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Original Article

Unveiling the impact of isolated phosphate-solubilizing fungi on soil fertility and sustainable agriculture: Insights from physicochemical analysis and plant growth trials

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Abstract

Soil phosphorus often becomes immobilized due to the formation of insoluble complexes. Phosphate-solubilizing fungi (PSF) help increase the availability of phosphorus in the soil, thus meeting the phosphate needs of plants. This study aims to evaluate the effectiveness of PSF as a biofertilizer. Pikovskaya's Agar medium was used to isolate fungal species from compost and open dumpsite soil. The phosphate solubilizing index and efficiency of each isolate were determined. Six PSF strains were isolated, comprising four species of *Aspergillus*, one species of *Fusarium*, and *Trametes*. The isolated strains demonstrated varying degrees of phosphate solubilization efficiency, with *Aspergillus niger* exhibiting the highest solubilization index of 2.1. A pot experiment was carried out to determine the effect of fungal strain on plant growth. The isolated fungal strain was introduced to the developed potting media which contained compost, coir-dust and soil at 1:1:1 ratio. *Vigna radiata* (Green grams) was selected as the experimental plant. Plant growth parameters (shoot and root length) were measured to determine the effects of fungal strains. Plant growth parameters were recorded after 30 days. According to the one-way ANOVA, pot experiments revealed significant increases compared to the control in the shoot ($p<0.05$) and root length ($p<0.05$) when inoculated with PSF strains, particularly *Aspergillus niger*, which yielded shoot and root lengths of 16.72±0.53 cm and 11.80±0.45 cm, respectively. These findings emphasize the potential of isolated PSF strains as effective enhancers of plant growth and highlight their role in improving soil fertility and agricultural sustainability.

Keywords: phosphate solubilizing fungi, biofertilizer, plant growth, phosphorus solubilization

1. Introduction

The global population has surged from 1.6 billion in 1900 to over 7 billion today, with projections of reaching 9 billion by 2050 (Soumare *et al.*, 2020). Feeding this rapidly growing population necessitates a significant increase in agricultural production. Chemical fertilizers are among the most effective strategies to boost global agricultural productivity. However, intensive farming practices that rely on these fertilizers are not only expensive but also pose environmental risks (Kumar & Sharma, 2023). The extensive use of chemical fertilizers in agriculture is currently debated due to environmental concerns and potential health risks for consumers. Overusing these chemicals can lead to a multitude of problems, including environmental pollution (affecting soil, water, and air), reduced input efficiency, decreased food quality, development of resistance in various weeds, diseases, and insects, soil degradation, micronutrient deficiencies, toxicity to beneficial organisms above and below the soil surface, and lower income from production (Jayathilake, Manage & Idroos, 2024b, Pahalvi, Rafiya, Rashid, Nisar, & Kamili, 2021; Srivastav *et al.*, 2023). Consequently, reducing

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the systematic use of chemical fertilizers and promoting sustainable agricultural practices has become a critical focus (Hou, Bolan, Tsang, Kirkham, & O'Connor, 2020; Singh, Bisht, Bhowmick, & Chauhan, 2024).

At present, biofertilizers have become a sustainable and alternative approach to chemical fertilizers. Biofertilizers are eco-friendly, cost-effective, non-toxic, and easy to apply; they help maintain the soil structure and biodiversity of the agricultural land (Jacob & Paranthaman, 2023). Bio-fertilizers contain living beneficial microorganisms that can colonize the rhizosphere and stimulate crop growth by increasing the supply of available nutrients to the host plant (Shahwar *et al.*, 2023). These bioinoculants infiltrate the rhizosphere and the interior of the plant when they are applied to the seed, plant surface, or soil. They enhance plant growth, crop productivity, and soil fertility. They also protect the plant from pests and diseases (Pandey, Bhattacharya, & Pandey, 2023).

