



ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2026): 4.82
 IJBS 2026; 8(7): 108-119
www.biologyjournal.net
 Received: 15-04-2026
 Accepted: 20-05-2026

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Piriformospora indica: A potent biofertilizer and biopesticides for plant growth promotion

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DOI: <https://www.doi.org/10.33545/26649926.2026.v8.i7b.939>

Abstract

The growing global population has increased food demand and put considerable pressure on the agricultural sector to increase crop production. Unfortunately factors such as drought heavy metal contamination and biotic stress have a negative impact on agricultural productivity. *Piriformospora indica* is an important endophytic fungus that has been proposed as a promising solution for sustainable agriculture. Its capacity to promote plant growth, reduce various levels of stress and improve soil health is important for bio fertilizers in sustainable crop production and environmental protection. *Piriformospora indica* is a beneficial root-colonizing fungus from the Basidiomycota group, which has significant attention for its various roles in promoting plant growth and stress resistance in sustainable agriculture. This study examines the interactions between *P. indica* and host plants and emphasizes its ability to improve seed germination, promote growth and increase overall yields. *P. indica* contributes to the acquisition of nutrients by increasing the absorption of essential elements such as nitrogen and phosphorus, there by addressing the deficiencies of common soils. In addition, it increases resistance to biotic and abiotic stresses such as pathogens, insects, drought, salinity and extreme temperatures. In pharmaceutical plants, *P. indica* increases the production of secondary metabolites and thus improves their therapeutic effects. The fungus also acts as a biological control agent, which helps to further reduce the biological stress on plants. In addition, the ability of *P. indica* to improve the structure of flowers, reproductive cycles and biomass production makes it a valuable asset for increasing crop productivity. As a bioinoculator, it is an ecologically friendly alternative to chemical fertilizers and supports sustainable agricultural practices.

Keywords: *Piriformospora indica*, Sustainable agriculture, Biofertilizer, Endophytic fungus, Plant growth promotion, Stress tolerance, Nutrient acquisition

Introduction

plants rhizosphere is reservoir of diverse group of microorganism this microorganism interact with plant in various way the interaction may be harmful in case of pathogenic microorganism and can be beneficial in case of plant growth promoting microorganism. The microorganisms which are beneficial for plants are may be bacteria, fungi arbuscular mycorrhizae fungi (AMF). AMF can colonize inside root and benefit plant in various way like growth promotion to resist biotic and abiotic stress^[1]. Such type of beneficial interaction found in fungi *P. indica* and plant. *P. indica* is a basidiomycota of order Sebaciales isolated from *Prosopis juliflora* in the desert of thar rajasthan by professor Ajit verma^[2]. *P. indica* is known for producing pear-shaped spores called chlamydospores, which come from thin-walled, hyaline, and septate hyphae^[3]. These chlamydospores play a crucial role in the survival and spread of *P. indica* across different environments. A distinctive feature of *P. indica* is its ability to be grown without plant material, on various artificial media. *Piriformospora indica* has been successfully grown on different types of media, such as Kafer and potato dextrose agar (PDA). Kafer medium serves as an effective substrate for the growth and maintenance of *P. indica*, which aids in research on its biology and agricultural applications. Likewise, PDA medium, widely used for fungal cultivation, supports the axenic growth of *P. indica* by supplying the essential nutrients needed for the fungus to develop its distinctive hyphal structures and chlamydospores^[3]. This capability to thrive on various media is crucial for the successful propagation and study of *P. indica*, setting it apart from other fungi that may depend on specific plant materials for growth.

The adaptability of *P. indica* to grow on different artificial media like Kafer and PDA also enhances its potential as a biofertilizer and biocontrol agent, allowing it to be cultured and scaled for agricultural purposes without relying on plant

hosts ^[4]. These characteristics is unique for *p. indica* because many other fungi, including arbuscular mycorrhizal fungi (AMF), which usually need a plant host for their cultivation



Fig 1: (a) *P. indica* colony in modified kafer meadia

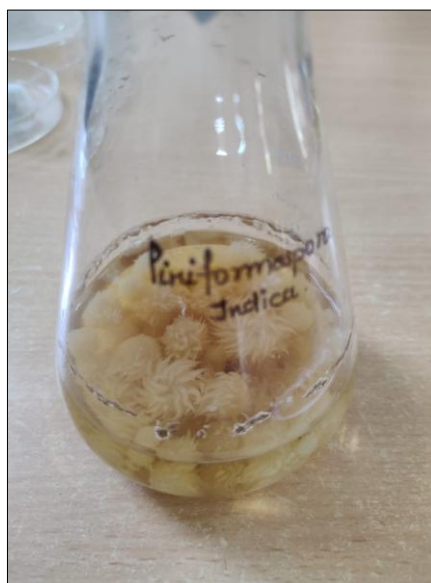


Fig 2: (b) *P. indica* chlamydospores in kafer media broth

P. indica colonize root of wide variety of plants like bryophyte pteridophytes monocot and dicot and benefit plant in various way like it promotes plant growth ^[5] promote root and shoot growth enhance seed size fruit

quality and yield ^[6]. *P. indica* also confer stress tolerance to plant by biotic factor like pathogen insect ^[7] and also help plant to tolerate abiotic factor like drought salinity stress high temperature freezing and heavy metal ^[8]

