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Screening of three fungal strain for plant-growth-promoting characteristics: An investigation into phosphate solubilization, IAA production, and siderophore synthesis

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Abstract
Phosphate-solubilizing fungus (PSF) makes phosphorus in soils far more available to plants. The goal of this study was to look at a number of soil fungus to see which ones help plants develop better. The focus was on their ability to break down phosphates, make siderophores, and make indole-3-acetic acid (IAA). We used rDNA sequencing of the ITS regions to figure out what kind of fungi they were, and then we tested how well they could make things grow in a lab setting. *Aspergillus sydowii*, *Aspergillus terreus*, and *Penicillium capsulatum* all dissolved phosphates at varying rates. *A. terreus* dissolved the most tricalcium phosphate (TCP). *A. terreus* produced the most IAA (17.82 µg/ml), followed *by P. capsulatum* and *A. sydowii*. Also, *A. terreus* produced the most siderophores, which suggests that it may help plants take in more iron. We tested *Vigna mungo*, *Sorghum bicolor*, and *Glycine max* in pots. We employed eleven treatments and three copies of soil that had been sterilized and dirt that had not been sterilized. The plants that got the biofertilizer grew a lot taller, had longer roots, and weighed a lot more when they were wet. It is important to correctly identify species and screen for PSF so that researchers can have a clearer idea of the taxonomic picture when choosing suitable taxa for future plant growth-promoting applications. These results show that these fungal strains, whether employed alone or in combination, have important traits that help plants grow and might be exploited as bioinoculants in sustainable farming.

Keywords: Phosphate solubilisation, plant growth promotion, biofertilizers, fungi

Introduction
Fungi may be found in tap water, sea water, industrial water and soil rhizosphere (Dayarathne, 2020; Doilom *et al.*, 2020; Dong *et al.*, 2020) [8, 10, 11]. Fungi are very important to the environment and the economy because they offer food, medicine, and help break down things (Hyde *et al.*, 2019) [15]. Fungi research might also help microbial biotechnology and other disciplines (Hyde *et al.*, 2018) [14]. Because of their special properties, fungi are very good in encouraging the development of plants. In contrast to bacteria, which have been extensively researched for their characteristics that promote plant development, fungi create vast networks of mycelial cells that expand their surface area for absorbing nutrients, which improves their capacity to solubilize phosphorus (Jiang *et al.*, 2021) [17]. Furthermore, fungal strains are stable and dependable options for the production of biofertilizers because they often maintain their growth-promoting characteristics even after many rounds of sub-culturing (Saranya *et al.*, 2022; Kumar & Prasher, 2023) [21, 32].

Insoluble phosphate (P) may be broken down by phosphate-solubilizing fungus (PSF) into accessible forms that plants can employ for a variety of metabolic functions (Dev *et al.*, 2023) [9]. They may also move nutrients around, produce organic acids that either reduce the pH of the soil to solubilize phosphorus or chelate its cationic partners (Yang *et al.*, 2022) [38], and increase the efficiency of phosphate fertilizers like phosphorite and single super phosphate (SSP) (Li *et al.*, 2015) [22]. Furthermore, fungi synthesize phosphatases like phytase, which catalyze the hydrolysis of organic phosphates, which releases phosphorus into forms accessible to plants (Moropana *et al.*, 2024) [26]. Numerous fungi generate IAA, a crucial phytohormone that is important for plant growth, including root elongation, cell division, and stress tolerance

(Rico *et al.*, 2023) [31]. another significant characteristic that fungus develop to aid in iron absorption in soils lacking in iron is siderophores (Zhang *et al.* 2018; Kabir & Bennetzen, 2024) [18, 40]. Therefore, the goal of this work is to find fungal strains that have the ability to synthesis siderophores, generate IAA, and solubilize phosphate qualities essential for plant nutrient absorption and growth (Nagrale *et al.*, 2023) [27].

According to Alori *et al.*, 2017; Kalayu, 2019 [4, 19], several phosphate-solubilizing fungal species, such as Aspergillus, Penicillium, Trichoderma, Rhizopus, Sclerotium, Talaromyces, and Fusarium have been isolated from plants and their root rhizospheres to date (Bao *et al.*, 2025) [5]. Further research on PSF would enhance the advancement of commercial PSF biofertilizers and the optimization of worldwide phosphorus fertilizer efficiency.

This study involved the screening of three fungal strains for their plant-growth-promoting traits phosphate solubilization, IAA production, siderophores synthesis and evaluate their potential as biofertilizers. Species are recognized through the application of phylogenetic analyses. We identify new species by examining morphological traits and conducting phylogenetic studies. This investigation offers fresh scientific perspectives on novel PSF extracted from the soil.

