INT. J. BIOL. BIOTECH., 17 (3): 599-606, 2020.

SIDEROPHORE PRODUCTION BY PHOSPHATE SOLUBILIZING FUNGI FROM RHIZOSPHERIC SOIL OF MEDICINAL PLANTS

Venkataramanaiah Jyothi, Hirakannavar Veeranna Sowmya and Basaiah Thippeswamy*

Department of P. G. Studies and Research in Microbiology, Bioscience Complex, Jnana sahyadri, Kuvempu University, Shankaraghatta – 577451, Karnataka, India.
*Corresponding Author: *thippeswamyb205@gmail.com*

ABSTRACT

In this work, we have isolated 66 phosphate solubilizing fungi from 75 rhizosphere soil of different medicinal plants around Malnad regions of Shivamogga district by serial dilution method using Pikovskaya's media. Identification of fungal colonies was done by morphology and microscopic observation using standard manuals and they were labeled as PSF 1 to PSF 66. Fungi showed good results in siderophore production and phosphate solubilization under laboratory conditions. Out of 66 isolates, 56 PSF were positive for siderophore production and 10 PSF cultures showed negative for siderophore production. After screening, 10 PSF were selected for maximum siderophore production at 7 days incubation and their nature was detected and P solubilization was tested. In P solubilization, Solubilization Index - 1.08 to 3.96, colour change from blue to yellow on agar plate and red to yellow in broth, pH - 5.48 to 3.0 from initial pH of 6.89, Titrable acidity - 10.7g/L to 37.76g/L and phosphate in the culture broth - 290µg/mL to 20µg/mL and 10 PSF showed positive result for IAA production. Due to P solubilization capacity of the fungi and production plant growth promoters, they can be recommended as Phosphate bio-inoculums in agricultural field.

Key words: Phosphate, Organic acid, Siderophore, Hydroxymate, Catecholate, Carboxylate.

INTRODUCTION

We are known that NPK (Nitrogen, Phosphorus, and Potassium) are the major plant nutrients, which are required for plant growth and development. So Phosphorus (P) places a second position of major plant nutrient next to the Nitrogen (Nisha *et al*., 2014). Hence the input of P into the agricultural field is very much necessary through fertilizers. In generally, soil contains 500 – 1000ppm of total phosphorus including organic phosphate and inorganic phosphate. But most of P is in a fixed with other chemicals which do not release the phosphorus for plants. Furthermore, soluble phosphorus in fertilizer or other nutrient source is quickly converted into less available forms when added to the soil (Naik *et al*., 2013). Praveen *et al*. (2012) have reported 49.3% of cultivated lands in India are deficient in available phosphorus. Therefore primary approach in agronomic management of phosphorus is to scavenge the native/fixed phosphorus and also to overcome the fixation of applied phosphorus fertilizer. The low cost practice to activate this objective is to inoculate soil with phosphate solubilizing fungi and bacteria inhabit soil, especially rhizosphere and play a significant role in the growth and development of plant.

Soil microorganisms solubilize the insoluble phosphate into the soluble form of phosphate to easily uptake by the plants such microbes are called Phosphate Solubilizers (Anand *et al*., 2016). These microbes can solubilize the phosphate through many process, they carry out the process of acidification, chelation, exchange reaction and production of organic and inorganic acids (Stephen and Jisha, 2011). The major mechanism of P solubilization is by the production of organic acids and phosphatase enzyme (Nisha *et al*., 2014). The P solubilizers not only provides soluble P to the plants but also facilitates the accessibility of other trace elements and by synthesizing important growth promoting substances (Mittal *et al*., 2008), including siderophore (Wani *et al*., 2007b) and antibiotics (Lipping *et al*., 2008) and providing protection to plants against soil borne pathogens (Hamdali *et al*., 2008).

Siderophores are small, high – affinity, iron – chelating compounds that are synthesized by microorganisms such are bacteria and fungi (Verma *et al*., 2012). Siderophores are secondary metabolites produced in order 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 (Gamit and Tank, 2014). Based on the functional group the siderophore can be divided into different types they are, hydroxamates nature of siderophore, Catecholate nature of siderophore and carboxylate nature of siderophore (Verma *et al*., 2012; Guan *et al*., 2001; Neilands, 1995). Fungi are the important siderophore producing microorganisms next to bacteria. While most fungi produce hydroxymate siderophore with high affinity. Some important siderophore producing fungi include *Aspergillus nidulans, A. versicolor, Penicillium chrysogenum, P. citrinum, Mucor, Rhizopus, Saccharomyces cerivisiae, Rhodotorula minuta* (Kannahi and Senbagam, 2014).

