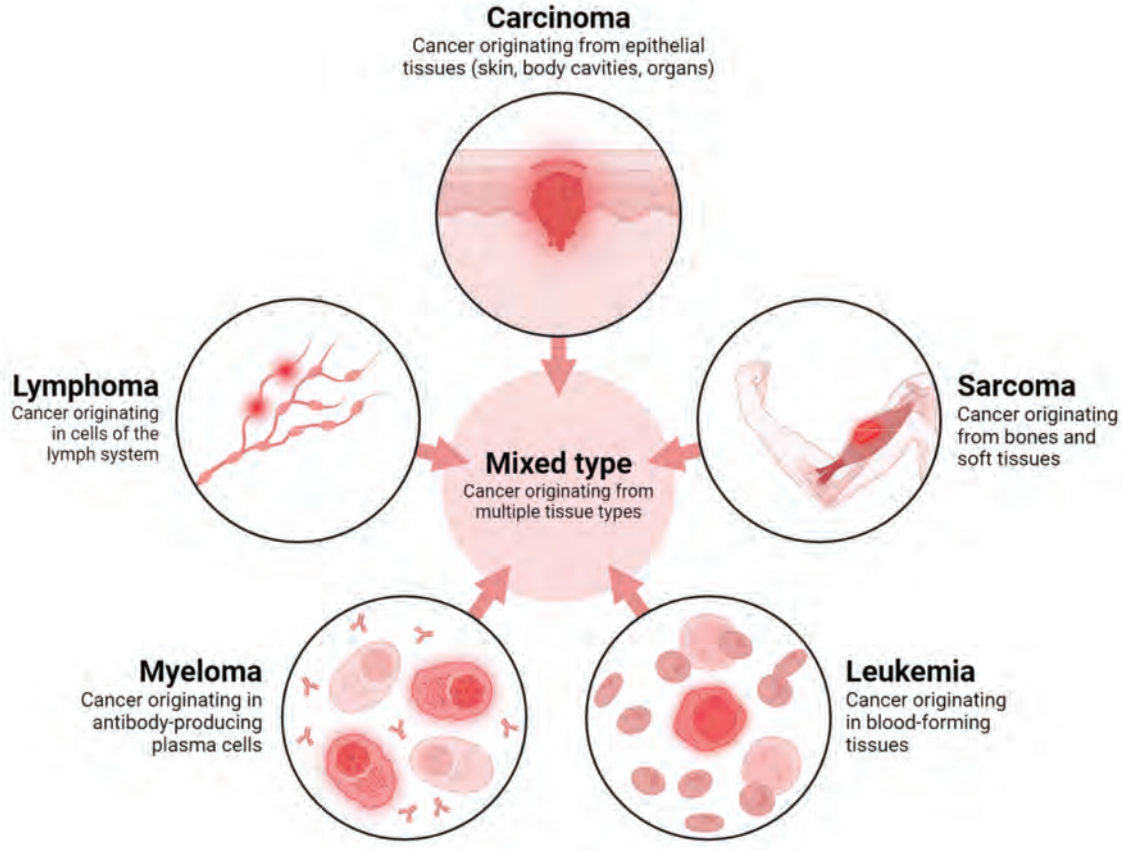


Figure 1: Type classification of Cancer cell based on Histology

Natural products, defined as small-molecule secondary metabolites, are derived from plants, marine organisms and microorganisms. Medicinal plants, widely distributed worldwide, have gained prominence in recent years for their significant role in disease treatment. These plants produce unique chemical compounds across taxonomically diverse organisms, serving critical biological functions that ensure survival. Their therapeutic efficacy is attributed to bioactive molecules, which form the core of a plant's chemical composition. For centuries, natural compounds have played a pivotal role in preventing and treating numerous ailments, serving as the cornerstone of traditional medicinal systems (Yuan *et al.*, 2016).

Natural products are highly valued for their "drug-like" pharmacological properties and "biologically compatible" molecular characteristics [Bindseil *et al.*, (2001)., Firn, R. D., & Jones, C. G. (2003), Vuorela *et al.*, (2004)] These features make them a rich source of chemical diversity and ideal candidates for further optimization using synthetic organic chemistry techniques. In modern drug discovery, natural products have proven particularly invaluable in the search for effective anticancer agents. Natural product drug discovery is a highly effective approach for developing new medical treatments, offering significant potential to enhance therapeutic success in today's rapidly advancing scientific landscape. It plays a vital role in tackling global health challenges and contributes to achieving health-related sustainable development goals by providing innovative solutions for medical interventions (Akram *et al.*, 2023). The structural complexity and bioactivity of this natural product provide excellent lead compounds that can be enhanced for therapeutic applications. This synergy between natural product chemistry and synthetic modification has enabled the development of innovative treatments, reaffirming nature's critical role in advancing healthcare.

Furthermore, terrestrial plants (e.g. *Artemisia annua* L., *Camptotheca acuminata* Decne., *Gingko biloba* L., *Curcuma longa* L., *Podophyllum peltatum* L., *Taxus brevifolia*, *Taxus baccata*, *Combretum caffrum*, *Euphorbia peplus* etc.) still dominate in current-day therapeutic

approaches, since the plant-derived compounds comprise a large portion of current pharmaceutical agents, most notably in the areas of antibiotherapy and chemotherapy (Cragg *et al.,* 2016).

Result and Discussion

The National Cancer Institute (NCI) has screened approximately 35,000 plant species for potential anticancer activities. Among them, about 3,000 plant species have demonstrated reproducible anticancer activity. Herbal drugs have a huge contribution to the inhibition of the progression of cancer. A large volume of clinical studies has reported the beneficial effects of herbal medicines on the survival, immune modulation, and quality of life (QOL) of cancer patients, when these herbal medicines are used in combination with conventional therapeutics (Sharma *et al.,* 2024). Many studies have focused on the chemoprotective properties of plants such as anticarcinogenic properties of *Abrus precatorius* on Yoshida sarcoma in rats, fibrosarcoma in mice and ascites tumor cells (Reddy, V. S., & Sirsi, M., 1969). Similarly, Dhar *et al*., have examined the anticancer properties of *Albizzia lebbeck* on sarcoma in mice and *Alstonia scholaries* on benzo[a]pyrene-induced forestomach carcinoma in humans. Other plants that have shown anticarcinogenic properties include *Anacardium occidentale* in hepatoma, *Asparagus racemosa* in human epidermoid carcinoma, *Boswellia serrata* in human epidermal carcinoma of the nasopharynx, *Erthyrina suberosa* in sarcoma, *Euphorbia hirta* in Freund virus leukemia, *Gynandropis pentaphylla* in hepatoma, *Nigella sativa* in Lewis lung carcinoma, *Peaderiafoetida* in human epidermoid carcinoma of the nasopharynx, *Picrorrhiza kurroa* in hepatic cancers and *Withania somnifera* in various tumors (Dhar, M. L. & Dhar, M. M., 1968)

The anticancer characteristics of a number of plants are still being actively researched and some have shown promising results. Some plants and plant products that have shown promise as anticancer agents are discussed in detail in the following sections.

Table 1:

Anticancer Activities of Major Chemical Constituents of Medicinal Plants (Desai *et al.,* 2008)

Name of Plant	Major Chemical Constituents	*In Vitro* Effects	*In Vivo* Effects
Tinospora cardifolia	20 β-hydroxyecdysterone, Cordioside, Columbin	Cytotoxic against HeLa cells	Significant tumor regression and survival in mice with Ehrlich ascites carcinoma
Ziziphus nummularia	Betulin, Betulinic acid	a. Cytotoxic, selectively towards cancer cells compared to normal cells b. Induces apoptosis through ROS generation, topoisomerase1 inhibition and MAP kinase activation c. Inhibits angiogenesis, modulates transcriptional activators, P53 and CD95 mediated cell death d. Induces loss of mitochondrial membrane potential, Cyt.c and Smac release	
Andrographis paniculata	Andrographolide	a. Alcoholic extract showed cytotoxicity against a panel of cancer cell lines. b. Increases antioxidant enzymes SOD, Catalase, GST; decreases LDH and MDA	Andrographolide regresses tumor in mice
Centella asiatica	Asiaticoside, hydrocotyline, vallerine, pectic acid, sterol, stigmasterol,	a. Leaf extract of *C. asiatica*, dose-dependently inhibits the proliferation of the transformed cell lines b. antitumor activity due to inhibition of	

