International Journal of Advanced Multidisciplinary Research
ISSN: 2393-8870
www.ijarm.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijamr Volume 10, Issue 2 -2023

Research Article

DOI: http://dx.doi.org/10.22192/ijamr.2023.10.02.002

"Isolation of Phosphofungi and Screening of their Silver Nanoparticles Biosynthesis"

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Abstract

Background: The biosynthesis of silver nanoparticles using microbes is considered to be environmentally friendly. Silver nanoparticles have been widely used in various fields including the medical field, industries, and agricultural fields. Agriculture is a major part of our India, nanotechnology in the field of agriculture focuses currently on target farming that involves the use of silver nanoparticles biological synthesis of silver nanoparticles is the best method.
Methods: Rhizosphere soil samples were collected at 10 - 15cm depth of roots of the medicinal plants. Phosphate Solubilizing Fungi were isolated from the sample by serial dilution method using Pikovskaya's agar medium.
Results and Conclusion: A total of 62 PSF were isolated and screened from 70 rhizosphere soil of medicinal plants. Among them 24 PSF showed a positive result for SNPs production and were further confirmed by UV – spectroscopic analysis, 10 PSF were selected with good peaks emerging between 400 – 500nm and the antibacterial property reveals the diameter of the zone of inhibition. And showed good results in different phosphate solubilization parameters under laboratory condition. Due to the observation of good results in the silver nanoparticles biosynthesis, antimicrobial activity, and phosphate solubilization, the selected PSF were recommended as phosphate bio inoculums in the agricultural field to improve plant growth and soil fertility.

Keywords

Silver nanoparticles, solubilization index, organic acid, siderophore, IAA.

Introduction

Silver nanoparticles are nanoparticles of silver, while frequently described as being silver some are composed of a large percentage of silver oxides due to their large ratio of surface to bulk silver atoms. Numerous shapes of nanoparticles can be constructed depending on the application at hand. Commonly used silver nanoparticles are spherical, but diamond, octagonal, and thin sheets are also common. Synthesis of nanoparticles by various methods has been done by several approaches viz., biotic and abiotic methods (*Bhattacharjee et al. 2017*). The biotic method

includes several microorganisms such as bacteria, fungi, and plant extracts for the synthesis of metal nanoparticles. Biological systems such as microorganisms (bacteria, fungi, algae, cyanobacteria, actinomycetes, and myxobacteria) and plants are being efficiently used either for intracellular or extracellular synthesis of different silver nanoparticles. Among the microbial systems, fungi are most commonly used because they are ubiquitously distributed in nature and play a crucial role in the synthesis of silver nanoparticles (*Yadav et al. 2015*). The biosynthesis of silver nanoparticles using microbes is considered to be environmentally friendly and is becoming more popular due to the choice of the solvent medium, reducing agent, and nontoxic material for the stabilization of the nanoparticles (*Bhattacharjee et al. 2017*). Silver nanoparticles have been widely used in various fields including the medical field, industries, and agricultural fields (*Yadav et al. 2015*).

Agriculture is a major part of our India, nanotechnology in the field of agriculture focuses currently on target farming that involves the use of silver nanoparticles biological synthesis of silver nanoparticles is the best method, it was found that silver nanoparticles were effective against fungal and bacterial infections and crop insect (*Arshad 2017*). Silver nanoparticles are widely used in a range of agricultural applications including diagnosis, target drug delivery, pest control, virus disease, the delivery system of pests, etc., Moreover, fungi have been reported to biosynthesize silver nanoparticles (*Siddiqi and Husen 2016; Verma et al. 2010*). Nanotechnology has the potential to revolutionize different sectors of agriculture (*Goel 2015; Rai and Ingle 2012*). Apart from their major application as antimicrobial agents for the management of plant pathogens, nanoparticles can serve as nano-pesticides, nano-insecticides, and nano-fertilizers. Many problems are associated with agriculture, such as excessive and continuous use of chemical fertilizers and water resources, a decrease in the fertility of the soil, and eventually crop production. Therefore, nano fertilizers can be the only alternative to regain and protect the fertility of the soil with minimum damage to the soil. The use of nano-fertilizers leads to an increase in nutrient efficiencies, reduces soil toxicity, and minimizes the potential negative effects associated with an overdose of chemical fertilizers. Hence, nanotechnology has a high potential for achieving sustainable agriculture, especially in developing countries (*Yadav et al. 2015*).

The rhizosphere is a site of higher microbial activity in and around the root of the soil, it harbors a great diversity of micro-organisms affecting plant growth and health. Medicinal plants play a vital role in human health care and have been used as a source of medicine since ancient times. A greater number of bacteria, fungi, and actinomycetes are present in the rhizosphere soil than in non-rhizosphere soil. (*Subba Rao 2011*). In other ways, soil microorganisms play a key role in soil P dynamics and the subsequent availability of phosphate to plants (*Sharma et at. 2013*). A diverse group of soil microflora was reported to be involved in solubilizing insoluble P complex enabling plants to easily absorb P (*Walpola and Yoon 2012*). Because phosphorus is one of the major nutrients essential for the growth and development of plants and microorganisms (*Khan et al. 2010; Achal et al. 2007*). It plays a significant role in plant metabolism and is important for the functioning of key enzymes that regulate metabolic pathways (*Nisha et al. 2014*).

Most of the microbial strains produce organic acids as their waste products. These acids decrease the pH of the medium that is responsible for the solubilization of the insoluble phosphates (*Nelofer et al. 2015*). Hence Phosphate Solubilizing Microorganisms convert these insoluble phosphates into soluble forms through special mechanisms. The major mechanism of mineral phosphate solubilization is the action of organic acid synthesized by soil microorganisms and by the action of phosphatase enzyme (*Achal et al. 2007*). Production of these organic acids resulted in the acidification of the microbial cell and its surroundings (*Nisha et al. 2014; Sharma et al. 2013*). Not only providing P to the plants the PSM also facilitates the growth of plants by

stimulating the efficiency of Nitrogen fixation, accelerating the accessibility of other trace elements, synthesizing important growth-promoting substances including siderophore and antibiotics, and providing protection to plants against soil-borne pathogens, fungi more active in solubilization phosphate than bacteria (*Alam et al. 2002*). Hence to overcome this object, the present study isolated the phosphate-solubilizing fungi and biosynthesis of silver nanoparticles from the fungi. In the present study, the PSF was isolated as a potential phosphate solubilizer from the rhizosphere soil of medicinal plants.