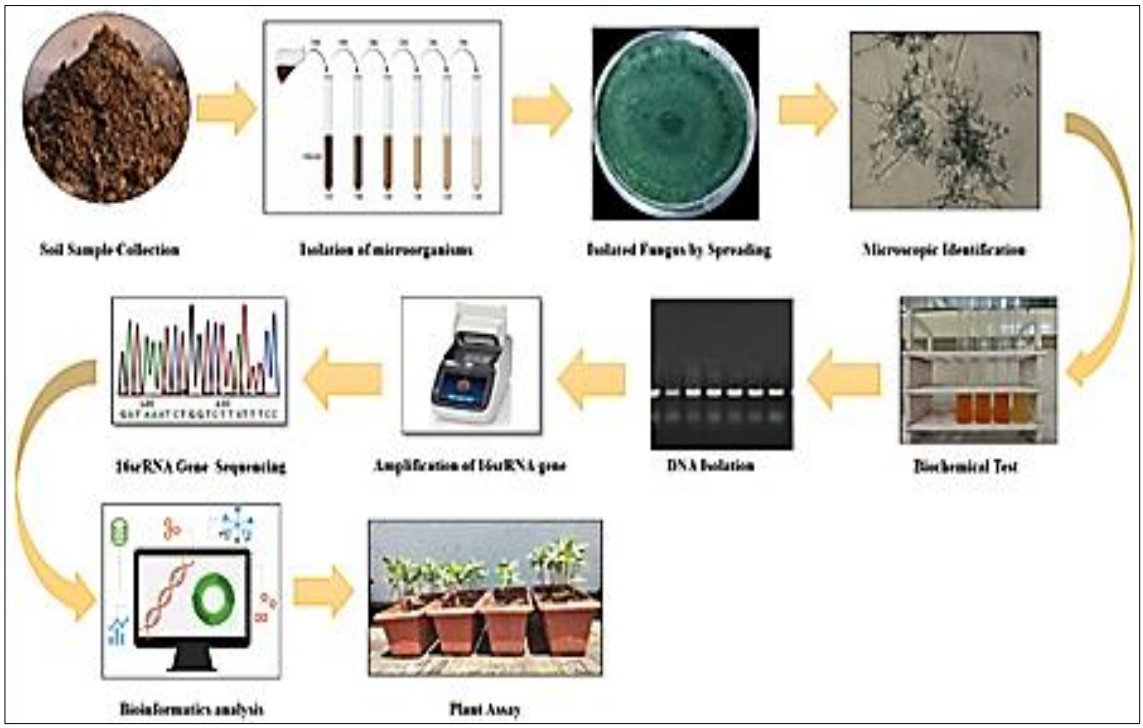

Fig 1: Graphical Abstract.

2. Materials and Methods

2.1 Isolation Method

In Conical flask, 1 gm of soil was mixed with 10 ml of sterile distilled water. We put the flasks with the soil suspensions in a shaking incubator overnight at ambient temperature and 160 rpm to let the microbes resuspend. After incubation, the suspensions were diluted in a series of steps up to 10−5 to lower the number of microbes, making it easier to separate different colonies. We placed 100 µl of each dilution onto PDA and PKVK agar medium plates. We found phosphate-solubilizing fungus because they produced a halo zone in PKVK media. TCP was in the PKVK medium, and since the PSMs could break down the less-soluble forms of phosphates, they used the phosphates in the culture media. The process created a clean zone surrounding the microbial colony. We placed the fungi that grew on PDA plates into a new culture and stored it at 4°C in a refrigerator.

2.2 Fungal strain identification

Arihant Bioscience (India) Pvt. Ltd. in Ankleshwar, Gujarat, India found the fungal isolates ABPL_F_002, ABPL_F_004 and ABPL_F_012. ITS DNA sequencing was used to figure out the strain. We followed the manufacturer's instructions to isolate and clean fungal DNA using SLS Research's gDNA Purification Kit (mini)Cat. No: #SCMR006. We used PCR forward and reverse primers to make the ITS1F-ITS4R region bigger for ITS DNA sequencing. The forward primer was ITS-1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and the reverse primer was ITS-4 (5′-TCC TCC GCT TAT TGA TAT GC-3′). The amplification was done using SafeAmp PCR Master Mix (CAT. No.: #SCMB010) in 30 µL reactions. The PCR process began with an initial denaturation at 95 °C for 5 minutes. Then, there were 35 cycles of 95 °C for 30 seconds (subsequent denaturation), 50 °C for 30 seconds (annealing), 72 °C for 50 seconds, and ended with a final extension at 72 °C for 7 minutes. After the last stretch, the temperature dropped to 4 °C. We utilized the obtained sequences to identify species by searching databases using http://www.ncbi.nlm.nih.gov/BLAST/. In order to better identify the fungal strains, a phylogenetic tree was inferred using a technique outlined in Makulana *et al.* (2024) [23]. Using MEGA6 software, the neighbor joining (NJ) technique was used to create the phylogenetic tree.