Chrome azurol sulfonate (CAS) assay is one of most used assay for the detection of siderophore produced by the microbes. CAS agar medium contains an iron CAS-HDTMA (Hexadecyltrimethyl ammonium bromide) complex which is blue colour, iron chelator siderophore produces by microbes decolourize the blue coloured ferric-dye complex, resulting in a yellow to orange halo around the colonies. So the present study aimed at siderophore production and P solubilization from rhizosphere soil samples of medicinal plants. The aim of the present work is to highlight on major phosphate solubilizing and siderophore producing fungi, which could be used for bio inoculums preparation in an eco friendly and profitable manner.

MATERIALS AND METHODS

Collection of rhizosphere soil samples

Different rhizosphere soil samples from 10 - 15cm depth of roots were collected from different medicinal plants around Malnad regions of Shivamogga district. The names of the plants are presented in Table 1. The collected soil samples were placed in sterile polythene covers and brought into the laboratory aseptically and maintained at 4°C for further uses (Lokesh *et al*., 2016).

Isolation of Phosphate Solubilizing Fungi

Phosphate Solubilizing Fungi (PSF) was isolated by serial dilution method using Pikovskaya's agar medium and incubation was done by room temperature for 7days. After incubation, plates were observed for solubilization zone around fungal colonies and they were sub cultured for further use (Verma and Ekka, 2015).

Microscopic Characterization of PSF

Identification of PSF was done by lactophenol cotton-blue (LPCB) mounting technique. Specimen was stained with LPCB stain, cover slip 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 the standard manuals (Aneja, 2009; Subramanian, 1983; Barnett, 1975; Booth, 1971).

Screening for siderophore production by PSF

About 60.5mg of Chrome azurol Sulfonate (CAS) in 50mL of distilled water and this was mixed with 10mL of Iron solution prepared 1mM Ferric chloride in 10mM Hydrochloric acid. The resultant solution was added to HDTMA solution (72.9mg of HDTMA in 40mL of distilled water) with constant stirring and sterilized. The sterilized dark purple liquid was added to sterile Pikovskaya's medium containing without Tricalcium phosphate to make CAS agar. PSF cultures were point inoculated on CAS agar plates and incubated at room temperature for 7days (Raval and Desai, 2015).

Detection of nature of siderophore

Sterilized Pikovskaya's both without Tricalcium phosphate was inoculated with PSF culture and incubated at room temperature for 7 days. After the period of incubation the contents of the flask was filtered through Whatman filter paper No. 1 and culture filterate was collected and used for the detection of nature of siderophore produced by phosphate solubilizing fungi.

Detection of Hydroxymate nature of siderophores

0.5ml of culture supernatant was added with a pinch of tetrazolium salt and 1 – 2 drops of 2N NaOH solution. A deep red colour was appearance instantly indicates the presence of Hydroxymate nature of siderophore (Ghosh *et al*., 2017; Xychung and Boyer, 1996).

Detection of Catecholate nature of siderophores

One mL of culture supernatant was hydrolyzed with 1mL of HCl (1N), to this 1ml of nitrite molybedate reagent (10g of sodium nitrite and 10g of sodium molybedate in 100mL of distilled water) was added. The Catecholate nature of siderophore presence was indicated by the development of a yellow colour and was confirmed by the addition of 1ml of 0.5N NaOH, which changes yellow colour to red colour within 5min. The un-inoculated medium was used as the control (Farokh *et al*., 2011).

Detection of Carboxylate nature of siderophores

Addition of 1ml culture supernatant to pink coloured solution of 3 drops of NaOH and 1 drop of phenolphthalein, results in disappearance of pink colour indicating presence of Carboxylate nature of siderophore (Ghosh *et al*., 2017; Vogel, 1992).

Parameters of Phosphate Solubilization by PSF

Solubilization Index (SI)

Pikovskaya's agar plates were point inoculated with PSF culture and incubated for 7days. Solubilization index of the selected cultures were calculated using following formula (Elias *et al*., 2016).