Materials and Methods

1. Collection of rhizosphere soil samples

Rhizosphere soil samples were collected from different medicinal plants available in different areas of the Shivamogga district. These samples were collected at 10 - 15cm depth of roots of the medicinal plants and the collected samples were placed in sterile polythene covers and then brought into the laboratory through the aseptic condition. In the laboratory, the soil samples were maintained at 4°C until further use of samples (*Chatli et al. 2008; Oliveira et al. 2008*).

2. Isolation of Phosphate Solubilizing Fungi

Phosphate Solubilizing Fungi (PSF) were isolated from the sample by serial dilution method using Pikovskaya's agar medium (containing Tri-calcium phosphate 5g, Glucose 10g, Ammonium sulfate 0.5g, Potassium chloride 0.2g, $MgSO_4$ $7H_2O$ 0.1g, $MnSO_4$ $7H_20$ trace, Ferrous sulfate trace, Yeast extract 0.5g, Distilled water 1L, Agar 20g and pH - 7.2) and incubation was done at room temperature for 7days. After incubation, plates were examined for solubilization zone around fungal colonies and they were subcultured for further use (*Nelofer et al. 2015; Wang et al. 2018*).

3. Microscopic Characterization

Identification of PSF was done by the lactophenol cotton-blue (LPCB) mounting technique. The specimen was stained with LPCB stain, the coverslip was placed above it and observed under the microscope at 40X magnification, and characters were noted by observing spore shape, spore size, spore arrangement, and arrangement of hyphae and identified by referring to the standard manuals (*Aneja 2009; Funder 1961*).

4. Extracellular Biosynthesis of Silver Nanoparticles (SNPs)

The isolated phosphate-solubilizing fungal culture was grown in Potato dextrose broth at room temperature for 14 days. After the growth, culture filtrate was collected by filtration through Whatman filter paper No. 1, and the filtrate was centrifuged at 1000rpm for 10min, and the supernatant was collected. An equal volume of supernatant and 5mM silver nitrate solution was mixed and kept for incubation at 37 for 24h (*Hamad 2019*). A colour change in the reaction mixture from colourless to reddish-brown indicates the formation of SNPs (*Tyagi et al. 2019; Chandra and Singh 2018*). The UV – spectroscopic analysis was carried out on the reaction mixture for confirmation of silver nanoparticle production at a wavelength of 200 – 600nm (*Tyagi et al. 2019; Elamawi et al. 2018*).

5. Antibacterial activity of Silver Nanoparticles

Microbial strains: The antibacterial activity of the biosynthesized silver nanoparticles from PSF were individually tested against five bacterial pathogens collected from the Microbial Type Culture Collection Center, Chandigarh, namely *Pseudomonas aeruginosa* (MTCC - 1934), *Pseudomonas syringae* (MTCC - 1604), *Xanthomonas campestris* (MTCC - 2286), *Klebsiella pneumonia* (MTCC - 7028) and *Escherichia coli* (MTCC - 1599).

Agar well diffusion method: The antibacterial activity was determined by using the agar well diffusion method against pathogenic bacteria (*Hamad 2019*). The bacterial cell suspension was swabbed on solidified Nutrient agar plates and wells were made by using a sterile cork borer. Wells filled with SNPs mixture care should be

taken so that the mixture could not overflow. Ciprofloxacin a standard antibiotic with of concentration 10mg/ml was used as a control. The plates were incubated at 37 for 24h. After incubation anti-bacterial property of SNPs was determined by measuring the zone of inhibition around the well in diameter (mm) (*Tyagi et al. 2019; Vishwanatha et al. 2018*).

6. Parameters of Phosphate Solubilization

Solubilization Index (SI): The fungal cultures were point inoculated on Pikovskaya's agar plates and incubated at room temperature for 7 days. The solubilization index was measured based on colony diameter and solubilization zone diameter formed around the colony of phosphate solubilizing fungi (*Tomer et al. 2017; Elias et al. 2016*) by using the formula,

$$SI = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

Assay of qualitative acid production by solid and liquid media: Pikovskaya's agar plate was supplemented with Bromophenol blue indicator (*Chadha et al. 2015*) and Pikovskaya's broth was supplemented with Bromocresol purple indicator was inoculated with PSF culture and incubated for 7 days (*Khan and Gupta 2015*).

Measurement of pH and Titrable acidity: The culture filtrate of PSF was centrifuged at 1000rpm for 10min to obtain the supernatant. The pH of the culture supernatant was measured by a pH meter as the final pH after 7 days of incubation. Initial pH was measured before inoculating the PSF culture into the broth. The uninoculated broth was used as a control (*Jain and Singh 2015; Kumar et al. 2014*). The amount of acid present in the filtrate was determined by titration of about 50ml of supernatant was titrated against 0.1N NaOH solution with a few drops of phenolphthalein indicator. The titrable acidity was expressed in g/L (*Wang et al. 2018; Khan and Gupta 2015*).

Estimation of Phosphate: Culture filtrate was centrifuged at 12,000rpm for 20min and supernatant was used for estimation of phosphate by Vanado – molybdate method (*Verma and Ekka 2015*), and it was expressed in terms of µg/ml. The optical density of the yellow-coloured supernatant was measured at 420nm and the phosphate present in the supernatant was calculated from a standard curve of KH_2PO_4 (*Kumar et al. 2014*).

7. Screening of Siderophore production

Siderophore production was detected by using Chrome Azurol Sulfonate (CAS) assay. 60.5mg of Chrome Azurol S was dissolved in 50ml of distilled water and mixed with 10ml of Iron solution (1mM Ferric chloride in 10mM Hydrochloric acid). While constantly stirring this solution was slowly added to HDTMA solution (72.9mg of HDTMA dissolved in 40ml of distilled water) and sterilized. The resultant dark purple liquid was added to sterilized Pikovskaya's medium containing no Tricalcium phosphate to make CAS agar. Then the CAS agar plates were spot inoculated with PSF culture and incubated at room temperature for 7 days (*Ghosh et al. 2017*).