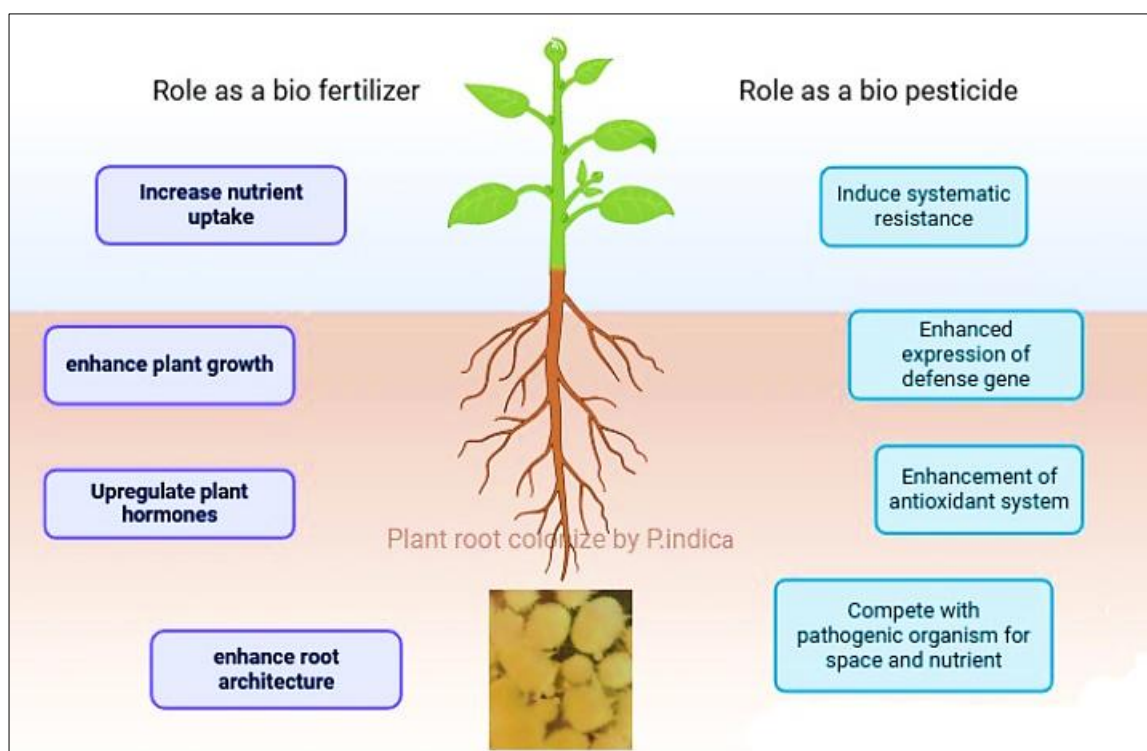


Fig 3: Piriformospora indicas role as bio fertilizer and bio pesticide

***P. indica* as a biofertilizer** **Promote plant growth**

In *in vitro* studies, *P. indica* has been found to significantly enhance seed germination and vigor across various crops, such as *Solanum melongena* (eggplant), *Capsicum annum*

(bell pepper), and *Abelmoschus esculentus* (okra) ^[9]. The fungus accelerates seed germination and boosts seedling vigor, which is crucial for developing strong plants. This effect has also been noted in other crops, including *Arabidopsis thaliana* ^[10], and orchids. These results highlight

P. indica's potential as a growth-enhancing agent, aiding in the successful establishment of vigorous seedlings.

Kumar *et al.* [11] report that *P. indica* can achieve nearly a 100% germination rate in various plant species. This is particularly important since seed germination is often the most critical stage in a plant's lifecycle, and factors affecting germination can influence overall crop yield. For instance, *P. indica* has been shown to enhance the germination and growth rates of Brassica oleracea (cabbage), Zea mays (maize), Tridax procumbens, Nicotiana tabacum (tobacco), and Oryza sativa (rice) [5]. These crops gain from the fungus's ability to improve seedling vigor, making them more resilient to early environmental challenges, which in turn supports better crop establishment and initial growth.

The advantages of *P. indica* extend beyond just the germination stage. Numerous studies indicate that this fungus increase strong vegetative growth.

The culture filtrate of *Piriformospora indica* promotes seed germination and plant growth in *Helianthus annuus* and *Phaseolus vulgaris* [12]. Research shows that *P. indica* enhances various growth parameters, including fresh and dry weight, height, leaf count, and both shoot and root development, leading to overall improved vegetative growth. Additionally, it stimulates the production of phytohormones and photosynthetic pigments [13]. The fungus also boosts nutrient uptake and efficiency, resulting in better plant growth, quality, and yield. Furthermore, *S. indica* enhances physiological traits such as inflorescence growth, longer blooming periods, and increased flower size and quantity. It accelerates flowering and reproduction by hastening the reproductive cycle of host plants. Moreover, *S. indica* has been found to enhance agricultural productivity by improving flower setting, increasing chlorophyll content, and ultimately raising overall crop yield through better plant health and growth. This symbiotic relationship between the fungus and host plants provides a sustainable method for enhancing crop productivity, particularly in less-than-ideal growing conditions.

The inoculation and colonization of *P. indica* are essential for boosting the growth and medicinal properties of various

medicinal plants [14]. Research has shown its presence in plants like *Coleus forskohlii*, *Bacopa monnieri*, *Stevia rebaudiana*, *Artemisia annua*, *Linum album*, *Trigonella foenum-graecum*, *Spilanthes calva*, *Withania somnifera*, *Chlorophytum borivilianum*, *Curcuma longa*, *Podophyllum peltatum*, *Azadirachta indica*, *Ocimum sanctum*, and *Lantana camara* [7][15]

The fungus has been found to enhance the production of chemical compounds and secondary metabolites in *C. forskohlii* [14]. In *B. monnieri*, *P. indica* promotes growth, increases bacoside levels, boosts antioxidant activity, and leads to nuclear hypertrophy [16]. Furthermore, its colonization improves the medicinal qualities of *A. indica*, *A. elegans*, and *H. annuus* [17]. In *L. album* cell cultures, *P. indica* enhances the production of the anticancer compound podophyllotoxin [15] while herbaceous plants like *S. rebaudiana* and *A. annua* show significant increases in leaf area and fresh biomass when grown with *P. indica* [18]. Field trials involving *P. indica* inoculation have also demonstrated positive growth responses in *S. calva* and *W. somnifera* [19]. Recent research has emphasized the advantages of *in vitro* co-cultivation with *P. indica* filtrates, which boost biomass productivity in *A. annua* and *W. somnifera* [19]. Additionally, cell suspension cultures of *W. somnifera* show promise for the continuous production of withaferin [19]. In summary, the symbiotic relationship with *P. indica* can be effectively harness. Inoculation with *P. indica* can significantly enhance the aboveground and total biomass of susceptible tomato variety "T07-1," while also promoting root growth in the TYLCV-resistant tomato "T07-4." Additionally, cherry tomatoes treated with *P. indica* have demonstrated marked improvements in fruit quality, yield, and storage longevity [2]. *Brassica napus* that is colonized by *P. indica* experiences accelerated growth and a substantial increase in both output and quality [20]. By stimulating the release of plant hormones, *Piriformospora indica* enhances root development. Research indicates that *P. indica* colonization can elevate IAA concentration and POD enzyme activity in the root system of *Dimocarpus longan*, which boosts the roots' nutrient absorption capabilities and supports the growth of longan cuttings [21].