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

Qualitative acid production assay by solid and liquid media

Pikovskaya's agar plate 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

Culture filtrate of PSF was centrifuged at 1000rpm for 10min to obtain supernatant. P^H of culture supernatant was measured by pH meter before inoculation and after the period of 7days incubation. Un-inoculated broth was used as control (Jain and Singh, 2015). Fifty mL of supernatant was titrated against 0.1N NaOH solution with few drops of phenolphthalein indicator. The titrable acidity was expressed in g/L (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 and it was expressed in terms of µg/mL. Optical density of the yellow coloured supernatant was measured at 420nm and the phosphate present in the supernatant calculated from standard curve of KH_2PO_4 (Verma and Ekka, 2015).

Screening of IAA production by PSF

Culture supernatant (2mL) was mixed with 2 drops of orthophosphoric acid and 4mL of Salkwoski reagent (50mL of 35% per chloric acid, 1ml of Ferric chloride solution) and allowed to stand in dark (Nenwani *et al*., 2010).

RESULTS AND DISCUSSION

Isolation and identification of Phosphate Solubilizing Fungi (PSF)

Seventy five rhizosphere soil samples were collected from different medicinal plants around Malnad regions of Shivamogga district. Sixty six PSF cultures were isolated by serial dilution method using Pikovskaya's agar medium. Then fungal colonies were point inoculated on fresh medium to make pure culture of the fungi for further studies of siderophore production and P solubilization. Isolates were named as PSF 1 to PSF 66 (Table 1). Then the fungal colonies were identified based on morphology and microscopic characters using standard manual. Lokesh *et al*. (2016) have isolated five different phosphate solubilizing fungi and screened from mine soil by serial dilution method.

Screening for siderophore production by PSF

Sixty six PSF cultures were screened using CAS agar medium for their ability to produce siderophore (Fig. 1). Among the 66 isolates, 56 PSF showed positive result for siderophore production. Out of 56 isolates, 46 PSF cultures showed minimum, 7 PSF cultures showed moderate and 3 PSF cultures showed maximum siderophore production during 7 days of incubation, and 10 PSF cultures showed negative for siderophore production (Table 2). The results were highlighted by earlier reports of Ghosh *et al*. (2017), who have reported that isolates of *Trichoderma* was showed siderophore production in CAS agar plate, *Trichoderma harzianum* produced maximum percentage of siderophores than *T. viride*, *T. asperellum* and *T. longibrachiatum.*

Detection of nature of siderophore produced by PSF

The 10 maximum siderophore producing PSF were selected and further the nature of siderophore produced was detected. PSF 15, 23, 29, 30, 37 and 52 showed positive for Hydroxymate nature of siderophore (Fig. 2), PSF 19 and 44 showed positive for Catecholate nature of siderophores (Fig. 3) and PSF 27 and 40 showed positive for Carboxylate nature of siderophores (Fig. 4) (Table 3)**.** Ghosh *et al*. (2017) have studied the typification of siderophores; it was found that *T. harzianum* recorded with maximum Hydroxymate and carboxylate production

whereas *T. viride*, *T. asperellum* and *T. longibrachiatum* recorded with lesser production of Hydroxymate and carboxylate as confirmed by color intensity.

Table 1. Isolation of Phosphate Solubilizing Fungi (PSF) from different medicinal plants.