8. Indole Acetic Acid (IAA) production

Phosphate solubilizing fungi were grown in potato dextrose broth supplemented with Tryptophan (1%). After complete growth, the culture filtrate was collected at 1000rpm for 10min. About 2ml of supernatant was mixed with 2 drops of orthophosphoric acid and 4ml of Salkwoski reagent (50ml of 35% perchloric acid, 1ml of Ferric chloride solution) and kept for incubation. The development of pink colour after 2h incubation at room temperature indicates indole acetic acid (IAA) production (*Nenwani et al. 2010*). The concentration of IAA production by PSF was estimated using a standard graph taking a concentration of standard IAA on the X-axis and Optical Density (530nm) on the Y-axis (*Pant and Agrawal 2014*).

Results

1. Isolation and identification of PSF

From the different medicinal plants, a total of 70 rhizosphere soil samples were collected in different regions of the Shivamogga district. Among the soil samples, 62 phosphate solubilizing fungal colonies were isolated and screened by using Pikovaskay's agar medium followed by the serial dilution method (Fig 1), pure cultures were prepared and they were named PSF 1 to PSF 62. Then the fungal colonies were identified based on morphology and microscopic characters using standard manuals (Fig 2).

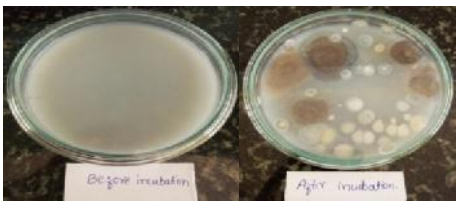

Fig 1: Isolation and screening of Phosphate solubilizing fungi by Pikovaskay's agar medium

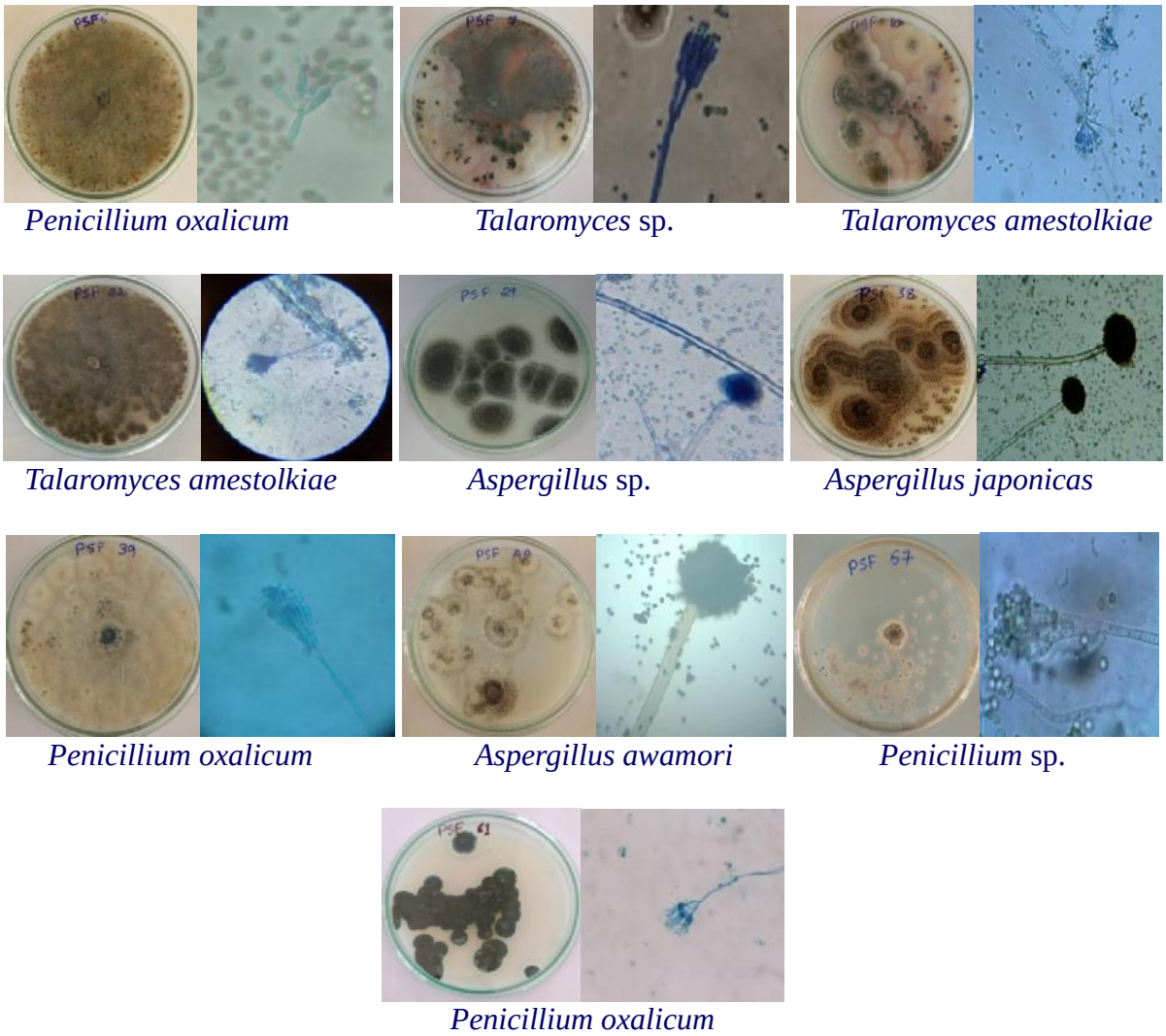

Fig 2: Pure cultures and their microscopic view of selected 10 Phosphate Solubilizing Fungi

2. Extracellular Biosynthesis of Silver Nanoparticles (SNPs) by PSF

The biosynthesis of silver nanoparticles (SNPs) was initially observed with the change of colour from colourless to reddish-brown with 5mM silver nitrate solution after 24h incubation (Fig 3). Among the 62 PSF isolates, a total of 24 phosphate-solubilizing fungi showed a positive result for SNPs production (Table 1). The remaining 38 phosphate solubilizing fungi showed negative results for SNPs production there is no colour change was observed. The positive results were further confirmed by UV – spectroscopic analysis with absorbance peaks emerging between 300 – 550nm, among them, 10 PSF were selected with good peaks emerging between 400 – 500nm (Fig 4) and they have further evaluated their antibacterial activity and phosphate solubilization efficiency under laboratory condition.

Table 1: Selection of isolated PSF showed positive result for biosynthesis of silver nanoparticle.