Table 1: Growth and Quality Improvement in Host Plants by *Piriformospora indica*.

Host Plant	Treatment method	Growth and Quality Benefit	Reference
Tomato (<i>Lycopersicon esculentum</i>)	Root treatment for seedlings	Promotes faster growth, boosts yield, and encourages early flowering. Fruits become firmer with a sweeter and more acidic taste.	[22]
Lettuce (<i>Lactuca sativa</i>)	Treating seedlings	Increases height, fresh and dry weight, and yield. Also boosts chlorophyll and improves nutrient levels, including nitrogen, phosphorus, and potassium.	[23]
Lettuce (<i>Lactuca sativa</i>)	Root treatment for seedlings	Enhances zinc and manganese levels for healthier growth.	[24]
Chinese cabbage (<i>Brassica oleracea</i>)	Root treatment for seedlings	Encourages stronger root and shoot growth, greater biomass, and more lateral roots.	[25]
Cabbage (<i>Brassica oleracea</i>)	Seed or substrate treatment	Leads to taller plants with more leaves and better fluorescence. Also boosts chlorophyll and carotenoids, improving the plant's antioxidant properties.	[26]
Bell pepper (<i>Capsicum annuum</i>)	Seed treatment	Promotes bigger plants and higher yields, leading to more robust growth.	[27]
Spinach (<i>Spinacia oleracea</i>)	Seed treatment	Results in taller plants with increased fresh and dry weight for a stronger harvest.	[28]
Taro (<i>Colocasia esculenta</i>)	Soil treatment	Increase plant height and leaf area, while increasing defense-related phenol content and enzymes.	[29]
Sweet potato (<i>Ipomea batatas</i>)	Seedling irrigation	Encourages greater biomass, more leaves, and stronger lateral roots. Enhances photosynthetic pigments and resilience to stress.	[30]
Wheat (<i>Triticum aestivum</i>)	Root treatment for seedlings	Results in increased dry biomass in shoots and higher uptake of key nutrients like nitrogen, phosphorus, and iron.	[31]
Rice (<i>Oryza sativa</i>)	Seed and root treatment	Encourages growth, larger biomass, and higher yield, while boosting NPK content and chlorophyll.	[32]

Rice (<i>Oryza sativa</i>)	Seed and soil treatment	Increases dry weight, while enhancing phosphorus and potassium uptake for healthier plants.	[18] [33]
Barley (<i>Hordeum vulgare</i>)	Seed treatment	Boosts overall crop yield, promoting better productivity.	[34]
Finger millet (<i>Eleusine coracana</i>)	Seed treatment	Increases growth, pod count, seed weight, and shelling efficiency.	
Green gram (<i>Vigna radiata</i>)	Seed treatment	Leads to more nodules, larger leaves, and better yields, along with improved mineral uptake and chlorophyll content.	[35]
Chickpea (<i>Cicer arietinum</i>)	Seed treatment	Boosts growth and yield, and increases phosphorus uptake for healthier plants.	[36]
Sugarcane (<i>Saccharum spp.</i>)	Plantlet treatment	Promotes growth, greater cane height, more tillers, and increased sugar content. Also enhances iron and copper absorption.	[37]
Rapeseed (<i>Brassica napus</i>)	Seedling treatment	Leads to higher biomass and yield, earlier flowering, and enhanced oil content, while reducing anti-nutrient levels.	[13]
Turmeric (<i>Curcuma sp.</i>)	Bud application	Boosts overall productivity, making plants more robust. Increases curcumin and oil content, enhancing the plant's medicinal properties.	[38]
Fennel (<i>Foeniculum vulgare</i>)	Seedling treatment	Encourages plants to grow taller with greater dry weight, especially in the fruits. Increases the essential oil content, particularly anethole.	[39]
Thyme (<i>Thymus vulgaris</i>)	Shoot application	Makes the plant grow taller and stronger, with improvements in both root length and biomass. Boosts the essential oil content, particularly thymol.	[39]
Bacopa (<i>Bacopa monnieri</i>)	Micro-propagated plants	Promotes healthy growth and strengthens the plant's antioxidant properties. It also increases the bacoside content, enhancing its medicinal value.	[16]
Aloe vera (<i>Aloe barbadensis</i>)	Root treatment	Leads to better overall biomass, longer shoots and roots, and more leaves. Boosts the plant's chlorophyll, gel, aloin, and phenol content, making the leaves even more beneficial.	[40]
Artemisia (<i>Artemisia annua</i>)	Seedling root treatment	Helps plants grow taller with more biomass and higher leaf yield. It also enhances the content of important compounds like chlorophyll, flavonoids, and artemisinin.	[41] [42]
<i>Centella asiatica</i> (<i>Centella asiatica</i>)	Root treatment	Promotes greater shoot and root growth, making the plant healthier overall. Increases the asiaticoside content, known for its medicinal benefits.	[43]
<i>Coleus forskohlii</i> (<i>Coleus forskohlii</i>)	Root treatment	Enhances the plant's aerial biomass and boosts flower development. It also helps with phosphorus uptake and improves chlorophyll content	[14]
<i>Lantana camara</i> (<i>Lantana camara</i>)	Suspension culture	Increases the production of valuable compounds, like triterpenoids (ursolic acid, oleanolic acid, betulinic acid), known for their medicinal properties.	[44]
Pine vines (<i>Aristolochia elegans</i> Mart.)	Substrate application	Promotes better plant height and leaf growth, resulting in greater biomass. Increases aristolochic acid levels in the leaves, which has medicinal uses.	[45]
Alfalfa (<i>Medicago sativa</i>)	Seedling treatment	Encourages biomass growth and increases dry weight in the shoots. It also enhances nutrient uptake and boosts antioxidant enzyme activity, improving plant health.	[46]
Sweet basil (<i>Ocimum basilicum</i>)	Plant treatment	Promotes better plant growth, larger leaf area, and increased yield. Increases oil content, including important compounds like geranial, neral, and estragole.	[47]
Gerbera (<i>Gerbera jamesonii</i>)	Root seedling treatment	Boosts both above and below-ground biomass, making the plant more robust. Enhances the plant's photochemical efficiency, supporting healthier growth.	[48]
Anthurium sp. (<i>Anthurium sp.</i>)	Root treatment	Promotes better plant and root growth, leading to stronger plants. Increases phosphorus uptake and enhances chlorophyll content for better overall health.	[49]
<i>Lolium multiflorum</i> (<i>Lolium multiflorum</i>)	Seed treatment	Increases plant height and improves overall biomass. Boosts chlorophyll content and helps the plant retain more water, making it more resilient.	[50]
Banana (<i>Musa acuminata</i>)	Root treatment for plantlets	Promotes better plant height and leaf growth, with larger stems. Increases the number of suckers and fingers per bunch, leading to higher yields. Enhances chlorophyll and nutrient content.	[51]
Passion fruit (<i>Passiflora edulis</i>)	Soil application	Helps plants grow better in the later stages and increases fruit size. Also enhances fruit quality and boosts secondary metabolites.	[52]
Trifolium orange (<i>Poncirus trifoliata</i>)	Substrate application	Encourages better plant height and leaf growth, leading to improved biomass overall. Increases nutrient content, including nitrogen, phosphorus, and magnesium.	[53]
Melon (<i>Cucumis melo</i>)	Substrate application	Leads to greater fresh and dry weight in plants, with better chlorophyll content for enhanced photosynthesis.	[54]
Pineapple (<i>Ananas comosus</i>)	Substrate inoculation in root zone	Helps plants grow taller and have more leaves. Boosts photosynthetic efficiency and improves nutrient content (N, P, K, Ca, Mg), leading to healthier, more productive plants.	[55]