2.3 Quantitative estimation of phosphate solubilization

We tested the three fungal strains, A. sydowii, A. terreus and P. capsulatum for both inorganic and organic forms of phosphate. We utilized a 1 cm agar block containing fungal isolates from PDA agar to inoculate 100 ml of PKVK broth with a pH of 6.5 in a 250 ml conical flask. Tri-calcium

phosphate was added to the broths separately as the only sources of insoluble P complex. We didn't put any fungus in the control flasks for each P source. Instead, we put them all in a rotary shaker at 150 rpm and incubated them at 28 °C for 6 days. Every day, 10 ml of each flask was taken out to measure the solubilized phosphate. We centrifuge the samples at 10,000 rpm for 10 minutes to get cell-free supernatants. We used the Olsen extraction technique as per Nelson & Sommers (1982); FAO (2021); Mayadunna *et al.* (2023) [24, 28] to quantify the soluble phosphate in the cell-free supernatants. The color-developing reagent was combined with the cell-free supernatant and put at room temperature for 30 minutes to see the color change to blue. We utilized solutions of monobasic potassium phosphate with known concentrations as a reference and measured the absorbance at 880 nm.

2.4 Indole-3-acetic acid (IAA) production: The fungal strains A. sydowii, A. terreus, P. capsulatum were screened for Indole-3-acetic acid (IAA) production using colorimetric assay method described by (Wang *et al.*, 2020; Vasava *et al.*, 2025) [36, 37], with some modifications. The fungal isolates were cultivated for five days at 28 °C in the dark in peptone broth (either without L-tryptophan or with 1 mg/L of L-tryptophan added). Following a 10-minutes centrifugation at 10,000 rpm to extract the cells from the culture medium, 1 ml of the supernatant was violently combined with one drop of OPA and 2 ml of Salkowski's reagent (50 ml of 35% perchloric acid and 1 ml of 0.5 M ferric chloride hexahydrate) and incubate for 30 minutes at RT. We used spectrophotometry at 530 nm to determine the quantity of IAA in the culture supernatant. We used solutions with known IAA concentrations as standards to figure out how much IAA was produced.

2.5 Siderophores production: The synthesis of siderophores was assessed using chrome azurol S (CAS) agar (Ferreira *et al.*, 2019; Qessaoui *et al.*, 2024) [13, 30]. The isolated fungus strain agar blocks was put onto CAS agar, incubated at 30 °C for 5-7 days. The orange-yellow halos surrounding the fungal colonies showed that they were making siderophores. Calculate the ratio (D/d) of the orange aperture diameter (D) to the colony diameter (d) to assess the iron-producing capability of the strain. We made CAS agar using the technique described by Schwyn & Neilands (1987) [33].

2.6 Evaluation of plant growth promotion by individual inoculation

The strain was put in PDB liquid medium with a 1% inoculum, put in a constant temperature oscillator at 28 °C and 150 r/min, and then let to grow for 5 days before being made into a suspension of 1 × 108 CFU/ml. We utilized seedlings as test subjects and put them in 30 x 15 cm containers with nutrient-rich soil as the growing medium. After a while of growing, each seedlings that had the same growth parameters were chosen for treatment. Each seedling had 30 ml of irrigation in every 7 days, for a total of 4 times. At the same time, fresh water was utilized as a control. Before the first treatment, the height and thickness of the stems were measured. We assessed the height, root length, wet weight, and dry weight of the plants after 40 days of treatment.

3. Results

3.1 Molecular identification of soil fungi

Three isolates A. sydowii, A. terreus and P. capsulatum were chosen for further research because they did a better job of breaking down phosphates in the quantitative analysis. These three fungal isolates were from a library of soil fungus that were gathered from the undersoil of decaying plant materials. They were selected because they may have features that help plants thrive. When the isolates were looked at under a microscope (40X magnification), it was clear that they were different in terms of coloration, sporulation, and hyphal development (Figure 2).