	flavonoids, thankunosides and ascorbic acid	DNA synthesis	
Curcuma longa	Curcumin	a. Inhibits cell proliferation in wide variety of cell lines b. Downregulates NFk-B, AP-1, EGR-1, COX-2, LOX, NOs, MMP-9, TNF, Chemokine, EGFR, HER2 c. Inhibits JNK pathway, serine/ threonine kinase pathway d. Inhibits metastasis by reducing MMP-2 e. Induces apoptosis *in vitro* by decreasing mito-chondrial membrane potential, release of Cyt c, activation of caspases 3 and 9, and downregulation of anti-apoptotic proteins Bcl-XL and Integrin associated Protein	a. Reduces VGEF and bFGF mediated angiogenesis b. Prevents colon and gastric cancers in rodents
Phyllanthus amarus	Nirtetralin, niranthrin, phyllanthin, phyltetralin	a. Inhibits cell proliferation in different cancer cells, inhibits the activity of cdc 25 tyrosine phosphatase b. Inhibits the activity of topoisomerase I and II in *Sacchromycescerviacae* mutant cell cultures c. Induces cell cycle arrest and interfere with DNA repair d. Purified lignins act as reversal agent of MDR	a. Oral administration of extract increases life span and reduced tumor size in mice bearing Dalton's lymphoma ascites (DLA) and Erlich ascites carcinoma (EAC) b. Plant extract decreases n-nitrosodiethylamine (NDEA)-induced tumor incidence c. Plant extract has anti-angiogenic effects in mice bearing Lewis lung carcinoma
Annona atemoya	Bullatacin	a. Cytotoxic against tumor cell lines. b. Induced cell death by chromatin margination and tumor cell condensation	
Mappia foetida	Camptothecin	a. Effective inhibitors of nucleic acid synthesis in HeLa cells and L-120 cells b. Inhibits topoisomerase-1 (topo "1) c. slows growth of human colon cancer cells and rhabdomyosarcoma cells. d. A potent chemotherapy drug against leukemia	a. Induces partial or complete remission of breast carcinoma in the xenograft model system b. The Phase II trials against lymphoma, leukemia, and solid epithelial tumors
Withania somnifera	Withaferin A	a. Induces apoptosis in variety of cancer cells through the rapid generation of ROS b. Up-regulation of apical death	a. *W. somnifera* formulation is highly effective in producing tumor regression by >50% in

		receptors and altered ratio of members of Bcl2 family of proteins leading to downstream activation of caspase-3 and PARP cleavage thereby producing nucleosomal DNA cleavage in HL-60 cells c. A combination of root and leaf extract of withaferin shows cytotoxicity against panel of cancer cell lines and induce apoptosis through RNOS generation	EAC and EAT models b. Enhances Th1 cytokine expression, CD3, increases T cell and CD40 expression in EAT mouse tumor model
Cedrus deodara	Lignans, Wikstromol, Matairesinol and dibenzyl butyrolactol	a. Lignan extracts from the stem wood of *cedrus deodara* exhibits cytotoxicity in a panel of human cancer cell lines b. Increases sub-G0 population in HL-60 and MOLT-4 cells c. Induces DNA ladder formation and nitric oxide (NO) formation that leads to apoptosis	Shows tumor regression in murine models
Boswellia serrata	Triterpenic acids	a. Induces apoptosis in various cancer cell lines b. Inhibits DNA synthesis and cell growth of HL-60 Inhibits topoisimerse I and II	Effective against brain tumors

Conclusion

In the current scenario, the primary focus is on developing efficient nanomedicines for cancer treatment. While stem cell therapy demonstrates the ability to target both primary and metastatic cancer foci, it also plays a significant role in repairing and regenerating injured tissues (Kaur *et al.,* 2023). Plant-derived compounds continue to serve as an inexhaustible source of bioactive molecules for discovering new chemotherapeutic agents. These compounds provide structural frameworks for the development of novel therapeutic agents against various human diseases. Even though a considerable number of natural compounds prove therapeutical efficacy in preclinical studies their number decreases dramatically until they reach the clinical trial phase. These plants have emerged as a promising and innovative tool in cancer treatment and drug discovery. Their natural bioactive compounds, derived from diverse chemical structures, serve as vital resources for developing effective and targeted anticancer agents. Through centuries of traditional medicinal use, plants have demonstrated significant therapeutic potential, which modern science continues to validate and harness. Exploring the diverse realm of plant-derived drugs reveals the intricate blend of traditional wisdom and advanced biotechnology shaping this field. The transformation of plant-based pharmaceuticals—from a seed in the soil to a pill reaching patients—is an extraordinary journey (Bhaskar *et al.,* 2024). Plant-derived compounds, such as alkaloids, flavonoids, terpenes, and polyphenols, exhibit potent anticancer properties, including anti-proliferative, apoptotic, and anti-metastatic effects. These molecules offer a unique advantage due to their “drug-like” characteristics, biological compatibility, and ability to interact with specific molecular targets.

Recent estimates suggest that over 85% of higher plants have yet to be systematically studied for their bioactive compounds (Pieters & Vlietinck, 2005). Currently, more than 60 plant-derived compounds are under investigation as potential anticancer agents [Saklani, A., & Kutty, S. K. (2008)., Harvey, (2008). Butler, (2008)]. Advances in multidisciplinary research methodologies have opened new avenues for drug discovery and development from plants. These include innovative approaches for compound isolation and structural characterization, improved high-throughput biological screening techniques targeting novel biological pathways, and advancements in synthetic chemistry to streamline the optimization of lead molecules.

Approximately 70"80% of the global population relies on traditional medicines for disease treatment. In rural regions

with limited access to advanced Western medical practices, traditional medications remain a vital part of inclusive healthcare solutions. Phytomedicines, rooted in centuries-old healing traditions, utilize natural resources and often address health holistically, considering psychological and ethical balance as essential determinants of recovery. While many preparations are directly derived from plants, some are enhanced through structural modifications. The immense plant diversity worldwide offers a vast reservoir of unidentified phytochemicals with unexplored biological activities (Chihomvu *et al.,* 2024).

As cancer remains a leading global health challenge, the exploration of plants and their metabolites holds immense potential for discovering novel chemotherapeutic agents. Integrating traditional knowledge with advanced scientific research ensures the sustainable utilization of plants as a source of innovative, effective and safer anticancer therapies. Thus, plants continue to be invaluable in the fight against cancer, paving the way for future breakthroughs in cancer treatment and prevention.

References

Akram, M., Rashid, A., Zainab, R., Laila, U., Khalil, M. T., Anwar, H. & Riaz, M. (2023). Application and Research of Natural Products in Modern Medical Treatment. *J Mod Pharmacol Pathol*, *1*(7), 2.

Bhaskar, K., Singh, S., Narkhede, S. & Kumar, M. (2024). *Innovative Approaches to Creating Plant-Derived Pharmaceuticals*. Book Saga Publications.

Bindseil, K. U., Jakupovic, J., Wolf, D., Lavayre, J., Leboul, J. & van der Pyl, D. (2001). Pure compound libraries; a new perspective for natural product based drug discovery. *Drug Discovery Today*, *6*(16), 840-847.

Butler, M. S. (2008). Natural products to drugs: natural product-derived compounds in clinical trials. *Natural product reports*, *25*(3), 475-516.

Cragg, G.M. & Pezzuto, J.M. Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. *Med. Princ. Pract.* 2016, 25 (Suppl 2), 41–59.

Chihomvu, P., Ganesan, A., Gibbons, S., Woollard, K. & Hayes, M. A. (2024). Phytochemicals in drug discovery—a confluence of tradition and innovation. *International journal of molecular sciences*, *25*(16), 8792.

Desai, A. G., Qazi, G. N., Ganju, R. K., El-Tamer, M., Singh, J., Saxena, A. K. & Bhat, H. K. (2008). Medicinal plants and cancer chemoprevention. *Current drug metabolism*, *9*(7), 581-591.

Dhar, M. L. & Dhar, M. M. (1968). Screening of Indian plants for biological activity. Part I.

Firn, R. D. & Jones, C. G. (2003). Natural products–a simple model to explain chemical diversity. *Natural product reports*, *20*(4), 382-391.

Harvey, A. L. (2008). Natural products in drug discovery. *Drug discovery today*, *13*(19-20), 894-901.

Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J. & Thun, M. J. (2007). Cancer statistics, 2007. *CA: a cancer journal for clinicians*, *57*(1), 43-66.

Kaur, R., Bhardwaj, A. & Gupta, S. (2023). Cancer treatment therapies: traditional to modern approaches to combat cancers. *Molecular biology reports*, *50*(11), 9663-9676.

Mathers, C. D. & Loncar, D. (2006). Projections of global mortality and burden of disease from 2002 to 2030. *PLoS medicine*, *3*(11), e442.

Parkin, D. M., Bray, F., Ferlay, J. & Pisani, P. (2001). Estimating the world cancer burden: Globocan 2000.

Pieters, L. & Vlietinck, A. J. (2005). Bioguided isolation of pharmacologically active plant components, still a valuable strategy for the finding of new lead compounds?. *Journal of ethnopharmacology*, *100* (1-2), 57-60.

Reddy, V. S. & Sirsi, M. (1969). Effect of Abrus precatorius L. on experimental tumors. *Cancer research*, *29*(7), 1447-1451.

Saklani, A. & Kutty, S. K. (2008). Plant-derived compounds in clinical trials. *Drug discovery today*, *13*(3-4), 161-171.

Sharma, A. N., Dewangan, H. K. & Upadhyay, P. K. (2024). Comprehensive review on herbal medicine: emphasis on current therapy and role of phytoconstituents for cancer treatment. *Chemistry & biodiversity*, *21*(3), e202301468.