Sl No.	Medicinal plant	Culture Code No.	Cultures	Result
1	*Achyranthus aspera*	PSF 5	*Penicillium oxalicm*	+
2	*Centella asiatica*	PSF 6	*Penicillium* sp.	+
3	*Asparagus racemosus*	PSF 7	*Talaromyces* sp.	+
4	*Costusingneus*	PSF 10	*Talaromycesamestolkiae*	+
5	*Wrightia tinctoria*	PSF 16	*Aspergillus niger*	+
6	*Ocimum sanctum*	PSF 22	*Talaromycesamestolkiae*	+
7	*Amaranthus viridis*	PSF 24	*Penicillium* sp.	+
8	*Ixora coccinea*	PSF 28	*Aspergillus* sp.	+
9	*Mimosa pudica*	PSF 29	*Aspergillus* sp.	+
10	*Solanum xanthocarpum*	PSF 34	*Aspergillus* sp.	+
11	*Ecliptaprostrata*	PSF 36	*Aspergillus* sp.	+
12	*Phyllanthus niruri*	PSF 38	*Aspergillus japonicus*	+
		PSF 39	*Penicillium oxalicum*	+
13	*Coleus* sp.	PSF 43	*Trichoderma* sp.	+
		PSF 44	*Aspergillus awamori*	+
14	*Ocimumbasillicum*	PSF 46	*Trichoderma* sp.	+
15	*Rauvolfia serpentina*	PSF 51	*Penicillium* sp.	+
16	*Justicia adhatoda*	PSF 54	*Penicillium* sp.	+
17	*Sansevieria trifasciata*	PSF 56	*Fusarium* sp.	+
18	*Pithecellobium*	PSF 57	*Penicillium* sp.	+
19	*Lantana camara*	PSF 58	*Fusarium* sp.	+
20	*Plumeria pudica*	PSF 59	*Alternaria* sp.	+
21	*Basella alba*	PSF 60	*Trichoderma* sp.	+
22	*Barlariaprionitis*	PSF 61	*Penicillium oxalicum*	+

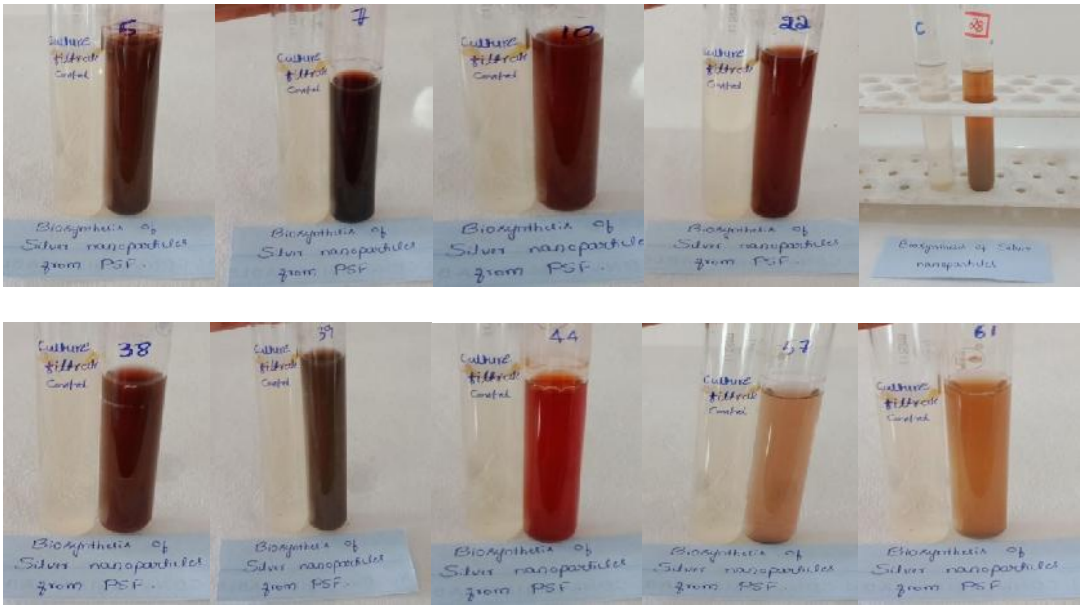

Fig 3: Biosynthesis of Silver Nanoparticles from selected 10 Phosphate solubilizing fungi

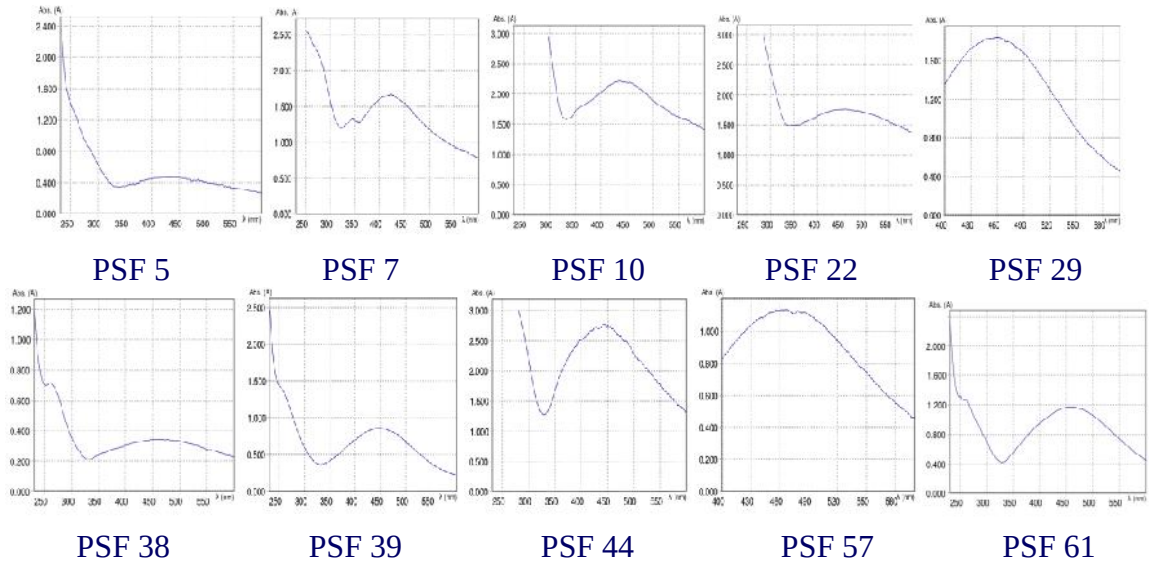

Fig 4: UV – spectroscopic absorbance peaks emerging between 400 – 500nm by phosphate solubilizing fungi

3. Antibacterial activity of SNPs synthesized by PSF

The antibacterial property of synthesized SNPs was determined by the diameter of the zone of inhibition by test bacteria compared with Ciprofloxacin as control. The test bacteria *E. coli* was more susceptible to SNPs synthesized by PSF 29 (26.7mm), *X. campestris* was more susceptible to SNPs synthesized by PSF 38 (24.5mm), *K. pneumoniae* was more susceptible to SNPs synthesized by PSF 7 (26.5mm), *P. aeruginosa* and *P. syringae* were more susceptible to SNPs synthesized by PSF 10 (30.9mm and 24.2mm respectively) compared with other phosphate solubilizing fungi (Table 2) (Fig 5).

