International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Talaromyces amestolkiae - A Potential Phosphate Solubilizer Isolated from Rhizosphere Soil of Medicinal Plants

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Abstract: ***In the present study, 13 PSF were isolated and screened by Pikovskaya's media from 15 rhizosphere soil samples of different medicinal plants and labeled as PSF1 to PSF13. The in-vitro study confirms PSF10 has more phosphate solubilizing potential and was identified as Talaromyces amestolkiae; morphologically and confirmed by molecular characterization. The 18s rRNA sequence was deposited at GenBank, NCBI (MN904859). PSF 10 has good results in SI (3.21) and reduced pH in culture filtrate (3.8 from 6.89), colour change of Bromophenol blue indicator to yellow on an agar plate and Bromocresol purple indicator to yellow in broth to acidic condition, TA (30.16g/L) and 45µg of phosphate was found in the culture broth. It produces siderophore and IAA. Due to the maximum phosphate solubilization efficiency of the fungus, Talaromyces amestolkiae compared to other fungal isolates it can be recommended for the development of phosphate solubilizing biofertilizers used in agricultural fields.***

Keywords: *Costus igneus*, Indole Acetic Acid, Solubilization Index, Siderophores.

1. Introduction

Phosphorus (P) is a vital nutrient available to the roots of the plant only in the form of a soluble state that is in short supply in the soil (Malviya et al.2011). It is estimated that about 98% of the Indian soil contains an insufficient amount of available P, which is necessary to support maximum plant growth (Nisha et al.2014). The two main categories of P in soils are organic and inorganic forms (Lokesh et al.2016). The organic form is found in humus and other organic materials, which are taken up by plants from the soil and utilized by animals that consume plants (Nisha et al.2014). An adequate supply of Phosphate in the early growth stages of a plant helps to promote the growth and productivity of the crops (Mahantesh and Patil 2011).

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). So the Phosphate Solubilizing Microorganisms (PSM) converts these insoluble phosphates into soluble forms through special mechanisms. That they carry out the acidification process, chelation, exchange reaction (Gulati et al.2010), and production of Gluconic acid (Nisha et al.2014). Production of these organic acids resulted in the acidification of the microbial cell and its surroundings (Nisha et al.2014). The organic and inorganic acids from the microbes, convert tri-calcium phosphate into di-and monobasic phosphates (Walpola and Yoon 2012) with the net result of enhanced availability of the element to the plants. The organic acid type produced by the microbes and their amounts differs with different organisms (Mahamuni 2012).

Not only proving 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, and by synthesizing important growth-promoting substances (Mittal et al.2008), including siderophores and antibiotics and providing protection to plants against soil-borne pathogens. Many bacterial, fungal, yeast, and actinomycetes species capable of solubilizing sparingly soluble P in pure culture have been isolated and studied (Pradhan and Sukla 2005). PSM includes various bacteria, fungi, and actinomycetes, which help to convert insoluble phosphate into a simple and soluble form of phosphate. Fungi are the best phosphate solubilizers compared to bacteria and other microorganisms.

The forests and hill regions of the Western Ghats are the treasure house of medicinal plants. Many of them are used for traditional and folk medicinal practices (Hemavani et al.2011). Medicinal plants have been used to a greater extent for the control of diabetes mellitus in various traditional systems of medicine worldwide as they are of natural origin. In India, *Costus igneus* popularly known as the insulin plant due to the consumption of its leaves helps to prevent diabetes mellitus by decreasing the blood sugar level (Laha and Paul 2019). The rhizosphere region of medicinal plants contains a variety of symbiotic microorganisms attracted by root exudates of medicinal plants and they help plants in various aspects like phosphate solubilization, nitrogen fixation, siderophores production, soil amendment, various mineral nutrients supply to plants, triggers the plant growth hormones and gave protection to the plants from invading pathogens. All these aspects promise rhizosphere soil of medicinal plants is a good source of phosphate-solubilizing microorganisms.

In the present study, *Talaromyces amestolkiae*-a potential phosphate solubilizer among the rhizosphere soil fungi of medicinal plants isolated from rhizosphere soil of medicinal

plants, characterized the phosphate solubilizing fungi and they were screened for solubilization efficiency and assessed for production of organic acids, which are involved in P solubilization, production of siderophores and plant growth promoter i. e., indole acetic acid (IAA).