Phosphorous (P) is an essential macronutrient and most soils contain high levels of P. However, its availability to plants is limited by the rapid immobilization of phosphorous compounds to insoluble forms and hence plant available forms of P in soils are found in low amounts (Jayathilake, Manage, & Idroos, 2024a). The depletion of phosphorus in soil and its limited availability for plants pose significant challenges to sustainable agriculture (Wendimu, Yoseph, & Ayalew, 2023). A greater part of soil phosphorus, approximately 95– 99%, is present in an insoluble form complexed with cations like iron, aluminum, and calcium, and cannot be utilized by plants (Bhatla, Lal, Kathpalia, & Bhatla, 2018). Among a vast range of biofertilizers, phosphate-solubilizing microbes play a vital role. In rhizosphere soil, phosphate-solubilizing fungi (PSF) can promote plant growth and increase plant production (Cheng *et al.*, 2023; Silva *et al.*, 2023). Phosphate-solubilizing fungi can enhance the solubilization of insoluble phosphate compounds (Sarmah & Sarma, 2023: Silva *et al.*, 2023). They also can mobilize and increase nutrient uptake, produce organic acids and increase the efficiency of phosphate fertilizers (Ibrahim *et al.*, 2022). Fungal species belonging to the phosphate-solubilizing genera *Penicillium, Aspergillus* and *Fusarium* are widely recognized for their potent capacity to dissolve intractable nutrients, such as phosphorus. The process by which these strains solubilize mineral phosphate is linked to the release of low molecular weight organic acids, which chelate the phosphate-bound cations by acting on their hydroxyl and carboxyl groups (Gurbanov *et al.*, 2021; Mehta *et al.*, 2019). This process transforms the phosphate into soluble forms. Extracellular phosphatase enzymes, which are produced by phosphate-solubilizing fungi, are capable of mineralizing organic P into inorganic P so that plants can use it (Arias, Heredia Abarca, del Carmen Perea Rojas, de la Cruz Elizondo & García Guzman, 2023; Rawat, Das, Shankhdhar, & Shankhdhar, 2021).

The present study records the phosphate-solubilizing and plant growth-promoting abilities of fungi isolated from compost and open dump sites. It offers new scientific insights into novel phosphate-solubilizing fungi from soil. Additionally, this research supports the development of environmentally friendly practices that enhance soil health, increase nutrient availability, and ensure long-term agricultural sustainability.

2. Methodology

2.1 Soil sample collection and analysis of soil samples

Soil samples were collected from compost sites and open dump sites in Karadiyana (6°48′ 51.8″ N, 79° 54′ 17.0″ E). About 50g of surface soil from each location was collected for the isolation process of phosphate-solubilizing fungi. Each sample was kept in a clean, sterile bottle, sealed, and transferred to the laboratory. Soil pH, electrical conductivity (EC), total soil carbon, soil organic nitrogen, and soil available phosphorus were determined following standard protocols and methods. (AOAC, 2000; Mussa, Elferjani, Haroun, & Abdelnabi, 2009; Walkley, & Black, 1934;).

2.2 Isolation of phosphate solubilizing fungi

Phosphate-solubilizing fungal strains were isolated using Pikovskaya's (PVK) Agar medium. Each soil sample (approximately 10 g) was transferred to an Erlenmeyer flask containing 90 mL of sterile water and shaken at 120 rpm for 60 min. Subsequently, a series of 10-fold dilutions of the suspension were prepared for each sample. Then, 200 µL of each dilution was plated on PVK agar, which contained 0.5 g of yeast extract, 10.0 g of dextrose, 5.0 g of tricalcium phosphate (TCP), 0.5 g of ammonium sulphate, 0.2 g of potassium chloride, 0.1 g of magnesium sulphate, 0.0001 g of manganese sulphate, 0.0001 g of ferrous sulphate, and 15.0 g of agar. The fungal isolates were purified by repeated culturing on potato dextrose agar (PDA) at 25˚C.