Mechanism of plant growth promotion

Increasing Nutrient Uptake: An Alternative to Chemical Fertilizers

Terrestrial plants need macronutrients like nitrogen, phosphorus, potassium, sulfur, magnesium, and calcium in large amounts, as well as micronutrients such as iron, manganese, copper, zinc, boron, molybdenum, chlorine, and nickel in smaller quantities for optimal growth. A lack of these vital nutrients can lead to both molecular and phenotypic abnormalities^[66]. Nutrient deficiencies are prevalent in natural soils, with only a small portion of

phosphorus (P) in soil and bedrock being accessible to plants^[67]. Likewise, almost half of the soils used for rice farming lack sufficient zinc (Zn)^[68]. Fertilization is a common approach to remedy these deficiencies, but its success is often limited by environmental conditions. For example, water-soluble zinc sulfate, a frequently used Zn fertilizer, can easily precipitate as hydroxides, carbonates, phosphates, or sulfides in the soil, leading to a low fertilizer use efficiency of just 1-5%^[68].

The environmental consequences of fertilizer production and use are a major concern. The industrial manufacturing

of nitrogen (N) fertilizers, for instance, accounts for 1-2% of the global fossil fuel energy output ^[69]. However, plants only take up about 30-50% of the nitrogen available in the soil, with the rest lost through nitrification and denitrification processes, which can result in eutrophication and other environmental issues ^[70]. Similarly, the extensive use of industrial P fertilizers not only depletes non-renewable geological P resources but also contributes to the eutrophication of freshwater and coastal ecosystems (Alewell *et al.*, 2020).

Deficiencies in key nutrients like nitrogen (N) and zinc (Zn) ^[71] can significantly impede plant growth and development. Nitrogen, an essential macronutrient, is mainly absorbed by plants as nitrate or ammonium, although some species depend on nitrogen fixation through partnerships with rhizobia. Mycorrhizal fungi play a crucial role in nitrogen delivery, preferring ammonium over nitrate and using amino acids as the primary method of nitrogen transfer to host plants ^[72].

Mechanism of *P. indica* mediated nitrogen acquisition

The contribution of *Piriformospora indica* to nitrogen acquisition has been emphasized in various studies. Sherameti *et al.* found that co-cultivating *Nicotiana tabacum* and *Arabidopsis* seedlings with *P. indica* boosts nitrogen transfer from agar media into the shoots ^[73]. This process involves the activation of NADH-dependent nitrate reductase (NR), a vital enzyme for nitrate assimilation. While the growth promotion linked to *p. indica* is associated with nitrate assimilation, the exact mechanisms are still not fully understood. Besides NR activation, *P. indica* also stimulates the expression of genes that encode glucan-water dikinase (SEX1), an enzyme important for starch breakdown during the early growth phases of *N. tabacum* and *Arabidopsis*. These actions seem to operate independently of heterotrimeric G-proteins and involve a homeodomain transcription factor that regulates the *Nia2*, *SEX1*, and 2-nitropropane dioxygenase genes. This coordinated regulation indicates a shared control mechanism for the pathways of nitrate and starch metabolism facilitated by *P. indica*.

P. indica improve phosphate uptake

Phosphorus (P) is an essential mineral nutrient, making up to 0.5% of the dry weight of plant cells. It is crucial for various regulatory, structural, and energy transfer processes ^[74]. Although phosphorus is abundant in soils, it is often not accessible to plants because it exists in poorly soluble complexes, which limits global crop production ^[74]. Plants take up phosphorus through two main methods: direct absorption using their transporters and indirect absorption through symbiotic relationships with organisms like mycorrhizal fungi ^[75].

Mechanism of *P. indica* mediated phosphorus uptake

The fungus *Piriformospora indica* has been shown to aid in phosphorus uptake. It helps transport phosphorus to the host plant through an energy-dependent mechanism, as demonstrated in studies using radiolabeled phosphorus ^[76]. Additionally, *P. indica* produces acid phosphatases that assist in accessing insoluble and complex phosphorus reserves in the soil, thereby improving phosphorus availability for the host plant. Recent research has concentrated on mycorrhizal relationships and their

significance in phosphorus acquisition ^[77]. High-affinity phosphate transporters have been identified and studied in various plant and fungal species, including *Arabidopsis thaliana*, *Medicago truncatula*, *Lycopersicon esculentum*, and *Neurospora crassa* ^[77]. However, due to the lack of stable transformation systems in arbuscular mycorrhizal (AM) fungi, comprehensive functional studies of phosphate transporters in these species are still limited ^[78].