S. No.	Plant name	PSF Culture	S. No.	Plant name	PSF Culture
1	*Datura fastuosa*	PSF 1	42	*Solanum xanthocarpum*	PSF 34
2	*Moringa oleifera*	-	43	*Eclipta prostrata*	PSF 35
3	*Leucus aspera*	PSF2			PSF 36
4	*Phyllanthus acidus*	PSF 3	44	*Brassica* sp.	PSF 37
5	*Argemone mexicana*	PSF 4	45	*Anacardium occidentale*	-
6	*Achyranthus aspera*	PSF 5	46	*Phyllanthus niruri*	PSF 38
7	*Centella asiatica*	PSF 6			PSF 39
8	*Asparagus racemosus*	PSF 7	47	*Phyllanthus* sp.	PSF 40
9	*Gymnema sylvestres*	PSF 8	48	*Acalypha indica*	PSF 41
10	*Tinospora cordifolia*	PSF 9	49	*Ricinus communis*	-
11	*Costus igneus*	PSF 10	50	*Tabernaemontana divaricata*	PSF 42
12	*Saraca asoca*	PSF 11	51	*Gloriosa superba*	-
13	*Calotropis* sp.	-	52	*Coleus* sp.	PSF 43
14	*Calotropis* sp.	PSF 12			PSF 44
15	*Vitex nigundo*	PSF 13	53	*Caesalpinia pulcherrima*	PSF 45
16	*Holorrhena antidysenterica*	PSF 14	54	*Nerium* sp.	-
17	*Eugenia jambolina*	-	55	*Ocimum basillicum*	PSF 46
18	*Clitoria ternatea*	PSF 15	56	*Mentha* sp.	-
19	*Wrightia tinctoria*	PSF 16	57	*Limonia acidissima*	PSF 47
20	*Phyllanthus amarus*	-			PSF 48
21	*Santalum album*	PSF 17			PSF 49
22	*Azadirachta indica*	PSF 18	58	*Portulaca oleracea*	PSF 50
23	*Aegle marvelous*	-	59	*Rauvolfia serpentina*	PSF 51
24	*Eucalyptus* sp.	PSF 19	60	*Plumbago zeylanica*	PSF 52
25	*Pongamia glabra*	PSF 20	61	*Oxalis corniculata*	PSF 53
26	*Vinca rosea*	PSF 21	62	*Justicia adhatoda*	PSF 54
27	*Ocimum sanctum*	PSF 22	63	*Sansevieria trifasciata*	PSF 55
28	*Solanum nigrum*	-			PSF 56
29	*Phyllanthus emblica*	PSF 23	64	*Pithecellobium* sp.	PSF 57
30	*Amaranthus viridis*	PSF 24	65	*Lantana camara*	PSF 58
31	*Amaranthus spinosus*	-	66	*Plumeria pudica*	PSF 59
32	*Alternanthera sessilis*	PSF 25	67	*Plumeria* sp.	-
33	*Euphorbia hirta*	PSF 26	68	*Basella alba*	PSF 60
34	*Euphorbia heterophylla*	PSF 27	69	*Barlaria prionitis*	PSF 61
35	*Ixora coccinea*	PSF 28	70	*Plectranthus amboinicus*	PSF 62
36	*Mimosa pudica*	PSF 29	71	*Annona reticulata*	PSF 63
37	*Cassia occidentalis*	PSF 30	72	*Cassia* sp.	-
38	*Punica granatum*	-	73	*Sida caordifolia*	PSF 64
39	*Asclepias curassavica*	PSF 31	74	*Annona muricata*	PSF 65
40	*Bauhinia purpurea*	PSF 32			PSF 66
41	*Momordica charantia*	PSF 33	75	*Butea monosperma*	-

Table 2. Screening for siderophore production by PSF.

S. No.	PSF cultures	Siderophore production	S. No.	PSF cultures	Siderophore production	S. No.	PSF cultures	Siderophore production
1	PSF 1	+	23	PSF 23	++	45	PSF 45	-
2	PSF 2	+	24	PSF 24	+	46	PSF 46	+
3	PSF 3	+	25	PSF 25	-	47	PSF 47	+
4	PSF 4	+	26	PSF 26	-	48	PSF 48	+
5	PSF 5	+	27	PSF 27	++	49	PSF 49	+
6	PSF 6	+	28	PSF 28	+	50	PSF 50	+
7	PSF 7	+	29	PSF 29	++	51	PSF 51	-
8	PSF 8	+	30	PSF 30	++	52	PSF 52	+++
9	PSF 9	+	31	PSF 31	+	53	PSF 53	-
10	PSF 10	+	32	PSF 32	+	54	PSF 54	+
11	PSF 11	+	33	PSF 33	-	55	PSF 55	-
12	PSF 12	+	34	PSF 34	++	56	PSF 56	+
13	PSF 13	+	35	PSF 35	+	57	PSF 57	-
14	PSF 14	+	36	PSF 36	+	58	PSF 58	+
15	PSF 15	++	37	PSF 37	+	59	PSF 59	+
16	PSF 16	+	38	PSF 38	++	60	PSF 60	+
17	PSF 17	+	39	PSF 39	+	61	PSF 61	+
18	PSF 18	+	40	PSF 40	+	62	PSF 62	-
19	PSF 19	++	41	PSF 41	+++	63	PSF 63	+
20	PSF 20	+	42	PSF 42	-	64	PSF 64	+
21	PSF 21	+	43	PSF 43	+	65	PSF 65	+
22	PSF 22	+	44	PSF 44	+++	66	PSF 66	+

Table 3. Detection of nature of siderophore produced by PSF.