Veeresham, C. Natural products derived from plants as a source of drugs. *J. Adv. Pharm. Technol. Res.* 2012, 3, 200–201.

Vuorela, P., Leinonen, M., Saikku, P., Tammela, P., Rauha, J. P., Wennberg, T. & Vuorela, H. (2004). Natural products in the process of finding new drug candidates. *Current medicinal chemistry*, *11*(11), 1375-1389.

Yuan, H.; Ma, Q.; Ye, L.; Piao, G. The traditional medicine and modern medicine from natural products. *Molecules* 2016, 21, 559.

Plant Science Research 46 (1&2) : 103-112, 2024

Plant Science Research

ISSN 0972-8546

Rhizophagus irregularis inoculation alleviates Zn toxicity in *Triticum aestivum* L. by improving biochemical response, antioxidative defense and nutrient uptake

Ankita Tripathy, Sarita Nalini Pradhan and Bandana Kullu[Ψ]

P.G. Department of Botany, Utkal University, Bhubaneswar, Odisha, India

ARTICLE INFO

Article history:
Received : 17 September 2024
Revised : 28 October 2024
Accepted : 9 November 2024

Keywords:

Rhizophagus irregularis
Arbuscular mycorrhizal fungi
Triticum aestivum
Zn toxicity
Nutrient uptake

ABSTRACT

Zn toxicity interferes with the normal physiological functioning of plants. Arbuscular mycorrhizal fungi (AMF) are among the beneficial root symbionts known to reduce abiotic stress in plants. The current study assessed the impacts of AMF *Rhizophagus irregularis* inoculation on the morphology, biochemical response, antioxidative enzyme activity and nutrient uptake of the experimental plant *Triticum aestivum* L. grown in Zn spiked soil (100-500 mg/kg soil). The toxic effect in *T. aestivum* was observed at Zn 300-500 mg/kg soil treatments adversely affecting morpho-physiology.AMF *R. irregularis* inoculation improved morphological features, and pigment content compared to non-mycorrhizal plants of this crop. However, inoculated plants' free amino acid and proline levels significantly decreased. Enhanced antioxidative enzyme SOD, CAT, GPX, and APX activities in mycorrhizal plants modulated redox state as evident from lower MDA and H_2O_2 with AMF inoculation. The macronutrients such as P, K, Ca, and Mg uptake notably enhanced with *R. irregularis* inoculation. The Zn immobilization in AMF colonized root attributed to reduced transportation to shoot, resulting in increased uptake of Fe, Cu and Mn. The findings highlighted the significant role of AMF *R. irregularis* in alleviating Zn toxicity by improving hydration status, oxidative stress management through antioxidative enzymes, and modulating nutrient uptake.

Introduction

Zn is a vital micronutrient, essential for several metabolic functions of living organisms. It is required for structural and catalytic processes like cell division and expansion, physiological processes such as osmolyte accumulation, stomata regulation, activation of enzymes, and carbohydrate metabolism in plants (Hamzah *et al.,* 2022). Globally, soil Zn deficiency is more prevalent than toxicity (Akther *et al.,* 2022). Nevertheless, human activities like smelting, mining, burning of fossil fuel, fertilizers, etc. introduced excessive Zn into agricultural soil (Alengebawy *et al.,* 2021). Being a heavy metal, Zn follows a wide range of heavy metal responses at a toxic level (Di Baccio *et al.,* 2005). Zn phytotoxicity causes stunted growth, altered photosynthesis, respiration rate and imbalanced ROS production (Kaur and Garg 2021). It also interferes with the absorption of other essential nutrients having similar ionic radii (Bankaji *et al.,* 2019). The Zn toxicity in plants is manifested as a change in physio-biochemical attributes because of Zn accumulation in plant tissue and/ or inadequate acquisition of nutrients and water. Through the internal enzymatic and non-enzymatic antioxidative defense systems, plants cope with oxidative stress caused by ROS accumulation.

Microorganisms are vital for aiding in plants' tolerance to environmental stresses. Among the beneficial microbes, arbuscular mycorrhizal fungi (AMF) are associated with approximately 72% of vascular plant roots. The obligate root symbiont obtains photosynthetic carbon and essential lipids from the host plant (Jiang *et al.,* 2017). AMF association has been proven to be an environment-friendly

[Ψ] *Corresponding author; Email:* bandkullu@gmail.com

strategy for the stress resilience of host plants (Begum *et al.,* 2019). The efficiency of AMF in reducing Zn toxicity in crop plants has been highlighted (Adeyemi *et al.,* 2021). The mechanisms suggested in AMF symbiosis for abiotic stress tolerance are elevated osmotic adjustment and stomatal regulation, plant hormone production, improved antioxidant activity, and accumulation of metals in AMF structures (Mitra *et al.,* 2021).

Triticum aestivum L. is the chief cereal crop that caters to about 20% of food calories consumed by the world's population (Erenstein *et al.,* 2022). There are reports on the toxic effects of excessive Zn on *T. aestivum* physiology (Li *et al.,* 2020). The *Rhizophagus irregularisis* a model AMF widely used for research and commercial applications. However, the effectiveness of AMF *R. irregularis* in subsiding Zn toxicity in *T. aestivum* has not yet been assessed. The most recent genome assembly data is available for *R. irregularis* (Manley *et al.,* 2023), which accentuates its suitability to comprehend the genome basis of host-AMF interactions in the future. Therefore, the current investigation evaluated the role of AMF *R. irregularis* inoculation in alleviating the Zn toxicity of *T. aestivum*. The mechanism of Zn stress mitigation by *R. irregularis* was elucidated by analyzing morphology, biochemical response, mineral uptake, and antioxidative enzyme activities.

Materials and methods

2.1. AMF inoculum

The pure culture of AMF *Rhizophagus irregularis* was mass propagated through the pot culture technique in maize as the host plant. The starter culture was inoculated into the roots of maize seedlings and allowed to grow under greenhouse conditions in sterilized soil and sand mix (2:1, v/v) combined with organic manure (3:1 v/v) under 60 ± 2% relative humidity (RH), 12h daylight of intensity ranging from 700-900 µmol m-2s-1 and 25 ± 2^0C temperature for 90 days. The quantity of AMF spore in the potting substrate was estimated (Gerdemann and Nicholson 1963) and recorded to be 200/100 g soil.

2.2. Pot culture and experimental design

The potting mix for the experiment was prepared by mixing sieved dried soil (2 mm sieve) and sand (2:1, v/v) into which organic manure was added (3:1, v/v). The potting mix was autoclaved to sterilize for 1 hour at 121^{0}C and 15 psi on alternate days thrice to eliminate any AMF contamination. The physicochemical properties of the potting mix are pH: 6.3, EC: 0.052 dsm^{-1}, OC: 16 g/kg, available Nitrogen: 148 mg kg^{-1}, Phosphorous: 93 mg kg^{-1}, Pottasium: 298 mg kg^{-1}, Zinc: 1.83 mg kg^{-1}, Iron: 8.9 mg kg^{-1}, Manganese:2.95 mg kg^{-1} and Copper: 0.75 mg kg^{-1}.

The pot culture experiment was a randomized 4 x 2 factorial design consisting of 4 levels of Zn addition (0, 100, 300 and 500 mg/kg soil) and 2 AMF inocula treatments such as non-mycorrhizal (NM) without AMF inoculation and mycorrhizal (M) with AMF inoculation. For pot culture 3 kg of sterilized potting mix was added to culture polybags (18 x 13 cm) with 30 g of mass propagated soil as AMF inoculum containing approx. 600 AMF spores. The NM treatment received the same amount of mass-propagated soil without AMF propagules to balance the nutrients. To each polybag, 10 numbers of surface sterilized *T. aestivum* seeds were sown. The poly bags were maintained in a greenhouse at 60±2% RH, 25±2^{0}C temperature, and 12h daylight (700-900 µmol $m^{-2}s^{-1}$). After 7 days of seed germination, the appropriate amount of ZnSO4 dissolved in deionized water was applied to the soil to attain the required Zn concentration. Treatment without Zn (Zn 0 mg/kg soil) receiving only deionized water was considered as control (C). For each treatment 4 replicate pots were maintained. The morphophysiological and mycorrhizal parameters under various treatments were analyzed after 45 days of growth. The second lower leaf and roots of *T. aestivum* were used for biochemical analyses.

2.3. Morphology

The test plants were uprooted carefully and washed thoroughly to remove the dirt. After bloat dry the individual shoot and root lengths were measured from base to tip. The plant biomass was dried at 80^{0}C and dry weight (DW) was determined. The RWC (%) of the leaf and root was estimated as per González and González-Vilar (2001).

2.4 Photosynthetic pigment content

The photosynthetic pigments such as Chl a, Chl b, total Chl, and carotenoid content of the leaf samples were estimated as per Arnon (1949).