Research has pinpointed and characterized a high-affinity phosphate transporter, PiPT, in *P. indica*. This transporter is vital for the translocation of phosphate to the host plant (Yadav *et al.*, 2010). Nevertheless, the exact mechanisms through which *P. indica* transfers phosphorus to plants are not fully understood. It is believed that two transporters are necessary: one for phosphate efflux from the host.

P. indica upregulate plant hormones that increase growth stem elongation and vigor

Piriformospora indica modulates the balance of plant hormones, which are directly involved in plant growth and development as well as stress tolerance. The level of phytohormones like auxins, cytokinins, gibberellins, and abscisic acid is regulated by *P. indica* for the promotion of root and shoot growth, uptake of nutrients, and strengthening of plant resilience during stress conditions.

- **Auxin:** Indole-3-acetic acid (IAA), a auxin that are essential for root elongation, cell division, and lateral root formation. ^[79] Demonstrated that in barley plant *P. indica* colonization in root promote higher level of synthesis of IAA which promote increase root hair density and lateral root formation. Similarly ^[80]. Found that in *Arabidopsis* plant *P. indica* colonization enhance root and shoot biomass by increasing IAA synthesis. Study show that *P. indica* increases polar auxin transport, which results in improved root architecture. This is especially advantageous for plants growing in nutrient-poor soils, where an extensive root system is necessary for optimal nutrient uptake ^[81].
- **Cytokinins:** A essential plant hormones that essential for shoot growth, cell division, delayed leaf senescence. In barley plant *P. indica* enhance cytokinins synthesis which contributes to increase shoot growth and promote prolonged chlorophyll retention and delayed senescence. ^[82]. Cytokinins contributes to nutrient mobilization in plant insuring proper distribution of nutrient in all part of plant which can enhance yields potential of crope plants ^[83].
- **Gibberellins:** A plant hormones essential for seed germination, plant elongation, and biomass accumulation. Gibberellins also increase after *P. indica* colonization in root which improves faster seedling emergence plant vigor and shoot elongation in *Arabidopsis* plant. ^[84] found that *P. indica* induce Gibberellins biosynthesis gene leading to higher synthesis of Gibberellins which promote cell division and growth in rice plant.
- **Absciscic acid (ABA):** Absciscic acid is a key hormone that produce in a plant after exposure to high temperature, drought, salinity and protect plant from such adverse condition. *P. indica* colonization also increase Absciscic acid biosynthesis and improve plant resistance for abiotic stress ^[85].

Promote growth even in stressed condition

Abiotic stress refers to harmful environmental conditions that inhibit the growth, development, and productivity of plants. These non-living stress factors include drought, salinity, extreme temperatures, flooding, and heavy metal toxicity. Unlike biotic stresses, which are caused by pathogens or pests, abiotic stress arises from environmental factors that disrupt the plant's physiological and biochemical processes [86].

Abiotic stress poses a significant challenge in agriculture, as it restricts crop yield and quality across various ecosystems. With climate change leading to more frequent and severe extreme environmental conditions, the effects of abiotic stress on global food security are becoming more pronounced [87][88]. Approximately 90% of arable land worldwide is impacted by at least one type of abiotic stress, resulting in considerable yield losses in essential crops like rice, wheat, and maize. *P. indica* colonization in plant root significantly enhances plants' ability to tolerate these abiotic factors [13].

Maintain growth in Drought Stress

Drought stress is a significant abiotic factor that greatly inhibits plant growth and agricultural productivity worldwide. It arises when there is not enough water available to satisfy a plant's requirements, often due to extended periods of low rainfall, water shortages, or changes in precipitation patterns caused by climate change [89]. The impact of drought on plants is substantial, affecting physiological processes such as water uptake, nutrient absorption, photosynthesis, and cell expansion. These disturbances can result in stunted growth, reduced yields, and, in severe cases, plant death [90].

In recent years, efforts to alleviate drought stress have concentrated on enhancing plant resilience through biotechnological methods and the application of plant growth-promoting microorganisms (PGPMs). *Piriformospora indica*, a fungus associated with roots, has attracted interest for its ability to mitigate drought stress. Research has demonstrated that it can improve drought tolerance by promoting root development, increasing water retention, and enhancing antioxidant defenses, thereby helping plants better withstand water scarcity. These beneficial microorganisms are essential in boosting plant stress tolerance, providing a sustainable approach to address drought stress in agriculture.

P. indica encourages root elongation and branching, which greatly improves the plant's ability to absorb water during drought conditions. Research has shown that it produces phytohormones such as gibberellins (Gas) and indole-3-acetic acid (IAA), contributing to stronger root systems and better drought resilience [56].

P. indica helps maintain osmotic balance in plants by boosting the levels of osmoprotective compounds like proline, soluble sugars, and glycine betaine. These substances are crucial for maintaining cell turgor and reducing the effects of dehydration. For example, Rajput *et al.* [2022] noted a significant increase in proline and sugar levels in barley plants inoculated with *P. indica* under drought stress [57].

Drought stress can cause oxidative damage in plants, leading to the formation of reactive oxygen species (ROS). *P. indica* helps alleviate this oxidative damage by enhancing the activity of antioxidant enzymes such as superoxide

dismutase (SOD), catalase (CAT), and peroxidase (POD). These enzymes play a vital role in reducing ROS accumulation, thereby protecting plant cellular structures [58].

Piriformospora indica plays a crucial role in influencing plant hormone signaling pathways, such as salicylic acid (SA), jasmonic acid (JA), and abscisic acid (ABA), which in turn enhances the plant's ability to respond to stress. It adjusts the SA and JA pathways to strengthen resistance against pests and pathogens, while ABA signaling is essential for improving tolerance to drought and heat. For example, *P. indica* has been found to activate genes related to SA, boosting systemic acquired resistance (SAR), and it regulates JA to help manage responses to biotic stress. At the same time, it influences ABA levels to enhance stomatal regulation and maintain osmotic balance during abiotic stress [83].