2.3 Determination of phosphate solubilization index

The fungal mycelium of each fungal strain was cultured on PDA at 28°C for 7 days. Subsequently, mycelial plugs were excised from the periphery of actively growing colonies using a sterile cork borer (5 mm^3). These isolated mycelium plugs were then transferred onto petri plates containing PVK agar, with uninoculated PVK agar plates serving as controls. On the seventh day of incubation, a comparative measurement of the solubilization index was conducted by assessing the clear zone and colony diameters in centimeters. The phosphate solubilization index was subsequently determined using the following formula (Doilom *et al.*, 2020).

$$\text{Phosphate solubilization index (PSI)} = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

2.4 Phosphate solubilization efficiency of fungal isolates

The phosphate solubilization activity test was conducted in 150 mL conical flasks containing 100 mL of PVK broth supplemented with 0.5% tricalcium phosphate (TCP) (pH = 7). Each fungal culture was inoculated with 10 mL of spore suspension (10^7 spores/mL), while sterile distilled water served as the control. The cultures were then incubated on a rotary shaker at 28°C and 130 rpm for 7 days. Aseptic aliquots of 1.5 mL of culture supernatant were

collected on the 2nd, 4th, 6th, and 8th days. Following the centrifugation at 12,000 rpm for 2 minutes to remove suspended solids and mycelial fragments, 0.1 mL of each culture supernatant was taken to estimate the phosphorus released from TCP. The available soluble phosphate in culture supernatants was assessed using the Bray extraction method at 882 nm (Doilom *et al.*, 2020).

2.5 Genotypic identification of fungal isolates and construction of phylogenetic tree

The DNA extraction was performed from the mycelium grown on PDA at 25°C for 7 days using the ZR Fungal/Bacterial DNA MiniPrep™ kit (Zymo Research, USA) according to the manufacturer's protocol. Subsequently, the extracted DNA samples were sent to Macrogen, Korea, for genotypic identification of fungal isolates based on Internal Transcribed Spacer (ITS) region. The phylogenetic tree was constructed using MEGA software (version 11) based on ITS region to determine the evolutionary relationships of the fungal isolates.

2.6 Determination of the effect of fungi on plant growth

The growth medium for the pot experiment consisted of a blend of compost, coir dust, and soil in a 1:1:1 ratio. The isolates were incorporated into this prepared potting mixture. Subsequently, 10 mL of spore suspensions (at a concentration of 10^7 spores/mL) from each isolate were inoculated into pots containing sterilized media. Green gram (*Vigna radiata*) seeds, obtained as a commercial product from Keells Super City (marketed under the Keells brand), were used for the growth trials. Plant growth parameters, including shoot length, root length, wet weight and dry weight, were recorded to evaluate the impact of the selected isolates on plant growth. A control pot treated with 10 mL of sterilized water was included. The pots were irrigated twice daily with 50 mL of water per day. The experiment followed a completely randomized block design with five replications per treatment. The physicochemical parameters of potting media were determined before seed germination and after 30 days of seed germination.

3. Statistical Analysis

Data were analyzed and graphed using Microsoft Excel. Data from different treatments were calculated and statistically analyzed with a one-way analysis of variance (ANOVA) using the Python 3.11 (VS code).

4. Results and Discussion

Soils are naturally rich in diverse microbial populations making it feasible to utilize these native communities to enhance crop productivity as a replacement to conventional chemical fertilizers (Bertola, Ferrarini, & Visioli, 2021; Dincă, Grenni, Onet, & Onet, 2022). In present study, soil associated with open dump site, and compost site soils, were selected to isolate and analyze the PSF.

Table 1 illustrates the soil quality parameters, showing that both compost and open dump site soils exhibit acidic pH values and high organic content. The majority of *Aspergillus* spp. and *Fusarium* spp. strains are associated with acidic environments and high nutrient availability. This might be due to their ability to produce organic acids and extracellular enzymes that facilitate nutrient acquisition and survival in such environments. Similar studies have demonstrated the correlation of *Aspergillus* spp. and *Fusarium* spp. strains with acidic and nutrient-rich environments (Šimonovičová *et al.*, 2021). These findings suggest a potential relationship between the isolated fungal strains and the soil conditions, indicating a propensity for phosphate solubilization in acidic, organic-rich environments.