S. No.	PSF Cultures	Culture code	Hydroxymate nature	Catecholate nature	**Carboxylate nature**
1	*Aspergillus* sp.	PSF 15	+	-	-
2	*Aspergillus* sp.	PSF 19	-	+	-
3	*Aspergillus* sp.	PSF 23	+	-	-
4	*Aspergillus* sp.	PSF 27	-	-	+
5	*Aspergillus* sp.	PSF 29	+	-	-
6	*Aspergillus* sp.	PSF 30	+	-	-
7	*Aspergillus carbonarius*	PSF 37	+	-	-
8	*Aspergillus niger*	PSF 40	-	-	+
9	*Aspergillus awamori*	PSF 44	-	+	-
10	*Aspergillus niger*	PSF 52	+	-	-

Table 4. Phosphate Solubilization by PSF.

S. No.	PSF cultures	SI	pH	Estimation of organic acid	Conc. of Phosphate (µg)	Production of IAA
1	PSF 15	1.08	55	10.7	290	+
2	PSF 19	1.24	5.48	12.4	280	+
3	PSF 23	2.37	4.9	20.1	155	+
4	PSF 27	2.87	4.4	24.33	75	+
5	PSF 29	2.86	4.39	25.38	75	+
6	PSF 30	2.65	4.7	22.23	110	+
7	PSF 37	3.54	3.6	37.76	30	+
8	PSF 40	3.91	3.1	32.8	20	+
9	PSF 44	3.96	3.0	33.89	20	+
10	PSF 52	3.87	3.6	31.82	25	+

Fig. 1. Screening for siderophore production by PSF.

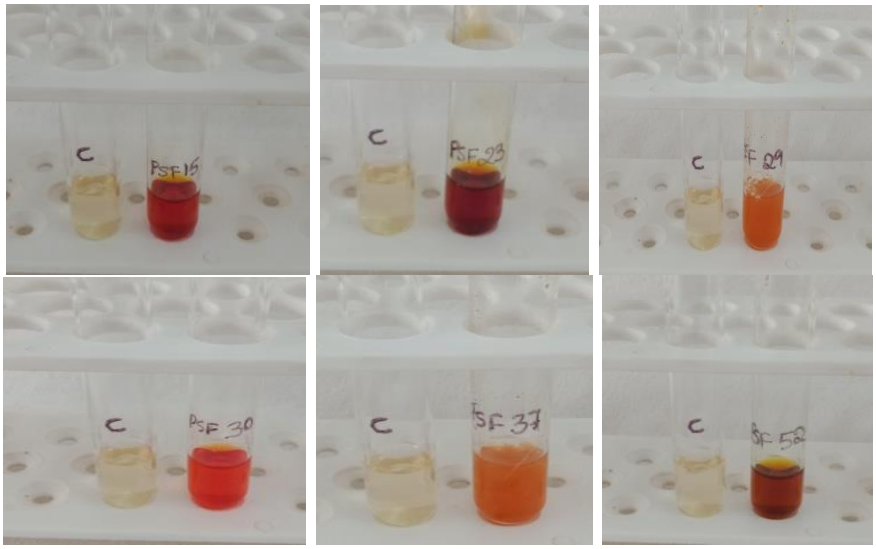

Fig. 2. Hydroxymate nature of siderophore produced by PSF.

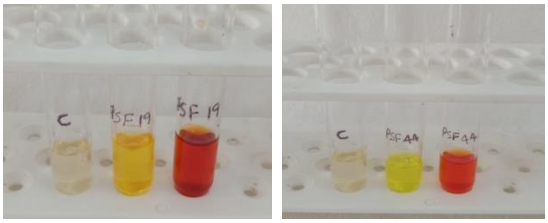

Fig. 3. Catecholate nature of siderophore produced by PSF.