Maintain growth in Salt stress

Salinity stress happens when plants are subjected to high levels of soluble salts, particularly sodium chloride (NaCl), in their soil or water. This condition creates osmotic stress, which restricts water absorption, and can lead to ion toxicity, especially from sodium and chloride ions. As a result, nutrient uptake is disrupted, cellular structures can be damaged, and oxidative stress increases, all of which negatively impact plant growth and productivity [59]. Additionally, salinity can hinder photosynthesis by causing stomatal closure, which further affects plant growth and yield [96]. Salinity stress poses a significant challenge to plant growth and productivity, primarily by causing osmotic imbalance, ion toxicity, and oxidative stress. The beneficial root-associated fungus *Piriformospora indica* (*P. indica*) has been shown to help mitigate the negative impacts of salinity on plants by enhancing their tolerance mechanisms. One key way that *P. indica* supports salinity stress tolerance is through osmotic adjustment. In saline environments, the fungus encourages the accumulation of compatible solutes like proline, sugars, and betaine in plants. These substances help preserve cellular integrity and function by stabilizing proteins and membranes, thus alleviating the osmotic stress caused by high salinity. For example, proline serves as an osmoprotectant, aiding in the maintenance of cellular water balance during salt stress. *P. indica* also enhances the plant's capacity to manage ionic homeostasis in saline conditions. It boosts the uptake of potassium ions (K^+) while reducing the absorption of harmful sodium ions (Na^+), which lessens the adverse effects of salinity on cellular functions. Additionally, the fungus aids in sequestering sodium ions into vacuoles, thereby mitigating the toxic consequences of excessive Na^+ accumulation in the cytoplasm [60]. This regulation of ions is crucial for maintaining proper cellular function and metabolism under salt stress.

The oxidative stress induced by salinity leads to the production of reactive oxygen species (ROS), which can damage cells. *P. indica* boosts the plant's antioxidant defense mechanisms by increasing the activity of enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX). These enzymes help neutralize ROS, reducing oxidative damage and assisting plants in maintaining cellular integrity under stress. *P. indica* also encourages root growth, which is essential for plants facing saline stress. The increased root biomass enables plants to

more effectively access water and nutrients, helping to mitigate the adverse effects of limited water availability in salty conditions. This improved root structure also aids the plant in excluding excess salt from the root zone ^[61].

Additionally, *P. indica* affects plant hormone signaling pathways, especially those related to abscisic acid (ABA), which is vital for responding to drought and salinity stress. The fungus elevates ABA levels in plants, promoting stomatal closure and reducing transpiration, which conserves water and limits salt absorption ^[62]. *P. indica* also influences other stress-related hormones, such as jasmonic acid (JA) and salicylic acid (SA), to boost stress resilience.

Maintain growth in Heat stress

Heat stress occurs when plants are subjected to temperatures that surpass their ideal growth range, usually above 30-35 °C, causing physiological and biochemical disruptions. This condition negatively impacts various plant functions, such as photosynthesis, respiration, and water management, leading to oxidative damage, stunted growth, and ultimately lower crop yields. It also triggers the buildup of reactive oxygen species (ROS), which can harm cellular components like membranes, proteins, and DNA ^[56]. Additionally, extended exposure to high temperatures can diminish enzyme activity, disrupt metabolic processes, and hinder germination, adversely affecting plant growth and productivity. The effects of heat stress are complex, as they not only disrupt cellular functions but also affect the regulation of plant hormones, resulting in changed growth patterns and decreased stress resilience. For example, the rise in abscisic acid (ABA) levels due to heat stress aids plants in conserving water by closing stomata, but it can also stunt growth. Furthermore, heat stress often hampers the plant's capacity to absorb and transport nutrients from the soil, intensifying the stress response.

Moreover, *P. indica* has been found to influence gene expression related to stress responses, particularly those involved in the production of heat shock proteins (HSPs). These proteins are crucial for safeguarding cellular proteins from denaturation caused by heat stress, and their increased production has been associated with better heat stress tolerance. Research suggests that inoculating plants with *P. indica* can boost the expression of HSPs, thereby improving the plant's resilience to heat-induced protein damage ^[63]. This molecular response highlights the role of *P. indica* in not only alleviating the physiological impacts of heat stress but also enhancing the plant's cellular mechanisms to manage stress at the molecular level.

Maintain growth in Heavy metal toxicity

Industrialization and the growing human population have led to a significant rise in the demand for natural resources, resulting in human activities that contribute to environmental degradation and ecological imbalances. A critical example of these activities is the contamination of soil and water with heavy metals, which poses a serious threat to ecosystems, biodiversity, and human health ^[56]. While activities such as mining, industrial processes, and waste disposal are major sources of heavy metal contamination, natural events like volcanic eruptions and rock weathering also contribute to the introduction of these toxic metals into the environment. The contamination of agricultural soils with heavy metals has become a pressing issue due to its harmful effects on crop growth and

productivity. These metals can disrupt essential plant functions, leading to stunted growth, impaired nutrient uptake, and decreased overall productivity. Additionally, the bioaccumulation of heavy metals in crops presents significant risks to human health, as these toxic substances can enter the food chain and result in various health issues, including organ damage, cancer, and neurological disorders ^[64]. Heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) are particularly concerning due to their persistent, bio accumulative, and toxic characteristics. Their presence in the environment has classified them as major pollutants, as they can linger in soil and water for extended periods without degrading ^[65]. The problem of heavy metal contamination in agricultural lands has received heightened attention in recent years, especially considering its negative impact on agro-ecosystems. Heavy metal toxicity in plants can significantly disrupt the physical, chemical, and biological processes essential for plant growth, including nutrient absorption, photosynthesis, and cellular respiration. This disruption not only hampers crop productivity but also affects the overall health of the ecosystem.

Heavy metal toxicity from elements such as cadmium (Cd), lead (Pb), and arsenic (As) disrupts crop growth by causing oxidative stress, inhibiting enzymes, and creating nutrient imbalances ^[66]. These metals produce reactive oxygen species (ROS), which can lead to lipid peroxidation, protein denaturation, and DNA damage. Crops affected by these toxins exhibit symptoms like stunted growth, chlorosis, and necrosis, ultimately resulting in reduced yield and quality.