The isolated PSF consisted of three *Aspergillus* spp. strains, one *Fusarium* spp. strain and one *Trametes* spp. strain. The genotyping identification confirmed that the fungal isolates as *Aspergillus flavus* (PV750653), *Aspergillus niger* (PV750652), *Fusarium proliferatum* (PV750648), *Aspergillus fumigatus* (PV750649), *Trametes cubensis* (PV750650) and *Aspergillus oryzae* (PV750651) from both compost site and open dump site soils as depicted in Figure 1. Previous studies have demonstrated the phosphate-solubilizing potential of fungal genera such as *Aspergillus* spp. and *Fusarium spp. as PSF strains* (Ahad, Sharma, Gulfishan, & Pandey, 2024; Attia, Salem, & Abdelaziz, 2024; Bashir *et al.*, 2024; Hussain *et al.*, 2024; Kumar & Sharma, 2023; Li *et al.*, 2024; Mukherjee, Roy, Parvin, & Dutta, 2024; Odoh *et al.*, 2020; Sarmah & Sarma, 2023; Xu *et al.*, 2024). However, there is currently no research studies on *Trametes cubensis* as a PSF strain for phosphate solubilization. Hence, this study contributes to the existing knowledge by suggesting that *Trametes cubensis* may possess unexplored phosphate-solubilizing potential and possible applications in sustainable agriculture.

Table 1. Physicochemical properties of collected soil samples

Parameter	Compost soil	Open dumpsite
Soil pH	3.13±0.02	6.73±0.02
Soil moisture (%)	56.70±1.2	34.70±0.6
Soil total organic carbon (%)	53.20±2.3	45.22±2.3
Soil total organic nitrogen (ppm)	0.46±0.01	0.39±0.01

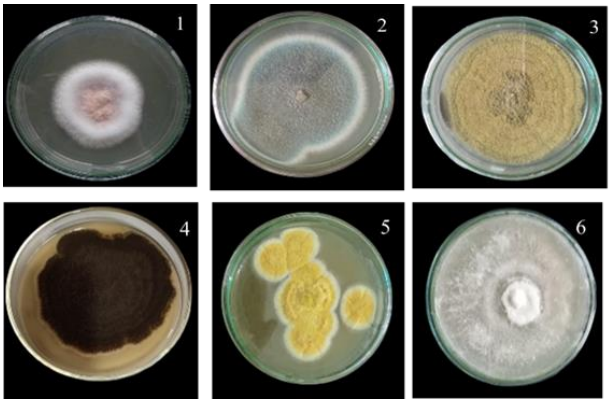

Figure 1. Isolated phosphate solubilizing fungal strains (1) *Fusarium proliferatum*, (2) *Aspergillus fumigatus*, (3) *Aspergillus oryzae*, (4) *Aspergillus niger*, (5) *Aspergillus flavus*, and (6) *Trametes cubensis*

Figure 2 illustrates the evolutionary relationships of the isolated PSF strains among *Aspergillus* species, *Fusarium proliferatum*, and *Trametes cubensis*. The evolutionary relationships among isolated PSF show diverse lineages within the *Aspergillus* genus and more distant phylogenetic ties to *Fusarium proliferatum* and *Trametes cubensis*. *Aspergillus flavus* and *Aspergillus oryzae* are closely related, sharing a recent common ancestor, while *Aspergillus niger* and *Aspergillus fumigatus* display greater divergence, indicating an earlier evolutionary split within the genus (Nargesi *et al.*, 2021). *Fusarium proliferatum* is phylo genetically more distant, branching out earlier than the *Aspergillus* species (Szabó *et al.*, 2024). *Trametes cubensis*, acting as an outgroup, is the most distantly related, suggesting a unique evolutionary path within the context of phosphate solubilization potential. These findings align with studies that highlight the phosphate-solubilizing abilities of *Aspergillus* spp. and *Fusarium* spp., although *Trametes cubensis* is less documented, indicating potential for further exploration in sustainable agriculture (Ahad, Sharma, Gulfishan, & Pandey, 2024; Attia, Salem, & Abdelaziz, 2024; Hussain *et al.*, 2024; Mukherjee, Roy, Parvin, & Dutta, 2024).