Table 2: Anti-bacterial activity of SNPs synthesized by PSF

SL No.	Culture	SNPs	Antibacterial activity (zone of diameter in mm)				
			Escherichia coli	***Xanthomonas campestris***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Pseudomonas syringae***
1	**Standard**	**-**	**47.6**	**46.1**	**50.3**	**50.7**	**51**
2	PSF 5	+	25	19	19	22	22
3	PSF 7	+	22	19	**26.5**	23.5	21.9
4	PSF 10	+	18.4	17.7	22.8	**30.9**	**24.2**
5	PSF 22	+	18	17.5	22.5	30.5	24
6	PSF 29	+	**26.7**	20.2	22.1	19.7	21.3
7	PSF 38	+	25.2	**24.5**	22	23	21.5
8	PSF 39	+	21	14.5	16.6	17	16.5
9	PSF 44	+	20.5	16	17.5	18.5	19.5
10	PSF 57	+	20.5	22.5	17.5	23.4	18.1
11	PSF 61	+	16.5	17	17	16.5	18.5

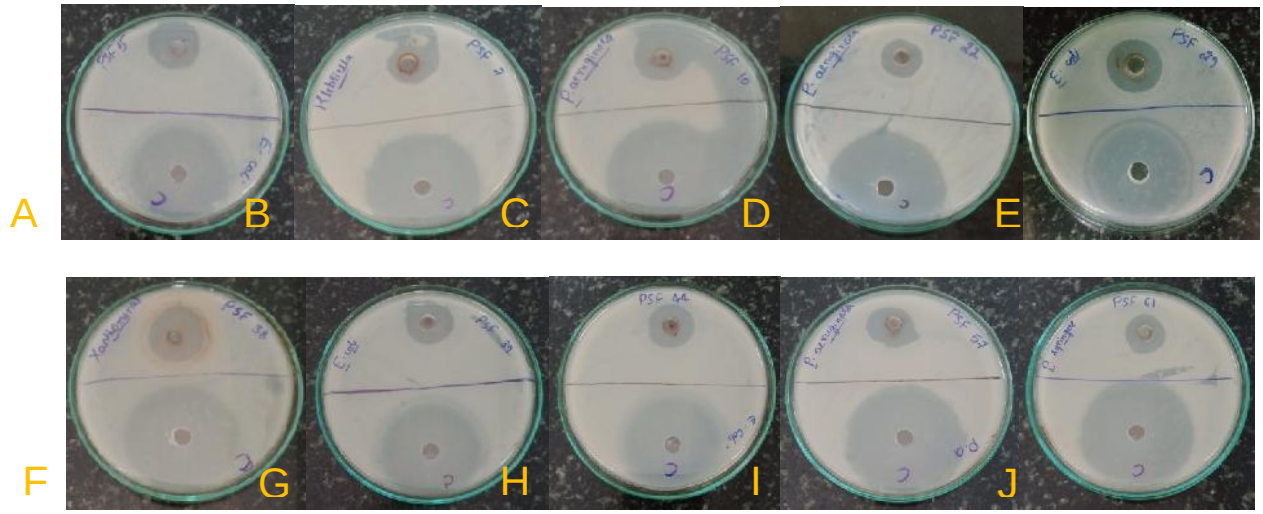

Fig 5: Antibacterial activity of SNPs synthesized by Phosphate Solubilizing Fungi (A: PSF 5 v/s *Escherichia coli*, B: PSF 7 **v/s** *Klebsiella pneumonia, C: PSF 10 v/s Pseudomonas aeruginosa, D: PSF 22 v/s Pseudomonas aeruginosa, E: PSF 29 v/s Escherichia coli, F: PSF 38 v/s Xanthomonas campestris, G: PSF 39 v/s Escherichia coli, H: PSF 44 v/s Escherichia coli, I: PSF 57 v/s Pseudomonas aeruginosa, J: PSF 61 v/s Pseudomonas syringae*)

4. Parameters of Phosphate Solubilization

Solubilization index (SI): The solubilization indices of the selected 10 PSF cultures ranged from 2.38 to 3.96, among them, PSF 44 (*Aspergillus awamori*) showed a maximum solubilization index of 3.96 (Table 3).

Assay of qualitative acid production by solid and liquid media: The selected 10 PSF showed colour change from blue to yellow on the agar plate when using Bromophenol Blue (Fig 6) and in the broth colour change from red to yellow was observed while using Bromocresol purple (Fig 7). Due to the production of organic acids by the PSF, the pH in the culture media reduces and was observed by using colour indicators.

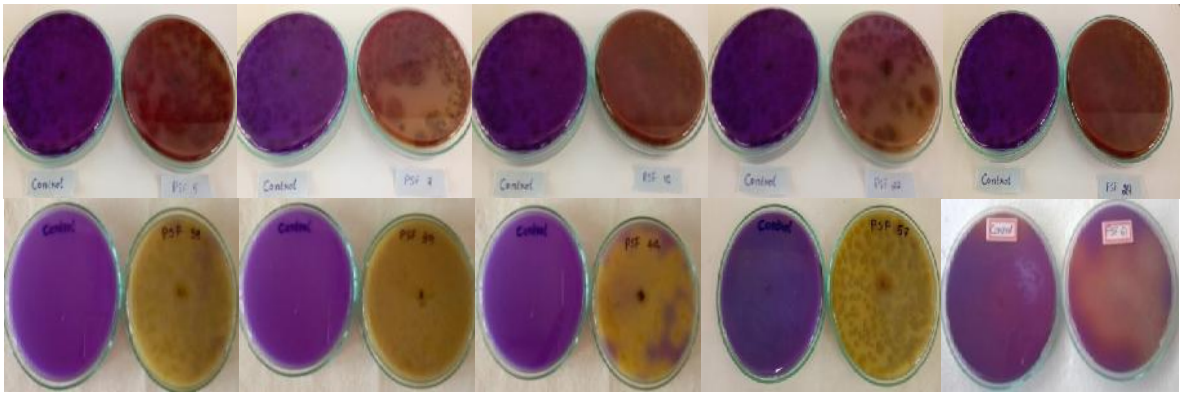

Fig 6: Assay of qualitative acid production on solid media by selected Phosphate solubilizing fungi

Fig 7: Assay of qualitative acid production in liquid media by selected Phosphate solubilizing fungi

Measurement of pH and Titrable acidity: Inoculation of selected 10 PSF to Pikovskaya's broth resulted in a decrease of pH ranging from 4.9 to 3.0 from the initial pH of 6.89. The measure of the amount of acid present in the culture broth ranged from 22.7g/L to 34.01g/L (Table 3).