2.3 Antioxidative enzyme activities

The antioxidative enzyme activity in leaf samples for SOD (Dhindsa et al. 1981), CAT (Aebi 1984), GPX (Hemeda and Klein 1990) and APX (Nakano and Asada 1981) were estimated following standard protocol.

2.4 Free amino acid and proline content

The free amino acid (Moore and Stein 1963) and proline (Bates *et al.,* 1973) content in leaf samples were determined.

2.5 Malondialdehyde (MDA) and hydrogen peroxide (H2O2) content

The MDA and H2O2 content in leaf samples were estimated as per Health and Packer (1968) and Alexieva *et al.,* (2001) respectively.

2.8 Mineral content

To estimate the mineral content in root and shoot, the oven-dried biomass (100 mg) was digested in 10 ml acid mixture ($HCl:HNO_3$, 3:1 v/v). The final volume of the acid-digested samples was adjusted to 50 ml with 2% nitric acid. The macronutrients (P, K, Ca and Mg) and the micronutrients (Zn, Cu, Fe and Mn) content in acid-digested samples were estimated through inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer, Avio 200) as per Chand and Prasad (2013).

2.9 Estimation of AMF root colonization and spore density

For assessment of mycorrhizal root colonization (%), cleaned root segments were stained with lacto glycerol trypan blue (0.05%) (Philips and Hayman1970). The stained roots under the microscope were observed in terms of hyphae, vesicles and arbuscules. AMF root colonization (%) was estimated by the grid-line intersect method (Giovannetti and Mosse, 1980) following the formula:

$$\text{Mycorrhizal colonization (\%)} = \frac{\text{(No.of root colonized with AMF)}}{\text{(Total no.of root inspected)}} \text{x } 100$$

$$\text{Occurrence(\%)of a particular AMF structure} = \frac{\text{(No.of root with a particular AMF structure)}}{\text{(Total no.of roots colonized with AMF)}} \text{ x } 100$$

To quantify the spore density in the potting soil, mycorrhizal spores were separated from 20 g soil (Gerdemann and Nicholson 1963; Daniel and Skipper 1982). The extracted spores were transferred to gridded filter paper and observed under a stereo-zoom microscope. The spores were counted and expressed as no. of spore/100 g of dry soil.

2.9. Statistical analysis

The impacts of AMF inoculation on *T. aestivum* under different Zn concentrations on measured parameters were statistically analyzed through two-way ANOVA. DMRT statistically tested the variations among the treatments. Data were represented as mean (4) ± SEM (standard error of mean).

3. Results and discussion

3.1. Root colonization and spore density of AMF

The AMF root colonization (%) was higher at Zn treatment 100 mg/kg soil (100%) than control (95%) which gradually decreased at 300 mg/kg soil (85%) and 500 mg/kg soil (76%) Zn treatment (Table 1). The hyphae, arbuscules and vesicles colonization rate also showed a similar trend as that of total mycorrhizal root colonization. However, the percentage of root colonization by hyphae (100-72%) was the highest followed by arbuscules (95-63%) and least by vesicles (90-55%). The spore density was increased at Zn level 100 mg/kg soil (134) and gradually decreased at 300 mg/kg soil (106) and 500 mg/kg soil (90) of Zn compared to the control (110). The Zn enrichment due to Zn treatment in the deficient soil (Zn 1.83 mg/kg in control soil) at 100 mg/kg soil of Zn treatment has attributed to the present observation. Reduced AMF root colonization in *T. aestivum* at high Zn concentrations (Coccina *et al.*, 2019) is similar to the present findings. The spore density of AMF *Funneliformis geosporum* associated with *T. aestivum* was adversely affected byincreasing Zn concentration (Abu-Elasoud *et al.*, 2017) supporting the current observation. Thus, the decline in root colonization (%) at 300 and 500 mg/kg of soil Zn treatment indicated the adverse impact of Zn toxicity on AMF root colonization and spore density.

3.2. Morphology

There was an improvement in the morphological parameters of *T. aestivum* such as shoot and root length, dry weight, and RWC at 100 mg/kg soil of Zn compared to the control (Table 2). However, adverse effects were observed on morphological parameters with subsequent increases in Zn concentration (300 and 500 mg/kg soil). Comparatively, M plants showed better morphology than the NM plants in all the treatments.The improvement in morphological features such as shoot and root length, and biomass (DW) at 100 mg/kg soil Zn than control was due to nutrient enrichment of soil after Zn treatment. The adverse impacts of Zn toxicity are inhibition of cell division, reduced water and nutrient uptake (Kaur and Garg 2021) supporting the present observation. AMF inoculation enhancing nutrient uptake, improving water relations and abiotic stress tolerance (Mitra *et. al.*, 2021) have contributed to better morphological parameters of M than the NM plants. AMF enhances water absorption in the host plant by increasing the surface area of absorption through extra radicle mycelium (Adeyemi *et al.*, 2021).

3.3. Photosynthetic pigment content

The Chl a, Chl b, total Chl and carotenoid content gradually declined beyond 100 mg/kg soil Zn treatment (Fig. 1). With AMF inoculation, there was an enhancement of Chl a (10-55%), Chl b (29-82%), total Chl (15-60%) and carotenoid

Table 1

AMF root colonization(%) and spore density of *R. irrgularis* in *T. aestivum* L. under different Zn treatments.

Treatment (Zn mg/kg soil)	Total AMF (%)	Arbuscules (%)	Vesicles (%)	Hyphae (%)	AMF Spore Density (No. /100 g of soil)
Control	95	73	68	94	110
100	100	95	90	100	134
300	85	80	66	86	106
500	76	63	55	72	90

Table 2

Morphological features of *T. aestivum* L. at different Zn treatments and *R. irrgularis* inoculation.

Zn treatment	Control		100		300		500	
(mg/kg soil)	NM	M	NM	M	NM	M	NM	M
Shoot length (cm)	30.1±0.7^{b}	34.4±0.6^{a}	30.1±0.8^{b}	34.1±0.5^{a}	28.5±0.3^{b}	32.5±0.6^{a}	21.4±0.2^{d}	26.2±0.6^{c}
Root length (cm)	24.2±0.2^{d}	28.3±0.1^{b}	27.6±0.1^{b}	30.8±0.04^{a}	23.1±0.08^{e}	25.7±0.4^{c}	16.1±0.07^{g}	19.1±0.6^{f}
Shoot DW (g)	0.98±0.1^{ab}	1.1±0.03^{a}	1.15 ±0.2^{a}	1.17±0.06^{a}	0.9±0.06^{ab}	1.06±0.07^{a}	0.68±0.1^{b}	0.95±0.03^{ab}
Root DW (g)	0.24±0.02^{bc}	0.28±0.01^{ab}	0.21±0.01^{c}	0.31±0.01^{a}	0.19±0.02^{cd}	0.24±0.02^{bc}	0.14±0.02^{d}	0.18±0.01^{cd}
Leaf RWC (%)	68.62±0.1^{c}	95±0.1^{a}	83.80±0.1^{b}	98.23±0.3^{a}	72.72±0.2^{c}	85.71±0.1^{b}	60.01±0.2^{d}	70.87±0.3^{c}
Root RWC (%)	60±0.4^{de}	66.66±0.2^{d}	74.41±0.1^{c}	96.55±0.1^{a}	65.51±0.8^{d}	80.76±0.08^{b}	54.55±0.1^{e}	65.83±0.2^{d}

NM: Non-mycorrhizal, M: Mycorrhizal, FW: fresh weight, DW: Dry weight, RWC: relative water content

Data (mean ± SE) is obtained from four replicates.Values within a row with different letters in the superscripts are significantlydifferent (p=0.05) tested through DMRT.

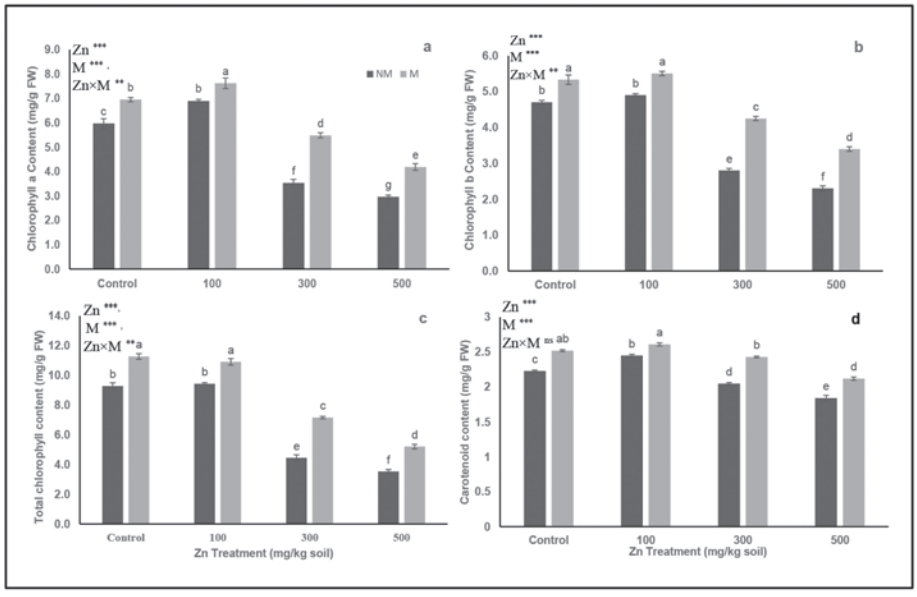


Figure 1: Effect of Zn treatment and AMF inoculation (NM: Non-mycorrhizal and M: Mycorrhizal) on a. Chlorophyll a content, b. chlorophyll b content, c. Total carotenoid content of *T.aestivum*. Data (mean±SE) is obtained from four replicates. Values in bars with different letters are significantly different at p=0.05 tested through DMRT. F values of Two-way ANOVA analysis denote significance at pe”0.05 (*), pe”0.01 (**), pe”0.001 (***), ns: nonsignificant. Zn: Zn concentration effect, M: AMF inoculation, Zn × M: Variable interaction effect.