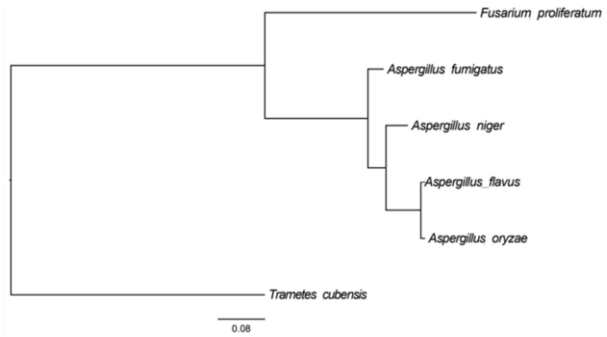

Figure 2. Evolutionary relationships of the isolated PSF strains

Figures 3 and 4 illustrate the phosphate solubilizing efficiency and pH variations during the incubation period. Among isolated PSF strains, *Aspergillus niger* showed the highest phosphorus availability, with 296.79 ± 0.21 µg/mL. Other isolated fungal strains including *Aspergillus flavus*, *Aspergillus fumigatus*, and *Fusarium proliferatum also* produced high phosphorus concentrations of 156.4 ± 0.3, 174.9 ± 0.3, and 78.9 ± 0.3 µg/mL in the second days of incubation period, respectively. In contrast, *Trametes cubensis* reached maximum phosphorus availability on the sixth day (160.7 ± 0.3 µg/mL), while *Aspergillus oryzae* peaked on the fourth day with 143.7 ± 0.3 µg/mL. These results align with previous studies demonstrating that *Aspergillus* species convert insoluble phosphate into plant available forms through organic acid secretion. The consistent pH reduction over the incubation period further supports the role of organic acids in enhancing phosphate bioavailability (Elias, Woyessa, & Muleta, 2016; Tian *et al.*, 2021; Zúñiga-Silgado *et al.*, 2020). Doilom *et al.*, (2020) have tested fungal strains for their ability to solubilize TCP on both solid qualitatively and in liquid Pikovskaya (PVK) media quantitatively. That study suggested that isolated *Aspergillus* spp. showed the most significant phosphate solubilizing activity on a solid PVK medium with the phosphate solubilization index (PSI) (2.58 ± 0.04) and the highest solubilized phosphates (1,523.33 ± 47.87 µg/mL) on a liquid PVK medium. As well, the study of Elias, Woyessa, and Muleta (2016) demonstrated a total of 359 fungal isolates that were obtained from 150 rhizosphere soil samples of haricot bean, faba bean, cabbage, tomato, and sugarcane. Among the isolates, 167 (46.52%) solubilized inorganic phosphate. The isolated phosphate solubilizing fungi belonged to genera of *Aspergillus* spp. (55.69%), *Penicillium* spp. (23.35%), and *Fusarium* spp. (9.58%). PSI ranged from 1.10 to 3.05. An isolate designated JUHbF95 (*Aspergillus* sp.) solubilized the maximum amount of P 728.77 µg/mL from TCP after 15 days of incubation. The highest (363 µg/mL) soluble-P was released from RP with the inoculation of JUHbF95 in the PVK broth after 10 days of incubation.

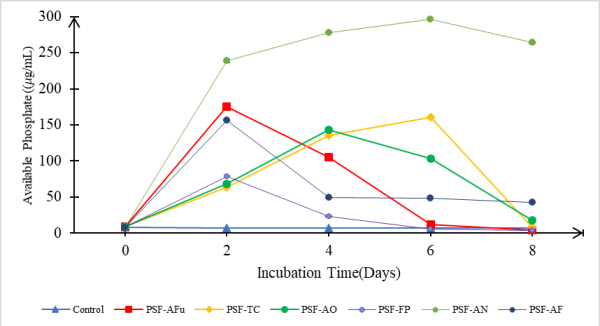

Figure 3. Solubilized P concentrations after 2, 4, 6 and 8 days of incubation in PKV broth inoculated with PSF isolates. (PSF-Afu *A. fumigatus*, PSF-TC -*T. cubensis*, PSF-AO- *A. oryzae*, PSF-FP- *F. proliferatum*, PSF-AN- *A. niger*, PSF-AF- *A. flavus*)