Estimation of Phosphate: The phosphate present in the culture filtrate of selected 10 PSF cultures ranged from 155µg/ml to 20µg/ml (Table 3).

Table 3: Phosphate Solubilization parameters

Sl No.	Culture Code	Culture	SI	SE	pH	Estimation of organic acid	Conc. of Phosphate (µg)
1	PSF 5	*Penicillium oxalic*	3.83	283	3.2	30.01	25
2	PSF 7	*Talaromyces* sp.	3.02	202	4.3	32.9	45
3	PSF 10	*Talaromyces amestolkiae*	3.21	221	3.8	30.16	45
4	PSF 22	*Talaromyces amestolkiae*	3.03	203	4.2	33.01	70
5	PSF 29	*Aspergillus* sp.	2.86	186	4.7	22.8	130
6	PSF 38	*Aspergillus japonicas*	3.61	261	3.4	37.54	30
7	PSF 39	*Penicillium oxalicum*	3.86	286	3.7	37.6	25
8	PSF 44	*Aspergillus awamori*	3.96	296	3.0	33.89	20
9	PSF 57	*Penicillium* sp.	2.38	138	4.9	22.7	155
10	PSF 61	*Penicillium oxalicum*	3.82	282	3.2	34.01	25

5. Screening of Siderophore production

The selected 10 PSF cultures were screened using CAS agar medium for their ability to produce siderophore. Among the 10 PSF cultures, 9 PSF cultures showed a positive result for siderophore production, after incubation colour change from blue to pink around the colony was observed, and PSF 57 (Penicillium sp.) showed negative for siderophore production, there is no colour change was observed around the colony (Table 4) (Fig 8).

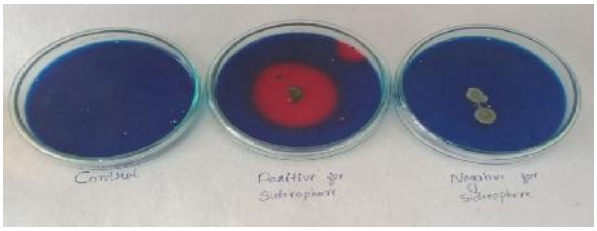

Fig 8: Siderophore production by Phosphate solubilizing fungi

6. Screening and Estimation of IAA production

In the screening of Indole Acetic Acid production, the selected 10 PSF showed positive results by the development of pink colour after the period of incubation with the addition of the Salkowaski reagent (Fig 9). The further detection of the concentration of IAA produced by PSF using a standard curve ranged from 40 to 190µg (Table 4).

Table 4: Siderophore and Indole Acetic Acid production

Sl No.	Culture Code	Culture	Siderophore production	IAA production	Conc. of IAA(µg)
1	PSF 5	*Penicillium oxalicm*	+	+	190
2	PSF 7	*Talaromyces* sp.	+	+	150
3	PSF 10	*Talaromycesamestolkiae*	+	+	140
4	PSF 22	*Talaromycesamestolkiae*	+	+	40
5	PSF 29	*Aspergillus* sp.	+	+	75
6	PSF 38	*Aspergillus japonicus*	+	+	90
7	PSF 39	*Penicillium oxalicum*	+	+	90
8	PSF 44	*Aspergillus awamori*	+	+	120
9	PSF 57	*Penicillium* sp.	-	+	115
10	PSF 61	*Penicillium oxalicum*	+	+	50

Fig 9: Indole Acetic Acid production by Phosphate solubilizing fungi

Discussion

Phosphorus plays a significant role in plant metabolism and is important for the functioning of key enzymes that regulate metabolic pathways. A diverse group of soil microflora was reported to be involved in solubilizing insoluble P complex enabling plants to easily absorb P. Hence the methods followed and the results obtained in our work were correlated with earlier findings of Nelofer et al. (2016) have serially diluted the soil samples and inoculated them in PVK agar by pour plate method. Among the 45 soil samples, 11 were given colonies with clear zones that were considered P solubilizing strains. While Wang et al. (2018) isolated 20 fungal strains from a wheat field sampling site, only eight of which (CS-1 to CS-8) showed phosphate-solubilizing capacity by the production of clear zones around colonies on the NBRIP medium.

Silver nanoparticles synthesized by phosphate-solubilizing microorganisms have become the theme of immense research interest in recent years due to their wide range of applications as antimicrobial agents. The earlier findings of Hamad (2019) has synthesized Ag-NPs by using 5mM AgNO3 solution showed a colour change from pale yellow to light brown after 24h of incubation, The colour change to brown was an indication of the formation of Ag-NPs in the medium, confirmed by UV–Vis spectroscopy technique bands of Ag-NPs were observed around 350–450nm. Antibacterial assays of biosynthesized Ag-NPs were studied against pathogenic strains of *E. coli*, *S. aureus*, and *P. aeruginosa* using the agar well diffusion method, and zone of inhibition was measured and Ciprofloxacin as standard.