content (11-13%) in different Zn treatments.The chlorophyll content is considered as an index for photosynthesis. Potentially noxious levels of Zn in soil cause phytotoxicity and impair photosynthesis (Kaur and Garg 2021). The AMF in oculum *Glomous aggregatum* and *Glomous intraradices* treatments elevating photosynthetic pigment (Nafady and Elgharably 2018) support current findings. AMF-mediated improvement in chlorophyll content is related to the enhancement of nitrogen (N) and magnesium (Mg) uptake (Mathur and Vyas 1995). The enhanced uptake of Fe with *R. irregularis* inoculation under Zn toxicity also contributed to enhanced photosynthetic pigment content.

3.4. Biochemical and anti-oxidative response

The free amino acid, proline, MDA and H_2O_2 contents gradually boosted with increasing Zn treatment (Fig. 2). Comparatively M plants accumulated less quantity of the metabolites than NM plants in all the Zn treatments. With AMF inoculation there was a decline in free amino acid (8 -12%), proline (1-10%), MDA (7-19%), and H_2O_2 (23 - 22%) at different levels of Zn. The antioxidative enzyme activity SOD, CAT, GPX and APX in the leaf of *T. aestivum* showed increasing trend with a rise in Zn concentration (Fig 3). The antioxidative enzyme activities showed enhancement with AMF inoculation and increased by 17-35%, 6-24%, 8-10% and 16-38%, for SOD, CAT, GPX and APX respectively at different Zn treatments. Protein degrades to free amino acids under abiotic stress (Batista-Silva *et al.,* 2019) which supports the present observation. The amino acid proline is an osmolyte that regulates the stress response by maintaining osmotic balance, stabilizing the membrane by preventing electrolyte leakage and balancing ROS in the host plant (Hosseinifard *et al.,* 2022). Decline proline content of M than NM plants indicated stress alleviating effect of AMF inoculation (Borde *et al.,* 2011) and supports the current observation. MDA and H_2O_2 level is the indication of oxidative stress. AMF symbiosis upregulates the defense mechanism by enhancing SOD, CAT, GPX and APX activities scavenge ROS, subsequently lowering the level of MDA and H_2O_2 in M plants (Chandrasekaran 2022) validated the present observation.

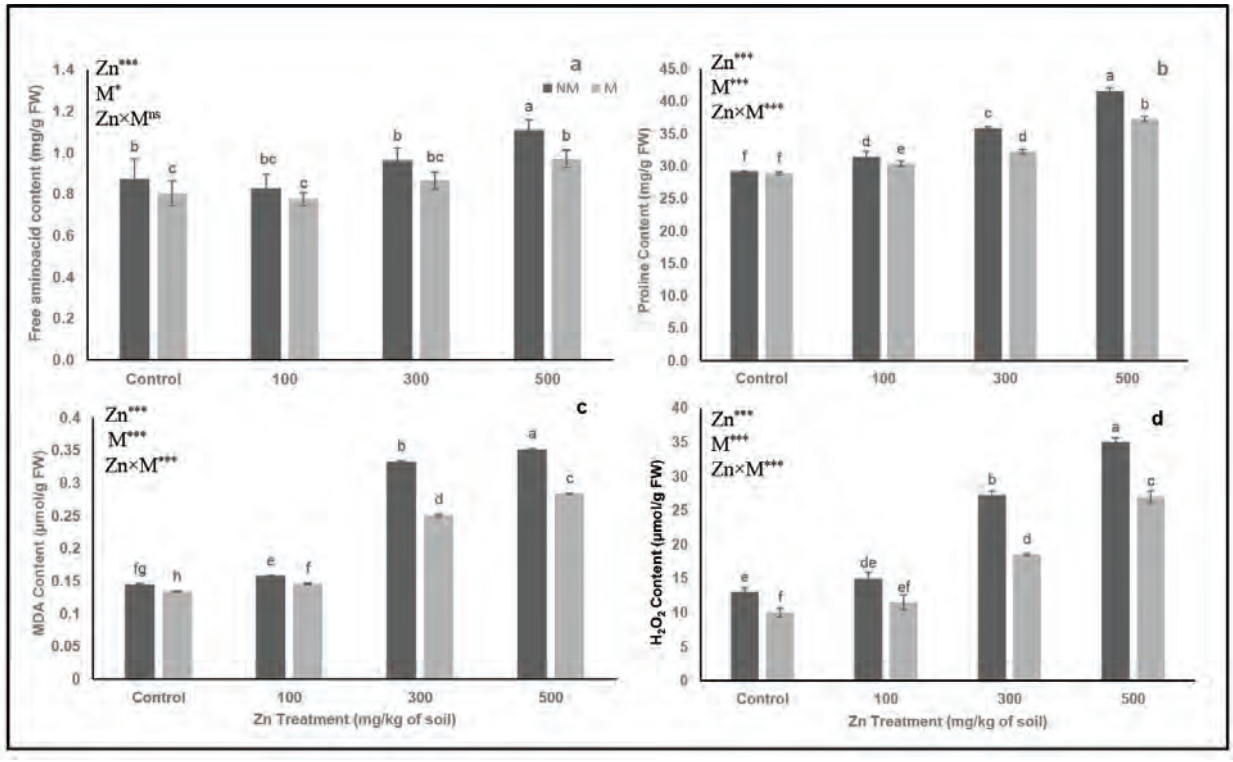


Figure 2: Effect of Zn treatment and AMF inoculation (NM: Non-mycorrhizal and M: Mycorrhizal) on a. Free amino acid content, b. Proline content, c. MDA content d. H_2O_2 (Hydrogen peroxide) content of *T. aestivum.* Data (mean±SE) is obtained from four replicates. Values in bars with different letters are significantly different at *p*=0.05 tested through DMRT. F value of Two-way ANOVA analysis denotes significance at p e" 0.05(*), p e" 0.01(**), p e" 0.001(***), ns: non-significant. Zn: Zn concentration effect, M: AMF inoculation effect, Zn x M: Variable interaction effect.