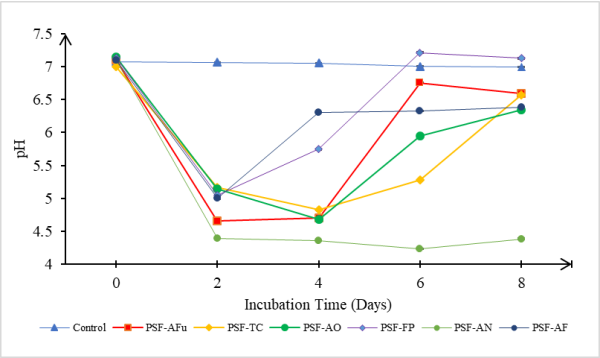

Figure 4. pH values of TCP containing PVK broth inoculated with PSF isolates after 2, 4, 6 and 8 days of incubation. (PSF-Afu *Aspergillus fumigatus*, PSF-TC -*Trametes cubensis*, PSF-AO- *Aspergillus oryzae*, PSF-FP- *Fusarium proliferatum*, PSF-AN- *Aspergillus niger*, PSF-AF- *Aspergillus flavus*)

Figure 5 shows the halo zone (clear zone) of *Aspergillus niger*, which has a phosphate solubilizing index (PSI) of 2.1. In contrast, *Fusarium proliferatum*, *Aspergillus fumigatus*, *Aspergillus oryzae*, *Aspergillus flavus*, and *Trametes cubensis* each have a PSI of 1 and did not exhibit a halo zone beyond their growth range. This low PSI value indicates that the visible zone of solubilization around microbial colonies was limited (Jokkaew, Jantharadej,

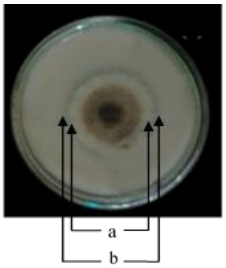

Figure 5. Clear zone formation by *Aspergillus niger* due to TCP solubilization on PVK agar after 7 days (a- colony diameter, b- halo zone diameter)

Pokhum, Chawengkijwanich, & Suwannasilp, 2022). However, this does not necessarily mean low phosphate-solubilizing potential. It suggests that the solid media environment may restrict the diffusion of organic acids and other solubilizing agents, thereby limiting zone formation. In contrast, *Aspergillus niger* exhibited a PSI of 2.15±0.01, showing a larger solubilization zone, likely due to its higher production of organic acids, which dissolve the insoluble phosphate into bioavailable forms. Moreover, Kumari, Senaratne, and Wijesinghe (2024) have shown that *Aspergillus niger* is a prominent PSF strain, exhibiting a clear zone due to solubilization of phosphorus.

Green grams (*Vigna radiata*) were used as the experimental model to study the influence of isolated phosphate-solubilizing fungi on plant growth. Green grams were selected for their numerous advantages including ease of cultivation, a short growth cycle, wide availability, adaptability to various environments, and sensitivity to nutrient levels (Favero *et al.*, 2021; HanumanthaRao, Nair, & Nayyar, 2016; Huppertz *et al.*, 2023). Figures 6 illustrates the variations in growth parameters observed among potting media inoculated with isolated PSF strains.

The inoculation of potting media with PSF strains remarkably enhanced the growth of green grams, with notable differences in shoot length, root length, wet weight, and dry weight compared to the control. Among the tested fungal strains, *Aspergillus niger* exhibited the highest shoot length (16.72 ± 0.53 cm) and root length (11.80 ± 0.45 cm), demonstrating its effectiveness in promoting plant growth. *Aspergillus oryzae* also demonstrated significant growth-enhancing potential ($p<0.05$), with a shoot length of 16.16 ± 0.34 cm and a root length of 10.51 ± 0.53 cm. The control plants showed lower shoot and root lengths of 9.96 ± 0.82 cm and 5.44 ± 0.32 cm, inferior to the plants grown in potting media treated with isolated fungal strains. The wet weight was highest with *Aspergillus niger* (0.69 ± 0.11 g), followed closely by *Aspergillus oryzae* (0.67 ± 0.12 g) and *Trametes cubensis* (0.68 ± 0.05 g), while the control exhibited a significantly lower wet weight of 0.34 ± 0.05 g. Dry weight was also highest with *Aspergillus oryzae* (0.12 ± 0.03 g) and *Aspergillus flavus* (0.12 ± 0.03 g), compared to the control's dry weight of 0.06 ± 0.02 g. Higher water absorption and early vegetative development are major reasons for the difference between wet and dry weights of mung bean plants grown under *A. niger* and *T. cubensis* treatments compared to higher structural biomass accumulation (Lubna *et al.*, 2018; Mundim *et al.*, 2022; Ouledali *et al.*, 2018). This might be due to enhanced nutrient availability and hormonal changes affecting