2. Materials and Methods

Collection of rhizosphere soil samples

The rhizosphere soil samples (10-15cm depth around the roots) were collected from different medicinal plants in dry deciduous forest of Western Ghats regions of Shivamogga district in sterile polythene covers and brought to the laboratory in aseptic condition and stored in a refrigerator at 4°C until used for the isolation of phosphate solubilizing fungi (Chatli et al.2008).

Isolation Phosphate Solubilizing Fungi

1g of rhizosphere soil was suspended in 9 ml of sterilized 0.85% of saline solution and serially diluted. The dilutions were plated on sterile solidified Pikovskaya's agar medium by spread plate method and incubated for 7 days at room temperature. After incubation plates were examined for solubilizing zone around fungal colonies and colonies showing solubilizing zone were selected and sub-cultured on fresh media for further use (Nelofer et al.2015; Gomes et al.2014).

Characterization of Phosphate Solubilizing Fungi

Microscopic Characterization: The specimen was stained with LPCB stain, a 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; Booth 1971).

Molecular Characterization: Isolated phosphate solubilizing fungi DNA was extracted by the CTAB method. The DNA concentration in the sample was estimated by recording absorbance at 260 and 280nm in a UV/ VIS spectrophotometer.

Solubilization Index (SI)

The isolated fungal cultures were point inoculated on Pikovskaya's medium and plates were incubated at room temperature for 7 days. After incubation, observed the formation of a zone around the colonies of fungi. The solubilization index was measured based on colony diameter and solubilization zone diameter formed around the colony of phosphate solubilizing fungi (Elias et al.2016). Using the formula,

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

Measurement of pH and Titrable acidity

PSF culture filtrate was centrifuged at 1000rpm for 10min and supernatant was collected. The P^H of the culture filtrate was measured by a P^H meter before inoculation and after incubation. Uninoculated broth served as a control (Jain and Singh 2015; Kumari et al.2010). The amount of acid production was estimated by titration using alkali.50ml of culture supernatant was titrated against 0.1N NaOH solution with a few drops of phenolphthalein indicator. The titrable acidity was expressed in g/L (Khan and Gupta 2015).

Assay of qualitative acid production

Assay of qualitative analysis for acid production on solid media: Pikovskaya's agar plates were prepared by supplemented with Bromophenol blue indicator. These plates were point inoculated with each fungal culture and incubated at room temperature for 7 days (Chadha et al.2015).

Assay of qualitative analysis for acid production in broth: Sterilized Pikovskaya's broth supplemented with Bromocresol purple indicator was inoculated with fungal culture and incubated for 7 days at room temperature (Khan and Gupta 2015).

Estimation of Phosphate

PSF culture was inoculated in Pikovskaya's broth and incubated at room temperature for 7 days at 100rpm in an orbital shaker incubator. The culture filtrate was collected and centrifuged at 3000rpm for 30min. Estimation of phosphate in the supernatant was done by the Vanadomolybdate yellow colour method, absorbance was measured at 420nm and it was expressed in µg/ml. The amount of phosphate was calculated from the standard curve of KH_2PO_4 (Verma and Ekka 2015; Sahoo and Gupta 2014).

Screening for siderophore production

The siderophore production was screened by using Chrome Azurol Sulfonate (CAS) agar medium.60.5mg of Chrome Azurol S was dissolved in 50ml of distilled water and mixed with 10ml of an Iron solution prepared by 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 solution was added to Pikovskaya's medium containing no tri-calcium phosphate to make CAS agar. Then the CAS agar plates were point inoculated with PSF culture and incubated at room temperature for 7 days (Ghosh et al.2017).

Screening for Indole Acetic Acid (IAA) production

PSF culture was grown in potato dextrose broth supplemented with 1% Tryptophan. After growth, culture filtrate was centrifuged at 1000rpm for 10min, and the supernatant was collected. About 2ml of culture supernatant was mixed with 2 drops of ortho-phosphoric acid and 4ml of Salkwoski reagent and allowed to stand in dark. After 2h incubation of the reaction mixture at room temperature, the development of pink colour indicates the production of indole acetic acid (Pant and Agrawal 2014; Priya et al 2013).