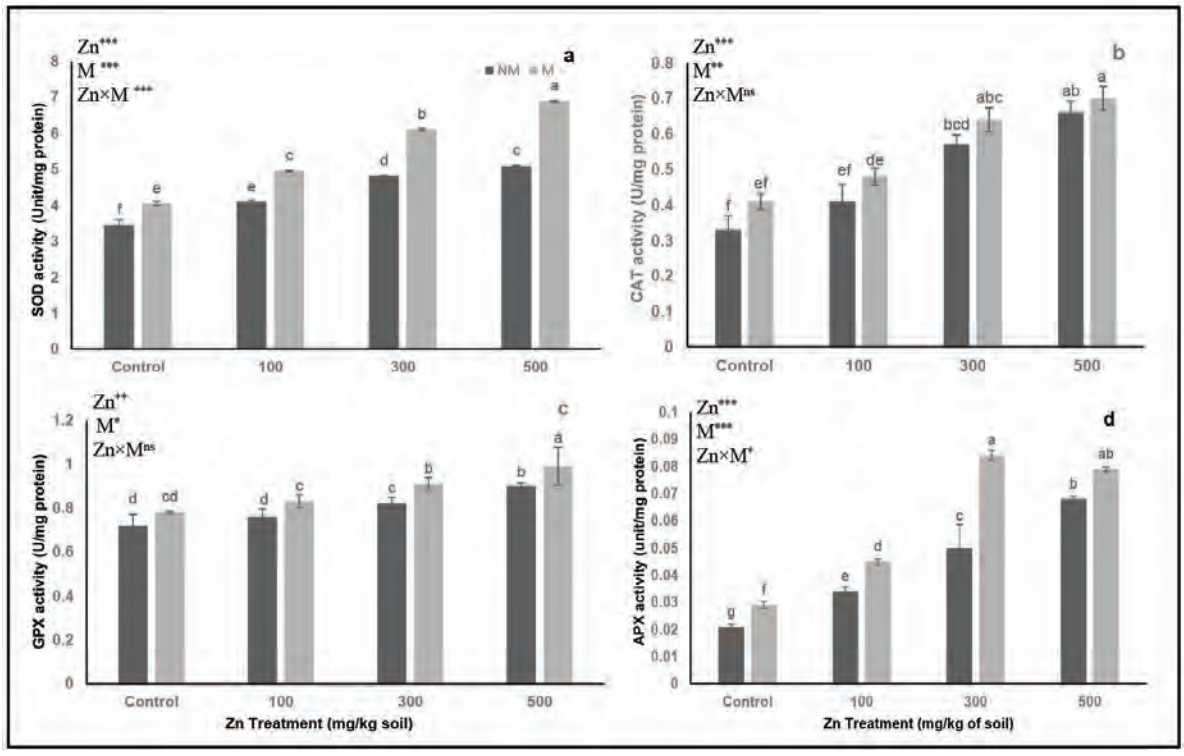


Figure 3: Effect of Zn treatment and AMF inoculation (NM: Non-mycorrhizal and M: Mycorrhizal) on a. SOD activity, b. CAT activity, c. GPX activity and d. APX activity of *T. aestivum*. Data (mean±SE) is obtained from four replicates. Values in bars with different letters are significantly different at *p*=0.05 tested through DMRT. F value of Two-way ANOVA analysis denotes significance at p e" 0.05 (*), p e" 0.01 (**), pe" 0.001(***), ns: non-significant. Zn: Zn concentration effect, M: AMF inoculation effect, Zn x M: Variable interaction effect.

3.5. Mineral content

The impact of AMF inoculation on the macro and micronutrient content of *T. aestivum* under different Zn treatments was summarised in Fig 4 and 5. The AMF inoculation resulted in the enhancement of P content in mycorrhizal (M) plants 2-4 times higher in root and 2-3 fold greater in shoot at different levels of Zn treatment compared to NM plants. With AMF-inoculation the K, Mg, and Ca content increased in the root by 7-34%, 15-29% and 4-24% respectively and in the shoot by 20-31%, 17-55%, and 6-33% respectively at different levels of Zn treatments. Zn content in the roots increased by 4, 54, and 126 fold higher in NM plants and 4, 85, and 135 fold higher in M plants compared to the control at Zn treatments 100, 300 and 500 mg/kg soil respectively. However, with AMF inoculation the Zn accumulation was enhanced (13-65%) in root and declined in shoot (16-25%) at different Zn treatments. The micronutrients Cu, Fe, and Mn content in NM plants were reduced in root and shoot at various Zn treatment levels (300-500 mg/kg soil Zn) compared to the control. In the M plants the Cu, Fe, and Mn content enhanced by 10-33%, 15-44%, and 10-41% respectively in root and 8-28%, 14-45%, 9-40% respectively in the shoot at different Zn treatments.

The current findings indicate that with an increase in soil Zn concentration, there was a corresponding upsurge in the uptake and accumulation of Zn in both root and shoot tissue. A previous report of a higher accumulation of Zn in the root compared to the shoot tissue of *T. aestivum* with AMF inoculation under excessive Zn (Saboor *et al.*, 2021) supports the present findings. The excess Zn in the soil hinders plant growth and metabolism by interfering with the uptake of other essential nutrients having similar ionic radii (Shri and Pillay 2017) justifying the present observation decreased uptake of Fe, Cu and Mn. The immobilization of Zn in the root by AMF structures (Kumar and Saxena 2019) and the increased surface area for absorption duo extensive extra radicle mycelium (Boorboori and Zhang 2022) have attributed to the improved uptake micro-(Fe, Cu, and Mn) and macronutrients (P, K, Ca and Mg), respectively.

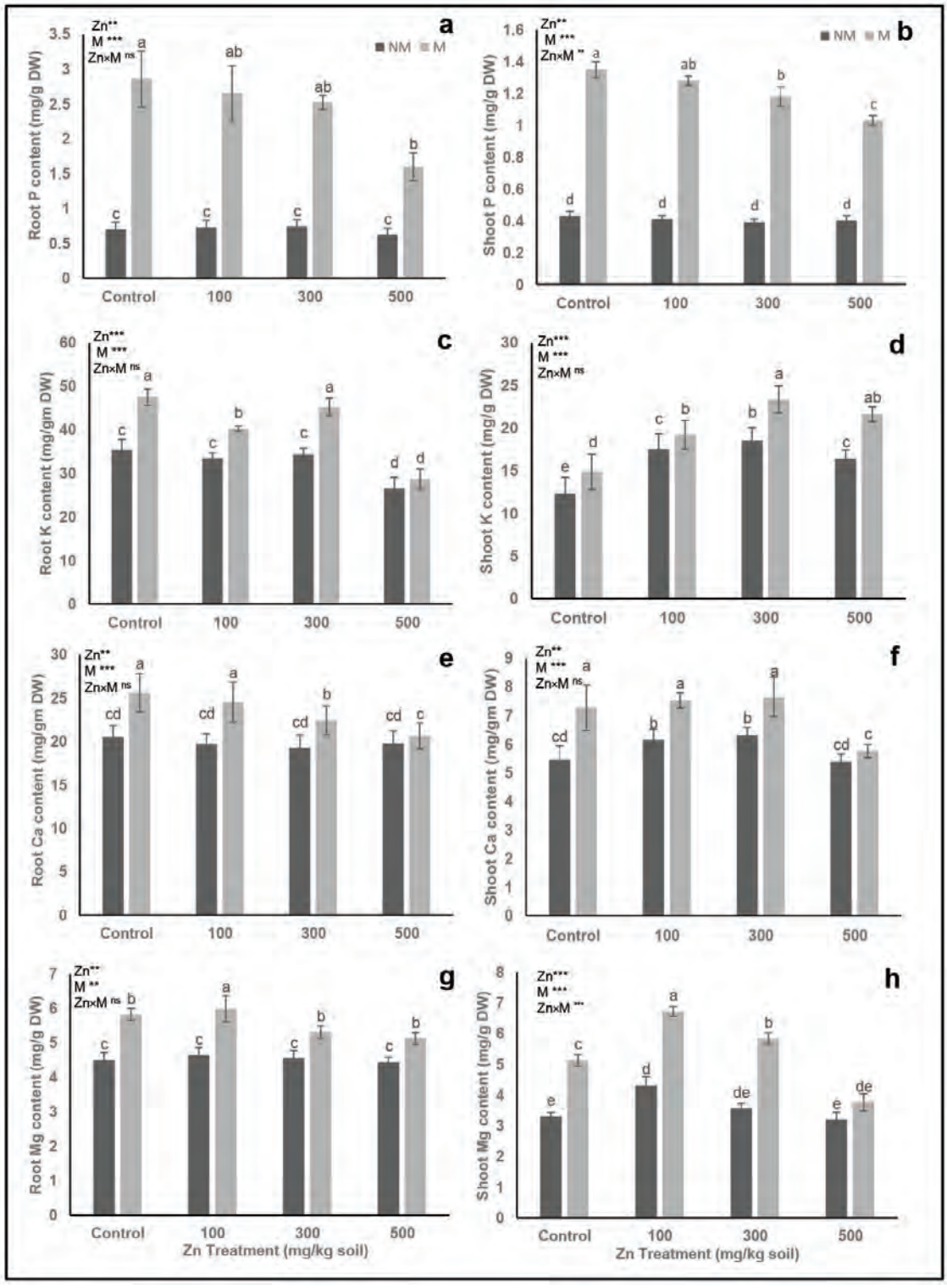


Figure 4: Effect of Zn treatment and AMF inoculation (NM: Non-mycorrhizal and M: Mycorrhizal) on a. Root P content, b. Shoot P content c.Root K content, d. Shoot K content, e. Root Ca content, f. Shoot Ca content, g. Root Mg content, h. Shoot Mg content of *T. aestivum*. Data (mean±SE) is obtained from four replicates. Values in bars with different letters are significantly different at *p*=0.05 tested through DMRT. F value of Two-way ANOVA analysis denotes significance at p e" 0.05 (*), p e" 0.01 (**), pe" 0.001(***), ns: non-significant. Zn: Zn concentration effect, M: AMF inoculation effect, Zn x M: Variable interaction effect.