Phosphate Solubilizing Microorganisms (PSM) converts these insoluble phosphates into soluble forms through special mechanisms. The results were highlighted by earlier reports by Tomer et al. (2017) have studied the solubilization index of three bacterial isolates ranging from 7.2 to 62mm, while Elias et al. (2016) obtained SI of 359 fungal isolates ranging from 1.10 to 3.05. Similar results were observed in earlier findings of Khan and Gupta, (2015) have observed colour change when fungal isolates were subjected to qualitative assay for acid production using 0.04% of Bromocresol purple, 29 acidophilic fungal isolates showed the formation of yellow coloration of the medium upon five days incubation. While Chadha et al., (2015) inoculated the fungal isolate with Bromophenol blue at a concentration of 0.003% and showed blue colour to yellow colouration upon incubation for seven days at 28°C. Similar results were recorded in earlier findings of Kumar et al. (2014) have observed a significant change in the pH of broth (5.5 and 4.8) shown by *B. megaterium* over control followed by *A. chlorophenolicus* respectively. Khan and Gupta (2015) checked the ability of acid production of 29 acidophilic fungal isolates isolated and 5 isolates LAK-2, BS-1.6, CM-2, DR-1, and DR-2 showed good acid production. Khan and Gupta (2015) checked the ability of acid production of 29 acidophilic fungal isolates isolated and 5 isolates LAK-2, BS-1.6, CM-2, DR-1, and DR-2 showed good acid production. Estimation of Phosphate by the Vanadomolybdate method was adopted in our work and the obtained results were correlated with earlier findings of Verma and Ekka (2015) were reported the concentration of phosphate in culture broth ranged from 219.16µg/ml to 59.17µg/ml.

Siderophores are secondary metabolites produced to scavenge iron from their surrounding making this essential element available to the cell. Thus, siderophores are the solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation. The results were highlighted by earlier reports by Ghosh et al. (2017), who reported that isolates of *Trichoderma* showed siderophore production in CAS agar plate, *Trichoderma harzianum* produced maximum percentage of siderophores than *T. viride, T. asperellum,* and *T. longibrachiatum*.

Phosphate solubilizing fungi not only proving P to the plants but also facilitate the growth of plants by synthesizing important growth-promoting substances, hence the earlier reports of Pant and Agrawal (2014) have isolated 6 bacterial cultures and used them to test the estimation of IAA by standard calibration curve with the help of Salkowski reagent. While Nenwani et al. (2010) reported that PSF was able to produce phytohormone IAA, isolate F1 was found to produce 11.45 $\mu g ml^{-1}$ of IAA which is significantly high.

Conclusion

The present study revealed that the rhizosphere soil serves as a good source for the growth and development of plants and phosphate-solubilizing fungi. These fungi were isolated on Pikovskaya's agar media containing tricalcium phosphate as a source of phosphate. The isolates showed good results in phosphate solubilization and biosynthesis of silver nanoparticles, also their antibacterial activity on pathogens. Due to the efficiency of the phosphate solubilization capacity of the isolated PSF and their ability to synthesize silver nanoparticles, these PSF can be recommended as biofertilizers or bioinoculums in the fields of agriculture and horticulture. Primarily for its use as an alternative to chemical phosphorous fertilizer and production of beneficial phosphofungi from rhizosphere soil resources may improve soil fertility, enhance plant growth and reduce the risk of environmental pollution and diminish the accumulation of phosphorous.

References

Achala, V., Savant, V. V. and Reddy, M. S. (2007). Phosphate solubilization by a wild type strain and UV-induced mutants of *Aspergillus tubingensis*. *Soil Biology & Biochemistry*. 39: 695–699. doi:10.1016/j.soilbio.2006.09.003.

Alam, S., Khalil, S., Ayub, N. and Rashid, M. (2002). *In vitro* solubilization of inorganic phosphate by phosphate solubilizing microorganism (PSM) from maize rhizosphere. *International Journal of Agriculture and Biology*. 4: 454–458.

Aneja, K. R. (2009). Experiments in Microbiology, Plant Pathology and Biotechnology.New age International Publishers. New Delhi. 4:157 – 162.

Arshad, A. (2017). Bacterial Synthesis and Applications of Nanoparticles, Nanoscience and Nanotechnology. *An Indian Journal*. 11(2): 1–30.

Barnett, H. L. (1975). Illustrated genera of imperfect fungi. 2: 1 – 225.

Bhattacharjee, S., Debnath, G., Das, A. R., Saha, A. K. and Das, P. (2017). Characterization of silver nanoparticles synthesized using an endophytic fungus, *Penicillium oxalicum* having potential antimicrobial activity. Advances in NaturalSciences: *Nanosciences and Nanotechnology*. 8: 1–6.

Booth, C. (1971). Illustrated the genus *Fusarium*. Common Wealth Mycological Institute. 1 – 237.

Chandha, N., Prasad, R. and Varma, A. (2016). Plant promoting activity of fungal endophytes associated with Tomato roots from central Himalaya, India and their interaction with *Piriformspora indica*. *International Journal of Pharma and Bio Sciences*. 6(1): 333–343.

Chandra, A. and Singh, M. (2018). Biosynthesis of amino acid functionalized silver nanoparticles for potential catalytic and oxygen sensing applications. *Inorganic Chemistry Frontiers*. 5(1): 233–257.

Chatli, A. S., Beri, V. and Sidhu, B. S. (2008). Isolation and characterization of phosphate solubilizing microorganisms from the cold desert habitat of *Salix alba* Linn. in trans Himalayan region of Himachal Pradesh. *Indian Journal of Microbiology*. 48: 267–273.

Elamawi, R. M., Al-Harbi, R. E. and Hendi, A. A. (2018). Biosynthesis and characterization of silver nanoparticles using *Trichoderma longibrachiatum* and their effect on phytopathogenic fungi. *Egyptian Journal of Biological Pest Control*. 28: 1–11. DOI 10.1186/s41938-018-0028-1.

Elias, F., Woyessa, D. and Muleta, D. (2016). Phosphate Solubilization Potential of Rhizosphere Fungi Isolated from Plants in Jimma Zone, Southwest Ethiopia. *International Journal of Microbiology*. Article ID 5472601, 1–11.

Funder, S. (1961). Practical mycology manual for identification of fungi. A. W. Broggers Boktrykkari A/S, Norway. 1 – 120.

Ghosh, S., Banerjee, S. and Sengupta, C. (2017). Bioassay, characterization and estimation of siderophores from some important antagonistic fungi. *Journal of Biopesticides*. 10(2) 105–112.

Hamad, M. T. (2019). Biosynthesis of silver nanoparticles by fungi and their antibacterial activity. *International Journal of Environmental Science and Technology*. 16: 1015–1024. (https://doi.org/10.1007/s13762-018-1814-8).

Jain, P. and Singh, D. (2015). Study on the Role of Phosphate Solubilizing Fungi in Phosphorus Bioavailability and Growth Enhancement of Potato. *Chemical Science Review and Letters*. 4(13): 101–108.