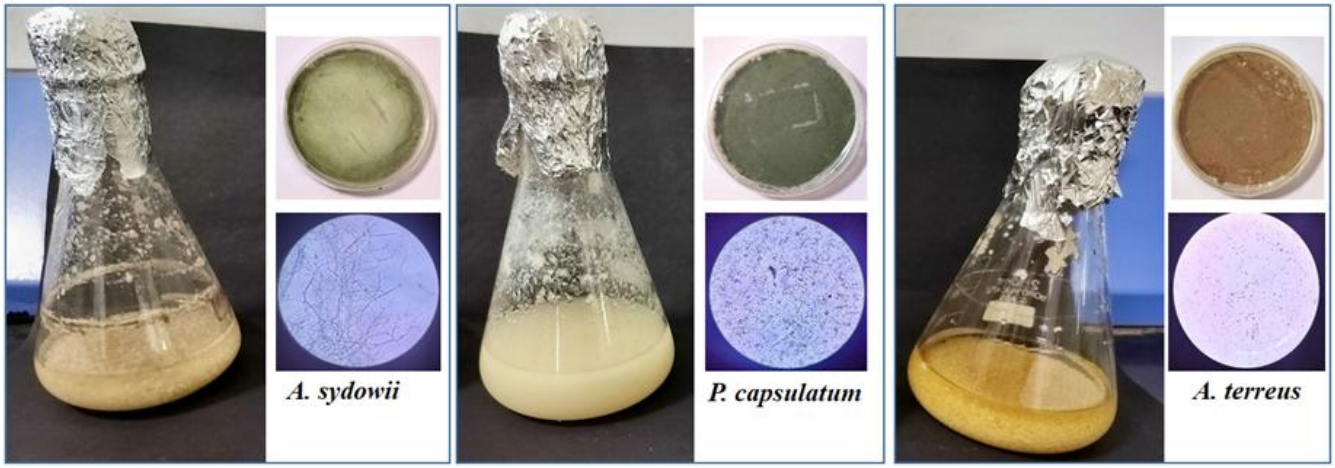

Fig 2: Pictures of the three fungal isolates that can dissolve P. They were cultivated on PDB medium on PDA plates, and their microscopic view.

After comparing the sequences of their ITS region with ones that are already in the NCBI database, it was clear who they were. The fungus ABPL_F_002 was 99% similar to Aspergillus terreus, ABPL_F_004 was 97% similar to Aspergillus sydowii, and ABPL_F_012 was 100% identical to Penicillium capsulatum (Table 1).

Table 1. Similarity between the sequence queried and the biological sequences within the NCBI database using BLAST.

Sample Name	ABPL_F_002	ABPL_F_004	ABPL_F_012
Predicated organism	*Aspergillus terreus*	*Aspergillus sydowii*	*Penicillium capsulatum*
Genebank Accession	PV659819	PV659831	PV659832
Identity	99%	97%	100%

3.2 Phylogenetic tree inferred for fungal strain identification

ABPL_F_002 is quite similar to Aspergillus terreus (LC593154.1), Aspergillus sp. (OR351419.1), and Aspergillus terreus (MT936887.1). A high bootstrap value (99%) backs this up, which means that there is a lot of confidence in this classification. The phylogenetic analysis shows that ABPL_F_002 is probably an Aspergillus terreus strain since it is quite similar to other Aspergillus terreus genomes. ABPL_F_004 Identification: ABPL_F_004 is quite similar to Aspergillus sydowii (HM016908.1), which makes it a separate group within the Aspergillus genus. This group has moderate bootstrap support (99%), which means that there is some confidence in this link. It is believed that ABPL_F_004 is a strain of Aspergillus species, maybe Aspergillus sydowii. ABPL_F_012 is quite similar to Penicillium capsulatum (JX841238.1), Penicillium capsulatum (MN031386.1), and Penicillium capsulatum (MZ568241.1) (Figure 3). MEGA6 was used to conduct evolutionary analysis.