Piriformospora indica, colonization in root plays a crucial role in alleviating heavy metal stress in crops. It enhances nutrient uptake, boosts antioxidant defenses, and promotes the production of phytochelatins and glutathione for metal chelation ^{[3][58]}. For example: Barley (*Hordeum vulgare*) under Cadmium Stress: Inoculation with *P. indica* led to reduced cadmium uptake and improved plant growth by activating antioxidant enzymes such as superoxide dismutase and catalase ^[3]. under Zinc Toxicity in zea maize *p. indica* increased zinc tolerance by modulating antioxidant activity and stress-related pathways, which enhanced root and shoot biomass. Moreover, *P. indica* helps to lower oxidative stress, modulates stress hormones (like salicylic acid), and immobilizes metals in the rhizosphere. By boosting crop resilience, *P. indica* offers a sustainable strategy for managing heavy metal stress and improving agricultural productivity in contaminated soils.

P. indica as biopesticides alternative of chemical pesticide

Numerous pests, parasites, and diseases cause significant biotic stress to plants. The two types of fungal parasites are biotrophic, which feed on living host cells, and necrotrophic, which destroy host cells by releasing toxins. Vascular wilts, leaf spots, and cankers are among the illnesses that these fungi can cause ^{[67][68]}. The majority of soil-borne diseases caused by nematodes, which consume plant tissues, produce nutritional deficits, stunted development, and indications of wilting ^[69]. Chlorosis and decreased plant growth are common symptoms of viruses, which can cause both regional and systemic harm ^[70]. Additionally, insects and mites harm plants by feeding by laying eggs or by piercing or sucking them. Certain insects act as vectors, carrying viruses and bacteria that can cause

additional harm to the plants [71]. Crop plants face threats from various attacks at every stage of their life cycle, from seedling establishment to maturation and the setting of grains or fruits. These diseases can hinder normal growth, survival, and productivity, affecting vital resources needed for a healthy and secure human existence. As a result, plant diseases frequently cause substantial decreases in crop yield. Pesticides are chemical compounds used to kill pests, such as: fungi, insect, bacteria, viruses, and nematodes. Pesticides can be classified by their origin into two main types: chemical and biological. Chemical pesticides are recognized for their rapid and effective pest control capabilities. They

are made from synthetic or inorganic compounds such as sulfur, copper sulfate, lime, and ferrous sulfate. These pesticides typically have straightforward chemical structures and are highly soluble in water, which enables pests to absorb them easily. This characteristic improves their effectiveness and longevity in the environment [72].

Using beneficial microorganisms for disease control is an eco-friendly alternative that supports sustainable agricultural development. This method not only improves crop yield and quality but also decreases dependence on chemical pesticides, promoting both food safety and environmental health. [12].

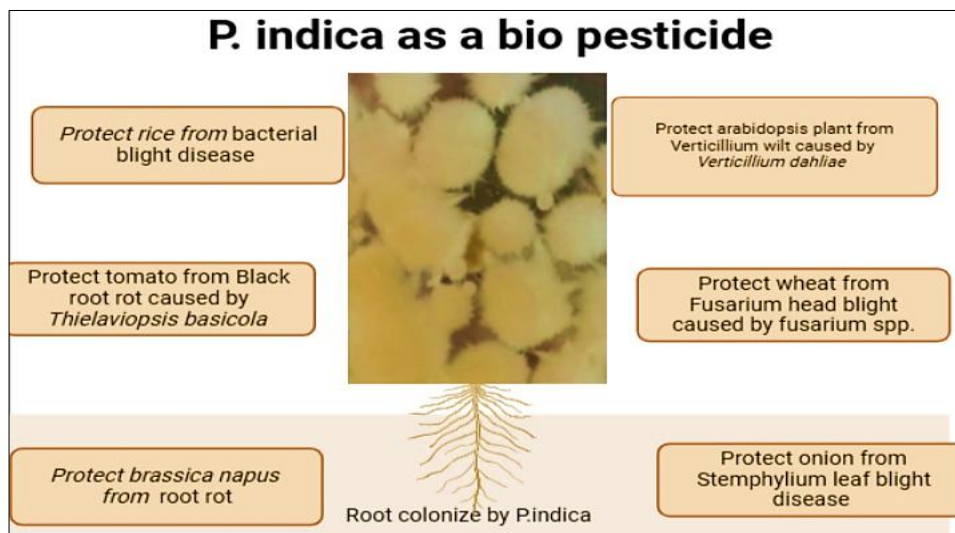


Fig 4: *piriformospora* as a bio pesticide

Piriformospora indica is considered one of the most promising microorganisms for plant protection due to its ability to regulate various physiological and biochemical processes. This includes the synthesis of antioxidants, secondary metabolites, osmolytes, and defense-related phytohormones [34]. Research has demonstrated that its colonization can enhance plant resistance to a variety of biotic stresses, such as bacterial, viral, and fungal diseases [7]. Numerous studies have highlighted its effectiveness as a biocontrol agent, providing resistance against diseases like black spot of cabbage caused by *Alternaria brassicicola* [83], Verticillium wilt caused by *Verticillium dahliae* [6] and root rot induced by *Fusarium graminearum*. [73]. These findings emphasize the important role of *P. indica* in alleviating biotic stresses across a diverse range of crops.

***P. indica* mediated biocontrol mechanism**

P. indica use various mechanism to protect plant from various pathogenic organism some of mechanism include

Induced systemic resistance

P. indica colonization in plant root induce induced systemic resistance which develop resistance for pathogenic attack and also activate defence pathway *P. indica* also induce expression of signaling pathway which regulates plant immunity these signaling molecules are salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) which activate defence gene know as pathogenesis-related (PR) proteins which codes for chitinases, glucanases, and peroxidases, which act as antifungal agents [74].

Shah *et al.*, 2011 demonstrated that *P. indica* inoculation in wheat plant induce increase activity of PR proteins and

other defense-related enzymes And reduced symptoms of Fusarium wilt caused by *Fusarium oxysporum*. [75].

Enhanced Expression of Defense Genes

In Rice plant infected with fusarium proliferatum *P. indica* colonization induce more rapid expression of pathogen related gene pR1, pR4, pR5 which suggests that *p.indoca* colonization accelerate defense mechanism. *P. indica* colonization in root also induce expression of transcription factor such as NPR1, WRKY and wRKY62, WRKY85 which are involved in regulating defense related gene and delay symptoms of *f.proliferatum*.infection in rice upto two weeks as compared to non-inoculated plant. [76].