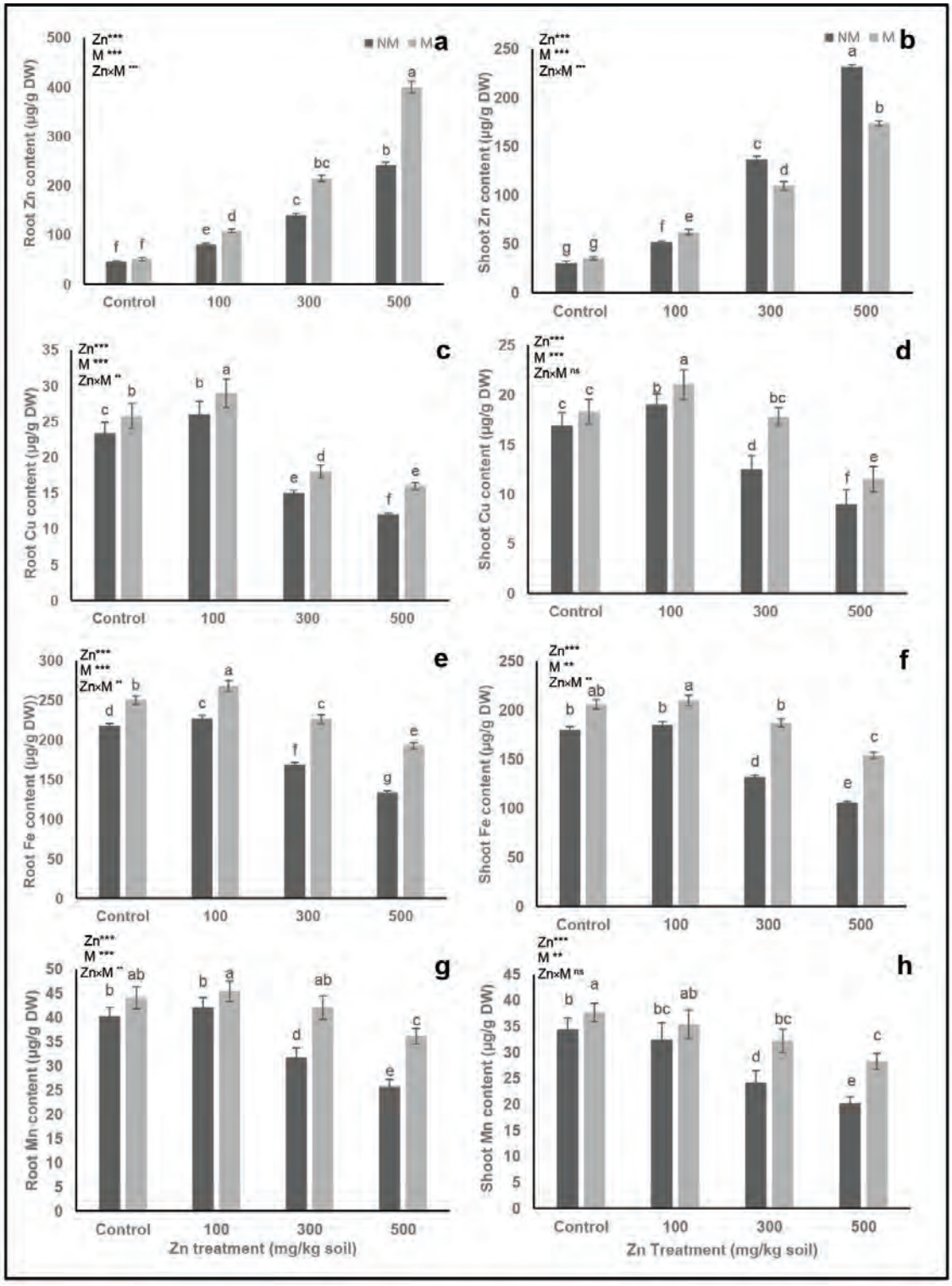


Figure 5: Effect of Zn treatment and AMF inoculation (NM: Non-mycorrhizal and M: Mycorrhizal) on a. Root Zn content, b. Shoot Zn content, c. Root Cu content, d. Shoot Cu content, e. Root Fe content, f. Shoot Fe content, g. Root Mn content, h. Shoot Mn content of *T. aestivum*. Data (mean±SE) is obtained from four replicates. Values in bars with different letters are significantly different at p=0.05 tested through DMRT. F value of Two-way ANOVA analysis denotes significance at $p \geq 0.05$ (*), $p \geq 0.01$ (**), $p \geq 0.001$(***), ns: non-significant. Zn: Zn concentration effect, M: AMF inoculation effect, Zn x M: Variable interaction effect.

Conclusion

The improvement of hydration status, enhanced pigment content and nutrient uptake of *T. aestivum* with *R. irrgularis* inoculation are linked to the better physiological condition of the plant. The modulation of the redox state through osmolyte proline and antioxidative enzyme activities are responsible for oxidative stress management under Zn toxicity. The immobilization of Zn in the mycorrhizal roots and limiting its transport to the shoot contributed to the mitigation of Zn stress. It was concluded that the *R. irregularis* inoculation in *T. aestivum* potentially alleviates Zn toxicity. Our study is the first report on *R. irregularis* mitigating Zn toxicity in *T. aestivum*. The study broadened the prospects of AMF *R. irregularis* for effective Zn stress management and sustainable agriculture. However, the omics approach to understand the host-AMF interaction under Zn toxicity needs to be undertaken.

Acknowledgments

The authors acknowledge Utkal University for providing the necessary facility to carry out the work and the RUSA 2.0 fellowship to Ankita Tripathy.

References

Abu-Elsaoud, A.M., Nafady N.A. & Abdel-Azeem, A.M. (2017). Arbuscular mycorrhizal strategy for zinc mycoremediation and diminished translocation to shoots and grains in wheat. PLOS One.12(11): e0188220https://doi.org/10.1371/journal.pone.0188220

Adeyemi, N.O., Atayese, M.O., Sakariyawo, O.S., Azeez, J.O., Sobowale, S.P., Olubode, A., Mudathir, R., Adebayo, R. & Adeoye, S. (2021). Alleviation of heavy metal stress by arbuscular mycorrhizal symbiosis in *Glycine max* (L.) grown in copper, lead, and zinc contaminated soils. Rhizosphere.18: 100325https://doi.org/10.1016/j.rhisph.2021.100325

Aebi H, Lester P (1984) Catalase *in vitro*. In: Packer L (ed) Methods in Enzymology, Academic Press, San Diego, pp 105, 121-126.

Akther, M.S., Das, U., Tahura, S., Prity, S.A., Islam, M. & Kabir, A.H. (2020). Regulation of Zn up-take and redox status confers Zn deficiency tolerance in tomato. Sci Hortic. 273: 109624.DOI:10.1016/j.scienta.2020.109624

Alengebawy, A., Abdelkhalek, S.T., Qureshi, S.R. & Wang, M.Q. (2021). Heavy metals and pesticide toxicity in agricultural soil and plants. Ecological risks and human health implications. Toxics.9(3): 42. DOI: 10.3390/toxics9030042

Alexieva, V., Sergiev, I., Mapelli, S. & Karanov, E. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell & Environment. 24(12): 1337-1344.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoxidase in *Beta vulgaris*. Plant Physio. 24: 1-151.

Bankaji, I., Pérez-Clemente, R.M., Caçador, I. & Sleimi, N. (2019). Accumulation potential of Atriplex halimus to zinc and lead combined with NaCl: Effects on physiological parameters and antioxidant enzymes activities. South African Journal of Botany. 123: 51-61.

Bates, L.S., Waldren, R.A. & Teare, I.D. (1973). Rapid determination of free proline for water stress studies. Plant Soil. 39: 205-208.

Batista-Silva, W., Heinemann, B., Rugen, N., Nunes-Nesi, A., Araújo, W.L., Braun, H.P. & Hildebrandt, T.M. (2019). The role of amino acid metabolism during abiotic stress release. Plant Cell Env. 42(5): 1630-1644.

Begum, N., Qin, C., Ahanger, M.A., Raza, S., Khan, M.I., Ashraf, M., Ahmed, N. & Zhang, L. (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Front Plant Sci. 10: 1068.

Boorboori, M. R. & Zhang, H. Y.(2022). Arbuscular mycorrhizal fungi are an influential factor in improving the phytoremediation of arsenic, cadmium, lead, and chromium. Journal of Fungi.8(2): 176. https://doi.org/10.3390/jof8020176.

Borde, M., Dudhane, M. & Jite, P. (2011) Growth photosynthetic activity and antioxidant responses of mycorrhizal and non-mycorrhizal bajra (*Pennisetum glaucum*) crop under salinity stress condition. Crop Prot.30(3): 265-271.

Chand, V. & Prasad, S. (2013). ICP-OES assessment of heavy metal contamination in tropical marine sediments. A comparative study of two digestion techniques. Microchemical Journal.111: 53-61.

Chandrasekaran, M. (2022). Arbuscular mycorrhizal fungi mediated alleviation of drought stress via non-enzymatic antioxidants: A meta-analysis. Plants.11(19): 2448. https://doi.org/10.3390/plants11192448

Coccina, A., Cavagnaro, T.R., Pellegrino, E., Ercoli, L., McLaughlin, M.J. & Watts-Williams, S.J. (2019). The mycorrhizal pathway of zinc uptake contributes to zinc accumulation in barley and wheat grain. BMC Plant Biology.19: 1-14.

Daniels, B.A. & Skipper, H.A. (1982). Methods for the recovery and quantitative estimation of propagules from soil. Methods & Principles of Mycorrhizal Research, American Phytopathological. St. Paul. pp 29-35.

Dhindsa, R.S., Plumb-Dhindsa, P.A.M.E.L.A. & Thorpe, T.A. (1981). Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and

decreased levels of superoxide dismutase and catalase. Journal of Experimental botany. 32(1): 93-101.