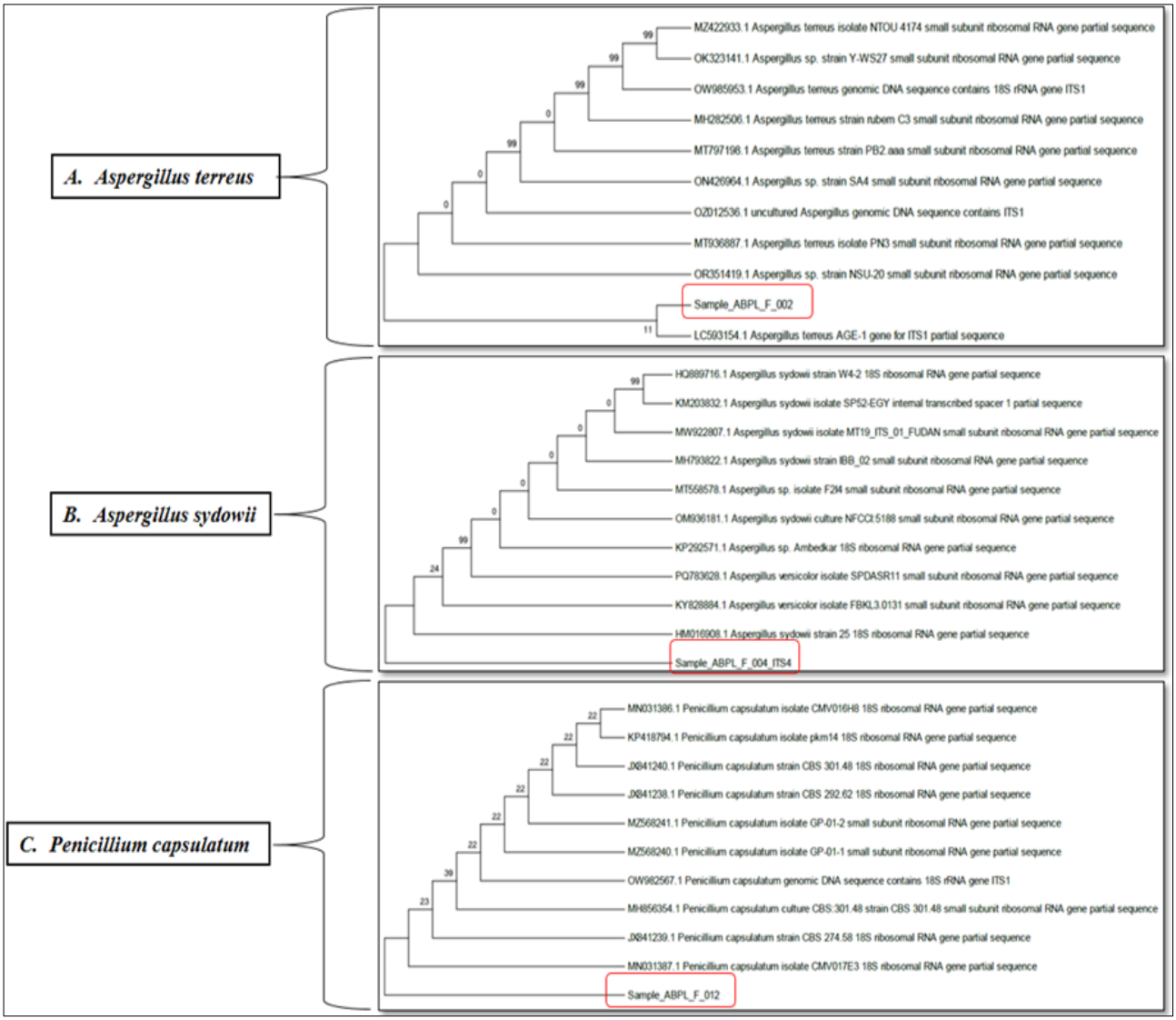

Fig 3: Neighbor-joining tree deduced using the ITS sequence of the three fungal strains isolated from the soil with reference fungal strains from NCBI.

3.3 Formation of clear zones and phosphate solubilization index on PKVK agar medium

The clear zone became visible in the isolates A. terreus and P. capsulatum on the 2nd day. The clear zone of the other fungal isolates became visible on the 3rd day and did not show the clear zone around colonies after the 7th day but solubilized TCP by removing white spots on PKVK agar (Figure 4). The isolate A. terreus showed the widest clear zone around the fungal colony (Figure 4).

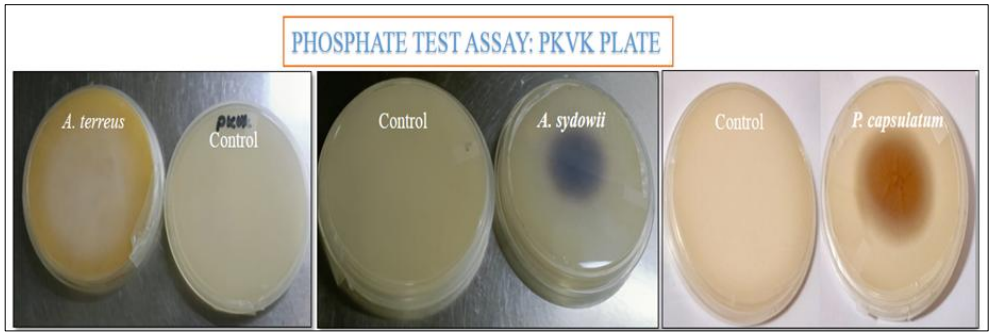

Fig 4: Colony features and clear zones formation for TCP solubilization on PKVK agar plates after 7 days.

In order to quantify the quantity of insoluble phosphate released into the medium upon inoculation of fungus and compare it with the uninoculated control, samples were taken every day for six days in a row. All three fungal strains in PKVK broths with both TCP supplementation had varying levels of insoluble phosphate throughout the study period, according to the findings in figure 5. When compared to the other strains, A. terreus dissolved the most phosphate over the six-day experimental period in TCP medium; on the sixth day of culture, the maximum soluble P of 162.32 µg/ml was reported. The capacity of the fungal strain A. sydowii to solubilize phosphate was modest. On the sixth day of culture, A. sydowii dissolved 141.20 µg/ml P, but P. capsulatum only dissolved 75.41 µg/ml P (Figure 5).

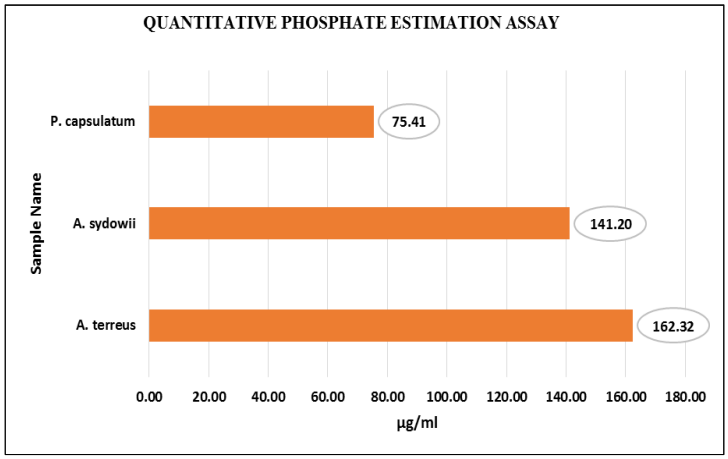

Fig 5: Solubilization of phosphate by the fungal strains in PKVK media supplemented with tricalcium phosphate (TCP) as an insoluble phosphate source for six days of culturing (A. sydowii, A. terreus and P. capsulatum).