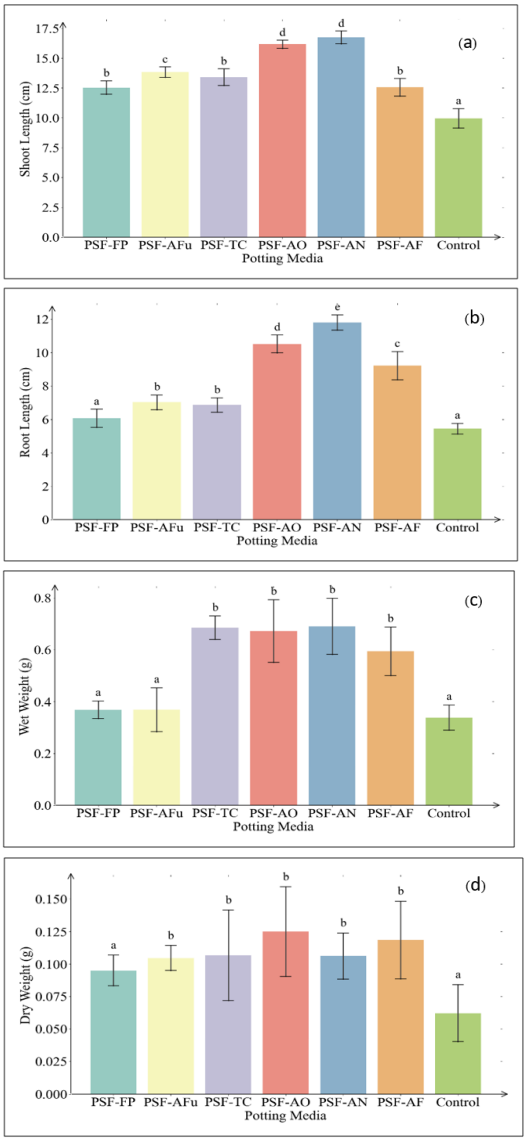

Figure 6. Variation in growth parameters of green grams, (a) shoot length, (b) root length, (c) wet weight, and (d) dry weight, on using potting media inoculated with isolated PSF strains. (PSF-Afu *A. fumigatus*, PSF-TC -*T. cubensis*, PSF-AO- *A. oryzae*, PSF-FP- *F. proliferatum*, PSF-AN- *A. niger*, PSF-AF- *A. flavus*)

water balance and tissue hydration. In the present study, *Trametes cubensis* exhibited significant growth-promoting effects on green grams. However, it has not been previously documented for such applications. These PSF strains are known to secrete organic acids, which enhance the availability of phosphate, a key secondary nutrient for plant growth, by lowering the pH of the soil and dissolving bound phosphates into plant-accessible forms. This ability of PSF strains to improve nutrient availability emphasizes their value as biofertilizers for sustainable agriculture. Previous studies have provided additional evidence supporting the growth-promoting abilities of both *Aspergillus* spp. and *Fusarium* spp. (Escobar Diaz, Gil, Barbosa, Desoignies, & Rigobelo, 2021;

Srivastava, Mehta, & Sharma, 2011). Furthermore, Kumari (2014) demonstrated that co-inoculation with *Aspergillus* and *Penicillium* species had a stimulatory effect on shoot and root lengths in *Vigna radiata*. This increase in growth could be attributed to the enzymes and organic acids secreted by PSF, which dissolve phosphorus, making it available for plant cell division and enlargement. Hence, this study reveals the potential of the isolated strains, including *Aspergillus* spp., *Fusarium* spp., and *Trametes* spp., as prominent phosphate solubilizing strains and analysis of the growth parameter data suggests that the isolated strains of *Aspergillus* spp., *Fusarium* spp., and *Trametes* spp. are the most effective in enhancing growth parameters.