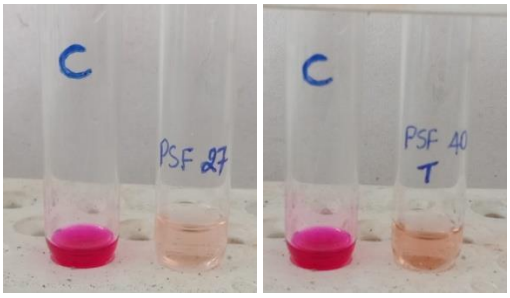

Fig. 4. Carboxylate nature of siderophores produced by PSF.

Parameters of Phosphate Solubilization by PSF

Solubilization Index (SI)

The solubilization index of selected 10 PSF cultures ranged from 1.08 to 3.96 (Table 4), the results were similar to the earlier findings of Elias *et al.* (2016) who reported SI ranging from 1.10 to 3.05.

Qualitative acid production assay

The selected 10 PSF showed colour change from blue to yellow on agar plate when using Bromophenol Blue, the similar result was observed in earlier findings of Chadha *et al.* (2015) and in the broth colour change from red to yellow was observed while using Bromocresol purple, same result was observed in earlier findings of Khan and Gupta (2015).

Measurement of pH and Titrable acidity

Inoculation of selected 10 PSF to Pikovskaya's broth resulted in decrease of pH ranging from 5.48 to 3.0 from initial pH of 6.89 (Table 4). These results were correlated with previous studies of Jain and Singh (2015) who have reported decrease in pH of broth, ranged 4.0 to 3.2 from initial pH of 7.5 ± 0.2, after the inoculation of phosphate solubilizing fungi.

The measure of amount of acid present in the culture broth ranged from 10.7g/L to 37.76g/L (Table 4). The same results were highlighted by Khan and Gupta (2015) have checked the ability of acid production of 29 acidophilic fungal isolates, and 5 isolates LAK-2, BS-1.6, CM-2, DR-1 and DR-2 showed good acid production.

Estimation of Phosphate

The phosphate present in the culture broth inoculated by selected 10 PSF cultures ranged from 290µg/mL to 20µg/mL (Table 4). The similar results were highlighted by Verma and Ekka (2015) who have reported the concentration of phosphate tricalcium amended broth by different PSF isolates gradually increases, which ranged from 219.16µg/mL to 59.17µg/mL.

Screening of production IAA by PSF

The selected 10 PSF cultures showed positive result for IAA production development of pink colour was observed after the period incubation. Nenwani *et al*. (2010) reported that PSF was able to produce phyto-hormone IAA, isolate F1 was found to produce 11.45 μgmL^{-1} of IAA which is significantly high.

CONCLUSION

Phospahte solobilizing fungi play a key role in soil P solubilization and subsequent availability of phosphate to plant. Phosphorus is a major plant nutrient available to plant roots only in soluble forms. The aim of the work was to highlight on major P solubilizing fungi and siderophore production for plant growth and development, which could be used for bio inoculums preparation in an eco friendly and profitable manner. Primarily, the preparation of bio inoculums will reduce dependency on chemical fertilizer and this will reduce the accumulation of Phosphorus in reservoirs and will make environment safe from pollution.

REFERENCES

Anand, K., B. Kumari and M.A. Mallick (2016). Phosphate Solubilizing Microbes: an effective and alternative approach as biofertilizers. *International Journal of Pharmacy and Pharmaceutical Sciences.* 8(2): 37- 40.

Aneja, K.R. (2007). *Experiments in Microbiology, Plant Pathology and Biotechnology*. New age International Publishers, New Dehli.

Barnett, H.L. (1975). *Illustrated genera of imperfect fungi*. Vol. 2. Pp.225.

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

Chadha, N., R. Prasad and A. Varma (2015). 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.

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

Farokh, R.Z., D. Sachdev, N. Kazemi-Pour, A. Engineer, K.R. Pardesi, S. Zinjarde, P.K. Dhakephalkar and B.A. Chopade (2011). Characterization of Plant-Growth-Promoting Traits of *Acinetobacter* Species Isolated from Rhizosphere of *Pennisetum glaucum. Journal of Microbiology and Biotechnology.* 21(6): 556 -566.

Gamit, S.K. and Tank (2014). Effect of Siderophore Producing Microorganism on Plant Growth of *Cajanus cajan* (Pigeon pea). *International Journal of Research in Pure and Applied Microbiology.* 4(1): 20 -27.