Joseph, S. and Jisha, M. S. (2008). Buffering Reduces Phosphate Solubilizing Ability of Selected Strains of Bacteria. *American-Eurasian Journal of Agricultural and Environmental Science*. 4(1): 110–112.

Khan, M. S., Zaidi, A., Ahemad, M., Oves, M. and Wani, P. A. (2010). Plant growth promotion by phosphate solubilizing fungi – current perspective. *Archives of Agronomy and Soil Science*. 56(1): 73–98. http://dx.doi.org/10.1080/03650340902806469.

Khan, R. and Gupta, A. K. (2015). Screening and Optimization of Organic acid Producers from Mine Areas of Chhattisgarh Region, India. *International Journal of Current Microbiology and Applied Science*. 4(2): 103–111.

Kumar, A., Maurya, B. R. and Raghuwanshi, R. (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). *Biocatalysis and Agricultural Biotechnology.* 3: 121 – 128. http://dx.doi.org/10.1016/j.bcab.2014.08.003.

Nelofer, R., Syed, Q., Nadeem, M., Bashir, F., Mazhar, S. and Hassan, A. (2016). Isolation of Phosphorus-Solubilizing Fungus from Soil to Supplement Biofertilizer. *Arab Journal of Science and Engineering.* DOI 10.1007/s13369-015-1916-2. DOI 10.1007/s13369-015-1916-2.

Nenwani, V., Doshi, P., Saha, T. and Shalini, R. (2010). Isolation and characterization of a fungal isolate for phosphate solubilization and plant growth promoting activity. *Journal of Yeast and Fungal Research.* 1(1): 009–014.

Nisha, K., Padma Devi, S. N. and Kumari, S. K. (2014). Role of Phosphorous Solubilizing Microorganisms to Eradicate P - Deficiency in Plants: A Review. *International Journal of Scientific and Research Publications.* 4(7): 1–5.

Oliveira, C. A., Alves, V. M. C., Marriel, I. E., Gomes, E. A., Scotti, M. R., Carneiro, N. P., Guimara, C. T., Schaffert, R. E. and Sa, N. M. H. (2008). Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. *Soil Biology and Biochemistry.* 1–6, doi:10.1016/j.soilbio.2008.01.012.

Pant, G. and Agrawal, P. K. (2014). Isolation and Characterization of Indole Acetic Acid producing plant growth promoting Rhizobacteria from Rhizospheric soil of *Withania somnifera. Journal of Biological and Scientific Opinion.* 2(6): 377–383.

Priya, S., Panneerselvam, T. and Sivakumar, T. (2013). Evaluation of Indole – 3 - Acetic Acid in phosphate solubilizing Microbes isolated from Rhizosphere soil. *International Journal of Current Microbiology and Applied Science*. 2(3): 29–36.

Rai, M. and Ingle, A. (2012). Role of nanotechnology in agriculture with special reference to management of insect-pests. *Applied Microbiology and Biotechnology.* 94: 287–293.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H. and Gobi, T. A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springer Plus*. 2: 587, 1–14. http://www.springerplus.com/content/2/1/587.

Siddiqi, K. S. and Husen, A. (2016). Fabrication of Metal Nanoparticles from Fungi and Metal Salts: Scope and Application. *Nanoscale Research Letters.* 11 98, 1–15, DOI 10.1186/s11671-016-1311-2. DOI 10.1186/s11671-016-1311-2.

Subba Rao, N. S. (2011). Soil Microbiology (Fourth Edition of Soil Microorganisms and Plant Growth), OXFORD & IBH Publishing Co. Pvt. Ltd, New Delhi, 82–112.

Tomer, S., Suyal, D. C., Shukla, A., Rajwar, J., Yadav, A., Shouche, Y. and Goel, R. (2012). Isolation and characterization of phosphate solubilizing bacteria from Western Indian Himalayan soils. *Biotechnology*. 7: 95, DOI 10.1007/s13205-017-0738-1.

Tyagi, S., Tyagi, P. K., Gola, D., Chauhan, N., Bharti, R. K. and Goel, A. (2015). Agricultural applications of nanotechnology. *Journal of Biological and Chemical Research*. 32 260–266. https://doi.org/10.1007/s42452-019-1593-y.

Verma, A. and Ekka, A. (2015). Assessment of Phosphate Solubilizing Potential of Fungal Isolates from Soils of Three Blocks of Raipur, Chhattisgarh, India. *Journal of Ravishankar University-B.,* 28(2): 44–50.

Verma, V. C., Kharwar, R. N. and Gange, A. C. (2010). Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus *Aspergillus clavatus.* Nanomedicine: Nanotechnology, *Biology and Medicine.* 5(1): 33–40.

Vishwanatha, T., Keshavamurthy, M., Mallappa, M., Murugendrappa, M. V., Nadaf, Y. F., Siddalingeshwara, K. G. and Dhulappa, A. (2018). Biosynthesis, characterization and antibacterial activity of silver nanoparticles from *Aspergillus awamori*. *Journal of Applied Biology and Biotechnology*. 6(05): 12-16.

Walpola, B. C. and Yoon Min-Ho. (2012). Prospectus of phosphate Solubilizing microorganisms and phosphorus availability in agricultural soils: A review. *African Journal of Microbiology Research*. 6(37): 6600–6605.

Wang, X., Wang, C., Sui, J., Liu, Z., Li, Q., Ji, C., Song, X., Hu, Y., Wang, C., Sa, R., Zhang, J., Du, J. and Liu, X. (2018). Isolation and characterization of phosphofungi, and screening of their plant growth-promoting activities. *AMB Express*. 8: 63 https://doi.org/10.1186/s13568-018-0593-4.

Yadav, A., Kon, K., Kratosova, G., Duran, N., Ingle, A. P. and Rai, M. (2015). Fungi as an efficient mycosystem for the synthesis of metal nanoparticles: progress and key aspects of research. *Biotechnology Letters*. 37: 2099–2120, DOI 10.1007/s10529-015-1901-6.

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	Subject: Nanotechnology
DOI:10.22192/ijamr.2023.10.02.002	

How to cite this article:
Jyothi, V., Nandish, G., Prakash, J and Basaiah Thippeswamy. (2023). "Isolation of Phosphofungi and Screening of their Silver Nanoparticles Biosynthesis". Int. J. Adv. Multidiscip. Res. 10(2): 7-21.
DOI: http://dx.doi.org/10.22192/ijamr.2023.10.02.002