3.4 Indole acetic acid production

The three types of fungi A. sydowii, A. terreus and P. capsulatum made enough IAA to be seen (Figure 6). The levels of IAA slowly increase from the day it was initially found in all three types of fungi. The maximum levels of IAA were 17.82 µg/ml with tryptophan and 12.30 µg/ml without it. These levels were reached by A. terreus on the sixth day of incubation. On the sixth day of incubation, the fungal strains A. sydowii and P. capsulatum produced the most IAA, with or without tryptophan, at 12.01 µg/ml, 5.16 µg/ml, and 6.87 µg/ml, 3.54 µg/ml, and 6.87 µg/ml, respectively.

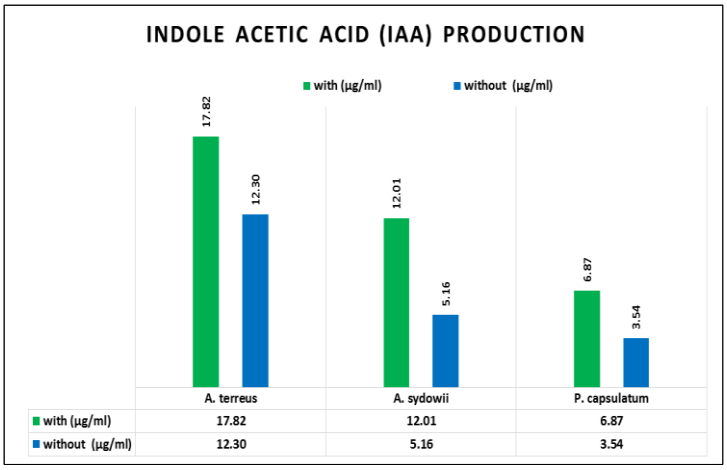

Fig 6: The production of auxin phytohormone (IAA) by the three fungal strains.

3.5 Siderophores production: Figure 7 shows that the fungal strain A. terreus generated more siderophores than A. sydowii and P. capsulatum. The three types of fungi produced the most siderophores on day six.

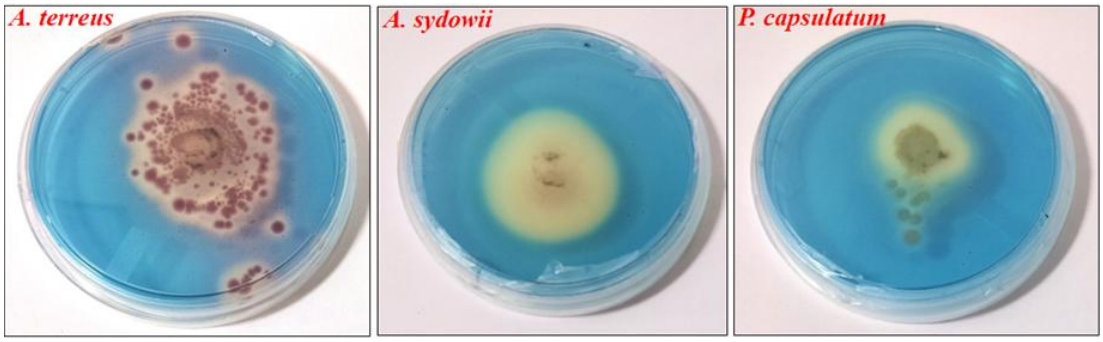

Fig 7: The production of iron-chelating molecules, siderophores, by the three selected fungal strains (A. sydowii, A. terreus and P. capsulatum) in a vitamin-free media.

3.6 Organic acid production: Figure 8 shows that the fungal strain A. terreus generated clearer zone than A. sydowii and P. capsulatum. The three types of fungi produced the most organic acid production on day six.

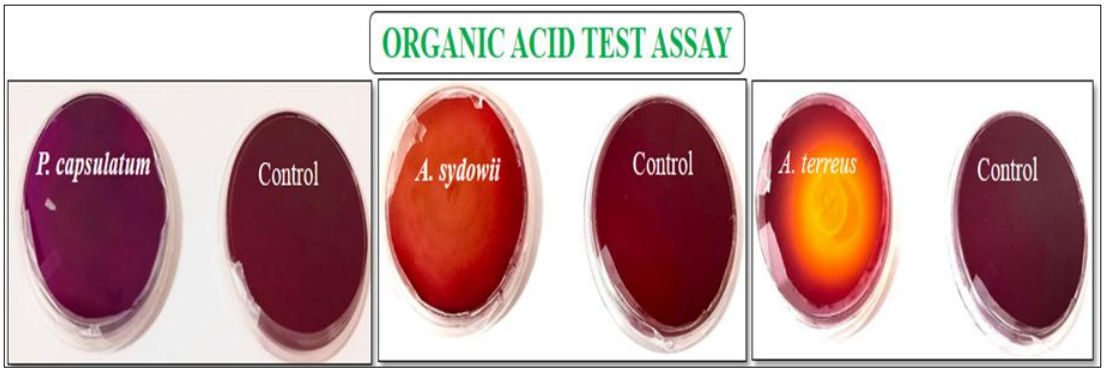

Fig 8: The production of organic acid by the three fungal strains.