Table 2 presents the variation in physicochemical parameters of the potting media before seed germination (Initial) and after 30 days of seed germination.

Initially, the potting mixture exhibited an acidic pH of 6.83 ± 0.02. Subsequently, the pH of all potting media inoculated with PSF strains and the control shifted towards alkaline. The control potting media showed the highest pH (8.23 ± 0.08), above the other media inoculated with PSF strains. The initial electrical conductivity (EC) of the potting media was measured at 1,040.67 ± 7.51 µS/cm. After 30 days of seed germination, the EC of PSF-inoculated potting media had decreased. Potting media inoculated with PSF-AN exhibited a higher available phosphate concentration (8.78 ± 0.09 mg/kg), possibly attributable to the phosphate solubilization ability of *Aspergillus niger*. The available phosphate concentration in potting media inoculated with isolated PSF strains was higher compared to the control potting media. This disparity can likely be attributed to the phosphate solubilization ability exhibited by these isolated fungal strains through the formation of organic acids. The chelating effect of organic acids facilitates the dissolution of phosphate minerals, making phosphorus more accessible to plants for uptake. As a result, potting media inoculated with PSF strains exhibit higher levels of available phosphate compared to control media. This increased availability of phosphate in the root zone promotes better nutrient uptake by plants, consequently enhancing their growth and development. Initially, the organic carbon content of the potting mixture was measured at 7.73 ± 0.13%. However, after 30 days of seed germination, the organic carbon contents had decreased. This reduction could be attributed to the utilization of organic carbon by the growing green grams and microbial activity in the potting media.

Potting media inoculated with these fungi exhibited increased availability of phosphate and demonstrated significant improvements in green gram growth parameters compared to control media. The findings highlight the importance of phosphate-solubilizing fungi, particularly *Aspergillus niger*, in enhancing soil fertility and agricultural sustainability through their role in improving plant growth and nutrient availability.

5. Conclusions

The present study emphasizes the importance of phosphate solubilization in enhancing plant growth parameters and highlights the potential of isolated fungal strains, particularly *Aspergillus niger*, *Fusarium* spp., and *Trametes* spp. Among the isolates, *Aspergillus niger* demonstrated the highest potential as a biofertilizer candidate, indicating its effectiveness in sustainable agricultural applications. Further research into the underlying mechanisms of phosphate solubilization by these fungi holds promise for developing innovative strategies to optimize nutrient uptake and enhance soil fertility.

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Table 2. The variation of physicochemical parameters of potting media before seed germination (Initial) and after 30 days of seed germination

Parameter	Before seed germination	After 30 day of seed germination						
	Initial	PSF-FP	PSF-AFu	PSF-AO	PSF-AN	PSF-AF	PSF-TC	Control
pH	6.83 ± 0.02	7.91 ± 0.08	7.94 ± 0.03	8.04 ± 0.03	7.92 ± 0.02	8.03 ± 0.02	8.02 ± 0.03	8.23 ± 0.08
EC (μS/cm)	1040.67 ± 7.51	163.83 ± 1.46	129.2 ± 1.06	120.0 ± 1.51	119.83 ± 0.81	115.67 ± 2.31	121.80 ± 1.87	115.23 ± 1.26
Available phosphorus (mg/kg)	1.74 ± 0.01	2.39 ± 0.07	3.18 ± 0.06	2.97 ± 0.06	8.78 ± 0.09	2.93 ± 0.04	2.82 ± 0.02	1.90 ±0.11
Organic carbon(%)	7.73 ± 0.13	5.89 ± 0.11	7.02 ± 0.15	5.56 ± 0.12	5.85 ± 0.29	4.07 ± 0.39	5.88 ± 0.26	7.01 ± 0.08

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