Di Baccio, D., Kopriva, S., Sebastiani, L. & Rennenberg, H. (2005). Does glutathione metabolism have a role in the defense of poplar against zinc excess? New Phytologist. 167: 73-80.

Erenstein, O., Jaleta, M., Mottaleb, K.A, Sonder, K., Donovan, J. & Braun, H. J. (2022). Global trends in wheat production, consumption, and trade. Wheat Improvement Springer, Cham : 47-66.

Gerdemann, J.W. & Nicolson, T. H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc. 46: 235-244.

Giovannetti, M. & Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84: 489-500.

González, L. & González-Vilar, M. (2001). Determination of relative water content. In Handbook of plant ecophysiology techniques, Dordrecht: Springer Netherlands:207-212.

Hamzah Saleem, M., Usman, K., Rizwan, M., Al Jabri, H. & Alsafran, M. (2022). Functions and strategies for enhancing zinc availability in plants for sustainable agriculture. Front Plant Sci. 7:13:1033092. doi: 10.3389/fpls.2022.1033092. PMID: 36275511; PMCID: PMC9586378.

Heath, R.L. & Packer, L.(1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of biochemistry and biophysics.125(1): 189-198.

Hemeda, H.M. & Klein, B.P. (1990). Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. J Food Sci.55(1): 184-185.

Hosseinifard, M., Stefaniak, S., Ghorbani Javid, M., Soltani, E., Wojtyla, £. & Garnczarska, M. (2022). Contribution of exogenous proline to abiotic stresses tolerance in plants: A Review. Int J Mol Sci.23(9): 5186. https://doi.org/10.3390/ijms23095186

Jiang, Y., Wang, W., Xie, Q., Liu, N.A., Liu, L., Wang, D., Zhang, X., Yang, C., Chen, X., Tang, D. & Wang, E. (2017). Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi. Science. 356(6343): 1172-1175.

Kaur, H. & Garg, N. (2021). Zinc toxicity in plants: a review. Planta. 253(6): 1-28.

Kumar, S. & Saxena, S. (2019). Arbuscular mycorrhizal fungi (AMF) from heavy metal-contaminated soils: Molecular approach and application in phytoremediation. Biofertilizers for sustainable agriculture and environment. 489-500.

Li, G., Li, C., Rengel, Z., Liu, H. & Zhao, P. (2020). Excess Zn-induced changes in physiological parameters and expression levels of TaZips in two wheat genotypes. Environmental and Experimental Botany. 177: 104133. https://doi.org/10.1016/j.envexpbot.2020.104133

Manley, B.F., Lotharukpong, J.S., Barrera-Redondo, J., Llewellyn, T., Yildirir, G., Sperschneider, J., Corradi, N., Paszkowski, U., Miska, E.A. & Dallaire, A. (2023). A highly contiguous genome assembly reveals sources of genomic novelty in the symbiotic fungus Rhizophagus irregularis. G3: Genes, Genomes, Genetics.13(6): jkad077https://doi.org/10.1093/g3journal/jkad077

Mathur, N. & Vyas, A. (1995). Influence of VA mycorrhizae on net photosynthesis and transpiration of *Ziziphus mauritiana*. J Plant Physio. 147(3-4): 328-330.

Mitra, D., Djebaili, R., Pellegrini, M., Mahakur, B., Sarker, A., Chaudhary, P., Khoshru, B., Del Gallo, M., Kitouni, M., Barik, D.P., Panneerselvam, P. & Das Mohapatra, P.K. (2021). Arbuscular mycorrhizal symbiosis: plant growth improvement and induction of resistance under stressful conditions. J Plant Nutri. 44(13):1993-2028.

Moore, S. & Stein, W.H. (1963). [117] Chromatographic determination of amino acids by the use of automatic recording equipment. Methods in enzymology, Academic Press. 6 (1963): 819-839.

Nafady, N.A. & Elgharably, A. (2018). Mycorrhizal symbiosis and phosphorus fertilization effects on Zea mays growth and heavy metals uptake. International Journal of Phytoremediation. 20(9): 869-875.

Nakano, Y. & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and cell physiology. 22(5): 867-880.

Phillips, J.M. & Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British mycological Society. 55(1): 158-IN18. https://doi.org/10.1016/S0007-1536(70)80110-3

Saboor, A., Ali, M.A., Husain, S., Tahir, M.S., Irfan, M., Bilal, M., Baig, K.S., Datta, R., Ahmed, N., Danish, S. & Glick, B.R. (2021). Regulation of phosphorus and zinc uptake in relation to arbuscular mycorrhizal fungi for better maize growth. Agronomy. 11(11): 2322. https://doi.org/10.3390/agronomy11112322

Shri, P.U., Pillay, V. (2017). Excess of soil zinc interferes with uptake of other micro and macro nutrients in *Sorghum bicolor* (L.) plants. Indian Journal of Plant Physiology. 22(3): 304-308.

Smith, S.E., Read, D.J. (2010). Mycorrhizal symbiosis. Academic Press.10.2136/sssaj2008.0015br.

INSTRUCTIONS TO AUTHORS

Plant Science Research publishes original review articles and results of scientific research in Botany, Plant Biotechnology, Biodiversity and other aspects of plant sciences as full papers or short communications in English. It is open only to the members of the Society. Publication is facilitated, if authors ensure that the manuscript and illustrations meet the requirements outlined below before they are submitted.

1. One hard copy of the manuscript along with the illustrations and a matching electronic version should be sent, preferably by email to:
   Dr. Debaraj Parida
   Associate Professor
   Department of Botany, Dhenkanal Autonomous College
   Dhenkanal-759001, Odisha, India
   Emial: odishabotanicalsociety1000@gmail.com
2. Text should be typed double spaced throughout with the left margin at least 40 mm wide. Each page of the article should be numbered.
3. The first page should carry only the title of the article, name(s) of author(s) and institution(s) where the work was done. An additional sheet should carry the *Keywords* (maximum six), a running title (not to exceed 50 letters and spaces) and the address for correspondence.
4. Papers should have an **ABSTRACT** in English not exceeding 200 words in the beginning and the usual sequence of Sections (1. Introduction, 2. Materials and methods, 3. Results, 4. Discussion and 5. References) should be followed for the text. "Results and Discussion" can also be combined into a single section, if necessary. A manuscript or review article should not exceed 20 (twenty) A4 size typed pages in double space including tables and figures. ***"Short communications"*** should have an abstract in the first paragraph but without any sub-headings like Introduction, Materials and Methods etc., and should not exceed 4 typed pages (A4 size) in double space.
5. Footnotes are not allowed, except under tables.
6. Symbols, units and nomenclature should conform to international usage. For all the numerical data metric system should be followed.
7. Correct plant names (in italics) with author citation (in normal fonts) must be provided.
8. Text references should be made using the author(s) name followed by the year of publication within parenthesis. Example: Murashige and Skoog (1962).
9. The references should be listed alphabetically at the end of the paper in the following pattern:
   i) *Journal:* Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.
   ii) *Edited volumes:* Bell, J. M. B. and Mudd, C. H. (1976). Sulphur dioxide resistance in plant: A case study of *Lolium perene*. *In*: Mansfield, T. A. (ed.) Effects of Air Pollution on Plant. Cambridge University Press, Cambridge, U.K., pp. 87-103.
   iii) *Books:* Halliwell, B. and Gutteridge, J. M. C. (2007). Free Radicals in Biology and Medicine. 4th Edn. Oxford University Press, New York. U.S.A.

   Unpublished materials and personal communications are accepted only as references in the text, not in the bibliography. The authors may refer to a latest volume of the journal "Plant Science Research" to ascertain the exact pattern of citation of references.
10. Tables should be typed on separate sheets furnished with short explanatory heading and be referred to in the text. They must be fit in a printed page within a length of either 8 or 16 cm.
11. Illustrations should be drawn on separate sheets in Indian ink and photographs be printed in black and white on glossy papers with strong contrast. They should be planned to appear with a maximum final width of either 8 cm or 16 cm. Letters, numbers and symbols must appear clearly, but should not be oversized. In diagrams, the top and right side should be boxed in by lines or the same type as the abscissa and ordinate. Each figure should be identified with author's name, figure number and indication of orientation. Combination of photographs and pen drawings in the same plate should be avoided. However, in exceptional cases, if photographs are required to be printed in colour in the journal then the author(s) will have to bear the expenses for the colour re-production.
12. Proofs of the articles will be sent to the authors by email and the same, after correction of printing errors, must be returned to the Managing Editor within seven days of receiving the proof. Corrections at the proof stage involving deviations from the original manuscript are not permissible and will be charged extra to the authors.
13. Printing cost at the rate of Rs.300/- (Rupees three hundred only) per page is charged to the authors.
14. A PDF of the article will be supplied gratis by email to the corresponding author.
15. Acceptance of any paper is subject to the recommendations of the reviewers, who are specialists in their own fields.
16. The Editorial Board reserves the right to reject any paper found unsuitable for the journal.

PLANT SCIENCE RESEARCH

Vol. 46, 2024

TABLE OF CONTENTS