3.7 Effect of three fungal strain on different plants growth: At the end of 40 days, there was a significantly higher growth of plants under sterilized soil conditions than for non-sterilized soil conditions (Table 2; Figure 9). T_0, T_4 and T_8 as a control in all three plants. T_1, T_5 and T_9 was treated with A. terreus strain. T_2, T_6 and T_{10} was treated with A. sydowii strain. T_3, T_7 and T_{11} was treated with P. capsulatum strain. T_1, T_5 and T_9 showed the highest growth among the eleven treatments. When considering the height of the plants, the highest was recorded in T_1, T_5 and T_9 as 15 cm, 23.9 cm and 17 cm respectively. The lowest height was recorded in control plants compared to other PSF strains. The highest root length identified in T_1, T_5, T_9 (26.2 cm, 35.4 cm and 41 cm, respectively) and the lowest root length were identified in T_2, T_6 and T_{11} as 22.5 cm, 30.4 cm and 18 cm respectively and the control T_0, T_4 and T_8 as 17.5 cm, 20.1 cm and 15.2 cm respectively. After 40 days, the highest wet weight was recorded in T_1, T_5 and T_9 as 4.33 gm, 5.88 gm and 9.05 gm respectively, the highest dry weight recorded in T_1, T_5 and T_9 as 0.598 gm, 1.13 gm and 0.75 gm respectively.

Table 2: Effect of three PSF strains on plant height, root length, wet weight and dry weight of each treatment used for the pot trials.

Black Gram (*Vigna mungo*)					
Treatment	No of leaves	PH (cm)	RL (cm)	WW (gm)	DW (gm)
T_0	5	13.2	17.5	2.53	0.444
T_1	6	15	26.2	4.33	0.598
T_2	7	13	22.5	5.21	0.512
T_3	7	14.6	25.3	2.95	0.427
Sorghum (*Sorghum bicolor*)					
Treatment	No of leaves	PH (cm)	RL (cm)	WW (gm)	DW (gm)
T_4	3	17.5	20.1	4.1	0.66
T_5	4	23.9	35.4	5.88	1.13
T_6	3	23.2	30.4	4.26	0.86
T_7	4	23.1	31.9	4.41	0.94
Soybean (*Glycine max*)					
Treatment	No of leaves	PH (cm)	RL (cm)	WW (gm)	DW (gm)
T_8	8	13	15.2	3.54	0.55
T_9	8	17	41	9.05	0.75
T_{10}	8	17	18.6	5.45	0.66
T_{11}	8	10.2	18	4.08	0.48

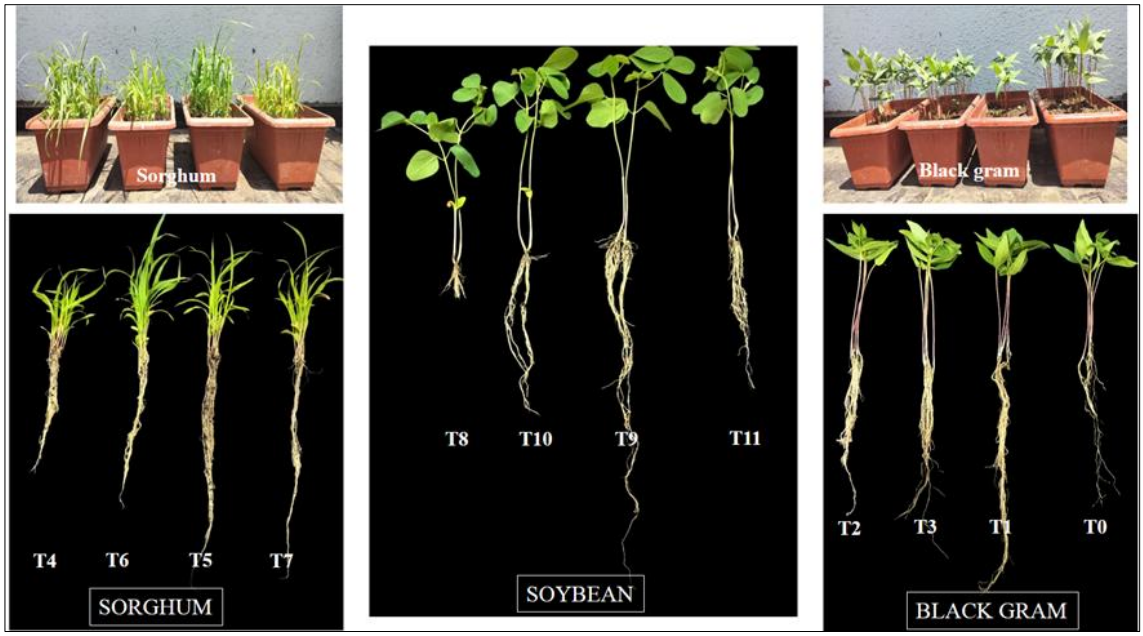

Fig 9: Pot assay with different treatments.