Modulation of Programmed Cell Death (PCD)

P. indica induce programmed cell death in plant for colonization. *P. indica* induce endoplasmic reticulum stress and triggered Caspase-Dependent Cell Death Further *P. indica* induce controlled form of programmed cell death in host root and prevent the uncontrolled spread of necrotrophic pathogen like fusarium spp. In Arabidopsis thaliana plant. [73].

Enhancement of Antioxidant Systems and ROS Regulation:

P. indica inoculation in plant increase the activity of antioxidants enzyme such as superoxide dismutase (SOD), catalase (CAT), and peroxidases which neutrillize excessive reactive oxygen species generated during pathogen attack and minimize damage caused by pathogen induced oxidative stress [74]. Ros is a important signaling molecules in plant their accumulation in plant leads to cellular toxicity. *P. indica* helps in ROS balance and

protect plant from ROS induced cell death.

Competition for Nutrients and Space

P. indica colonize in plant roots cortex and reduce the space

available for pathogen colonization ^[18] Further *P. indica* enhance nutrient uptake in host plant that strengthens plant defenses and enhanced nutrient uptake make less nutrient available for pathogenic organism and inhibit their growth.

Table 2: Biotic Stress Protection Mediated by *Piriformospora indica*

Host plant	disease	pathogen	Effect of <i>P. indica</i> inoculation	reference
<i>Triticum aestivum</i>	Fusarium head blight	<i>Fusarium</i> spp.	Protection against disease	[77]
<i>Lycopersicon esculentum</i>	Verticillium wilt	<i>Verticillium dahliae</i>	Protection against disease	[6]
<i>Arabidopsis thaliana</i>	Verticillium wilt	<i>Verticillium dahliae</i>	reduction in disease development	[78]
<i>Arabidopsis thaliana</i>	Verticillium wilt	<i>Verticillium longisporum</i>	Protection against disease	[79]
<i>Arabidopsis thaliana</i>	Root rot	<i>Rhizoctonia solani</i>	Protection against disease	[79]
<i>Arabidopsis thaliana</i>	Leaf blight	<i>Alternaria brassicae</i>	Protection against disease	[80] [81]
<i>Hordeum vulgare</i>	Rhizoctonia root rot	<i>Rhizoctonia solani</i>	Protection against disease	[73]
<i>Lycopersicon esculentum</i>	Fusarium wilt	<i>Fusarium oxysporum</i>	Protection against disease	[73]
<i>Lycopersicon esculentum</i>	Black root rot	<i>Thielaviopsis basicola</i>	Protection against disease	[73]
<i>Lycopersicon esculentum</i>	Yellow leaf mosaic	<i>Pepino mosaic virus</i>	Protection against disease	[6]

P. indica mediated synthesized nanoparticle as a bio fertilizer and bio pesticide

Over the last few years, nanoparticle synthesis has experienced a major change towards more eco-friendly and sustainable routes. Conventional nanoparticle synthesis is predominantly based on toxic chemicals and energy-requiring processes, which are of environmental concern and produce toxic by-products. On the other hand, green synthesis processes provide a potential alternative through the utilization of bio-based resources such as microorganisms, plants, and agricultural waste as environmentally friendly sources for nanoparticle fabrication (Xu *et al.* 2024). Several studies have illustrated that these green synthesis processes are capable of fabricating nanoparticles with favorable properties. Microbial-mediated synthesis has proven to be very promising since microorganisms are able to reduce metal ions to produce nanoparticles. In addition, fungi-algae synthesis techniques have come to be used as a possible solution, which are sustainable and scalable in terms of producing nanoparticles (Subramaniam *et al.* 2015).

The synthesis of nanomaterials through biological methods, particularly using fungi like *P. indica*, has become a focus due to its environmentally friendly and effective nature. *P. indica* is a beneficial fungus associated with plant roots that can promote growth and enhance stress tolerance. Meanwhile, nanoparticles have unique characteristics that can increase nutrient availability and offer protection against environmental challenges. Recent research has examined the combined effects of *P. indica* and nanoparticles for agricultural purposes. For example, Wahab *et al.* 2024) studied the application of silver nanoparticles (AgNPs) produced alongside *P. indica* to increase the levels of essential plant compounds such as chlorophyll, carotenoids, and flavonoids in black rice. This combination not only improved plant growth but also enhanced the production of secondary metabolites, showcasing the positive impact of AgNPs when used with fungi. In a similar way Singh *et al.* (2017) looked into the effects of zinc oxide (ZnO) nanorods in conjunction with *P. indica* to enhance the growth and nutritional profile of broccoli. Their findings indicated that this partnership led to better plant productivity and nutrient density, underscoring the potential of nanoparticle-fungal collaborations in agricultural management.

Additionally, chemically synthesized AgNPs, and when paired with *P. indica* improved the nutritional quality of

black rice, presenting a viable option for enhancing crop quality within sustainable farming frameworks. These studies indicate that using *P. indica* for nanoparticle synthesis, or integrating it with commercially available nanoparticles, could provide a novel method for improving agricultural productivity and resilience

Challenges and Limitations as biofertilizer and bio pesticide

Host Specificity: *P. indica* can colonize wide range of plant but its colonization efficacy is vary with plant like it poorly colonize in legumes plant and these make its role as biofertilizer and as a biopesticide less effective in some plants

Scalability: for the application as bio fertilizer and as bio pesticides mass production of *p. indica* culture required. These task is full field by development of cost-effective and optimized fermentation protocols.

Competition with Other Microorganisms

In the rhizosphere other microorganism already present which can affect or compete for *P. indica* colonization ^[10]

Conclusion

As an eco-friendly alternative to chemical fertilizer and pesticides. It Enhances plant resistance against diverse biotic stresses (fungi, bacteria, viruses, nematodes). Triggers defense pathways, boosts antioxidant systems, and competes with pathogens for nutrients and space. *P. indica* can be better alternative for synthetic chemical fertilizer and pesticide for sustainable agriculture research.

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