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

Guan, L.L., K. Kanoh and K. Kamino (2001). Effect of Exogenous Siderophores on Iron Uptake Activity of Marine Bacteria under Iron-Limited Conditions. *Applied and Environmental Microbiology.* 67(4): 1710-1717.

Hamdali, H., M. Hafidi, M.J. Virolle and Y. Ouhdouch (2008). Rock phosphate solubilizing Actinomycetes: Screening for plant growth promoting activities. *World Journal of Microbiology and Biotechnology.* 24: 2565-2575.

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

Kannahi, M. and N. Senbagam (2014). Studies on siderophore production by microbial isolates obtained from rhizosphere soil and its antibacterial activity. *Journal of Chemical and Pharmaceutical Research.* 6(4): 1142-1145.

Khan, R. and A.K. Gupta (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.

Lipping, Y., X. Jiatao, J. Daohong, F. Yanping, L. Guoqing and L. Fangcan (2008). Antifungal substances produced by *Penicillium oxalicum* strain PY-1-potential antibiotics against plant pathogenic fungi. *World Journal of Microbiology and Biotechnology.* 24: 909-915.

Lokesh, S.T., K.J. Naveen Kumar and B. Thippeswamy (2016). Screening of efficient phosphate solubilizing fungi from mine soil and effect of phosphofungi on seed germination and vigour index of ground nut (*Arachis hypogaea* L.) and green gram (*Vigna radiata* L.). *International Journal of Biological Research.* 4(2): 288-294.

Mittal, V., O. Singh, H. Nayyar, J. Kaur and R. Tewari (2008). Stimulatory effect of phosphate solubilizing fungal strains (*Aspergillus awamori* and *Penicillium citrinum*) on the yield of chickpea (*Cicer arietinum* L. cv. GPF2). *Soil Biology and Biochemistry.* 40:718-727.

Naik, S.K., S. Maurya, R. Kumar, K. Sadhna, S. Gagrai, B. Das, S. Kumar and B.P. Bhatt (2013). Inorganic phosphate solubilization by phosphate solubilizing fungi isolated from acidic soils. *African Journal of Microbiology Research*, 7(34): 4310-4316.

Neilands, J.B. (1995). Siderophores: Structure and Function of Microbial Iron Transport Compounds. *Journal of Biological Chemistry,* 270(45): 26723-26726.

Nenwani, V., P. Doshi, T. Saha and R. Shalini (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., S.N. Padma Devi, S. Vasandha and K. Sunitha Kumari (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.

Praveen, M.P., V.B. Kuligod, N.S. Hebsur, C.R. Patil and G.N. Kulkarni (2012). Effect of Phosphate Solubilising Fungi on growth, yield and nutrient content in Maize (*Zea mays*). *Karnataka Journal of Agricultural Science,* 25(1): 58 – 62.

Raval, A.A. and P.B. Desai (2015). Screening and Characterization of Several siderophore Producing Bacteria as Plant Growth-Promoters and Biocontrolling agents, *International Journal of Pharmacy and Life Sciences,* 6(10-11): 4803 – 4811.

Stephen, J. and M.S. Jisha (2011). Gluconic acid production as the principal mechanism of mineral phosphate solubilization by *Burkholderia* sp. (MTCC 8369). *Journal of Tropical Agriculture,* 49 (1-2): 99-103.

Subramanian, C.V. (1983). *Hyphomycetes taxonomy and biology*. Academic Press. London.

Verma, A. and A. Ekka (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., K. Joshi and B. Mazumdar (2012). Study of Siderophore Formation in Nodule-Forming Bacterial Species Research. *Journal of Chemical Sciences*, 2(11): 26-29.

Vogel, A.L. (1992). In: *Elementary Practical Organic Chemistry.* CBS Publishers, pp.2190.

Wani, P.A., M.S. Khan and A. Zaidi (2007). Synergistic effects of the inoculation with nitrogen fixing and phosphate solubilizing rhizobacteria on the performance of field grown chickpea. *Journal of Plant Nutrition and Soil Science*, 170: 283-287.

Xychung, H. and G.L. Boyer (1996). Siderophore-Mediated Aluminum Uptake by *Bacillus megaterium* ATCC 19213. *Analytical Chemistry,* 68: 1812.

(*Accepted for publication June 2020*)