4. Discussion

PSF are advantageous fungi that can solubilize inorganic phosphorus from insoluble substances. Phosphorus (P) is a primary necessary macronutrient for plants and is used in soil as phosphate fertilizers. A significant amount of soluble inorganic phosphate added to the soil as chemical fertilizer is quickly immobilized and rendered inaccessible to plants.

Three fungal strains (Aspergillus sydowii, Aspergillus terreus, and Penicillium capsulatum) that showed the capacity to solubilize inorganic phosphates. On PKVK medium, all three strains demonstrated normal fungal growth, with differing levels of mycelial development and clear zone formation. These findings suggest that the strains are capable of adapting to and using non-bioavailable phosphorus sources as their exclusive supply of phosphorus (Alam *et al.*, 2023; Alikhani *et al.*, 2023) [2, 3].

Quantitative studies of Aspergillus terreus showed variations in soluble phosphate levels, which illustrate how phosphate solubilization is dynamic. These variations are influenced by variables such enzymatic activity, pH, nutritional availability, and fungal development stage (Zhang *et al.*, 2023; Prasad *et al.*, 2023) [29, 39]. Aspergillus sydowii dissolved TCP (141.20 µg/ml), but Aspergillus terreus dissolved the most TCP of all the strains (162.32 µg/ml). P. capsulatum had a somewhat reduced but still noteworthy solubilization capability of 75.41 µg/ml. These results demonstrate the strains' strong phosphate solubilization processes and are in line with earlier research on related fungal species, suggesting their potential for use in sustainable agriculture (Bononi *et al.*, 2020; Kong *et al.*, 2023) [7, 20].

In addition to improving nutrient bioavailability, soil fungus have other functions in promoting plant development. They generate compounds such cytokinin, auxins, gibberellins, and siderophores that promote growth (Tandon *et al.*, 2020) [34]. In particular, siderophores and IAA synthesis are essential for promoting plant development. While siderophores improve iron absorption by generating iron siderophores complexes in soils lacking iron, IAA promotes cell proliferation and root elongation (Ahmad *et al.*, 2023) [1]. Compared to the other two strains in this investigation, A. terreus generated the most IAA, indicating its potential as a bioinoculant to promote root growth. The most promising strain for enhancing iron bioavailability in nutrient-poor soils was A. terreus, which also showed the maximum siderophore synthesis. The efficiency of fungi that produce siderophores and IAAs in fostering plant development and battling phytopathogens for vital nutrients like iron has also been shown in earlier research (Behera *et al.*, 2017; Iqbal *et al.*, 2024) [6, 16].

According to a previous research, T. purpureogenus was able to support the development of wheat seedlings under both normal and stressed circumstances by producing high quantities of IAA (405 mg/L when fed with L-tryptophan) (Zhou *et al.*, 2023) [41]. This emphasizes how crucial it is for biofertilizers to be able to adapt to their surroundings. Fungal biofertilizers have a lot of potential as sustainable substitutes or additives to conventional fertilizers, but a number of environmental conditions may affect how well they work. When creating biofertilizer formulations, it is important to take into account the distinct environmental conditions of various geographic locations (Turaeva *et al.*, 2020; Mohamed *et al.*, 2022) [25, 35]. As a result, scientists are constantly separating fungus from different areas in order to find biofertilizers that are tailored to certain conditions. As far as we are aware, this is the first research to separate fungal biofertilizers from the soils of the Ankleshwar (Gujarat) area, which are rich in biodiversity. These fungal strains are expected to function best in areas with comparable ecological traits since they have adapted to the local environmental circumstances.

5. Conclusions

In summary, this research showed that Aspergillus sydowii, Aspergillus terreus, and Penicillium capsulatum have the potential to be used as biofertilizers because of their capacity to synthesis siderophores, solubilize phosphate, and synthesize indole-3-acetic acid (IAA). Aspergillus terreus produced the most IAA and siderophores and was the best at solubilizing inorganic phosphorus (TCP). Fungal consortia have the potential to improve the performance of biofertilizers; further studies, including field tests, will confirm their effectiveness. Overall, this research advances the use of fungal biofertilizers as sustainable substitutes for

chemical fertilizers, which may increase plant growth, improve nutrient availability, and promote ecologically friendly farming methods.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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