3. Results and Discussion

Isolation Phosphate Solubilizing Fungi

A total of 15 rhizosphere soil samples of medicinal plants in the Western Ghats regions of Shivamogga district were collected. From them, 13 fungal colonies among the isolates showed a solubilization zone on Pikovskaya's agar medium and they were subcultured and labeled as PSF1 to PSF13

listed below in Table 1. The results were correlated with earlier findings of Lokesh et al. (2016) have isolated 5 phosphate solubilizing fungi from the mine soil in Bellary, Karnataka, India. Gomes et al. (2014) tested 59 isolates from maize rhizosphere; the genera were dominant *Burkholderia* and *Bacillus* in the group of the most efficient bacteria and *Talaromyces* and *Penicillium* in the fungi group.

Characterization of Phosphate Solubilizing Fungi

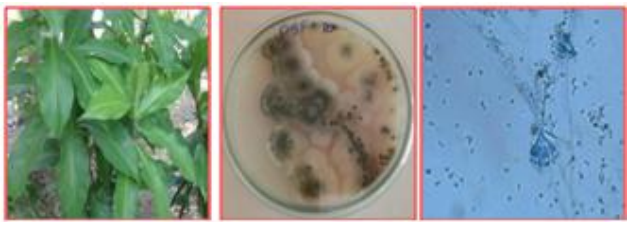
Microscopic characterization: Isolated PSF was identified as *Aspergillus* sp., *Alternaria* sp., *Penicillium* sp., and *Talaromyces* sp. based on morphological characteristics regarding the standard fungal manuals (Table 1).

Table 1: Isolation of PSF from rhizosphere Soil Samples of Medicinal Plants

S. No.	Medicinal plant name	Culture code	Cultures
1	*Datura fastuosa*	PSF 1	*Aspergillus* sp.
2	*Moringa oleifera*	-	-
3	*Leucus aspera*	PSF 2	*Aspergillus* sp.
4	*Phyllanthus acidus*	PSF 3	*Alternaria*sp.
5	*Argemone mexicana*	PSF 4	*Aspergillus* sp.
6	*Achyranthus aspera*	PSF 5	*Penicillium*sp.
7	*Centella asiatica*	PSF 6	*Penicillium*sp.
8	*Asparagus racemosus*	PSF 7	*Talaromyces* sp.
9	*Gymnema sylvestres*	PSF 8	*Aspergillus* sp.
10	*Tinospora cordifolia*	PSF 9	*Penicillium*sp.
11	***Costus igneus***	**PSF 10**	***Talaromyces amestolkiae***
12	*Saraca asoca*	PSF 11	*Aspergillus* sp.
13	*Calotropis procera*	-	-
14	*Calotropis gigantea*	PSF 12	*Penicillium*sp.
15	*Vitex nigundo*	PSF 13	*Aspergillus* sp.

Molecular Characterization: PSF 10 isolated from rhizosphere soil of *Costus igneus (*Fig 1A) was selected based on its maximum phosphate solubilizing efficiency and its molecular characterization was done by18s rRNA sequencing and confirmed the culture PSF10 was confirmed as ***Talaromyces amestolkiae (***Fig 1B and 1C) and its 18s rRNA gene sequence given below was deposited at GenBank, NCBI with reference code **MN904859**.

GCGGAAGATGGGGGGCCCTCGTGGCCAACCTCC CACCCTTGTCTCTATACACCTGTTGCTTTGGCGG GCCCACCGGGGCCACCTGGTCGCCGGGGGACAT CTGTCCCCGGGCCCGCGCCCGCCGAAGCGCTCT GTGAACCCTGATGAAGATGGGCTGTCTGAGTAC TATGAAAATTGTCAAAACTTTCAACAATGGATCT CTTGGTTCCGGCATCGATGAAGAACGCAGCGAA ATGCGATAAGTAATGTGAATTGCAGAATTCCGT GAATCATCGAATCTTTGAACGCACATTGCGCCCC CTGGCATTCCGGGGGGCATGCCTGTCCGAGCGT CATTTCTGCCCTCAAGCACGGCTTGTGTGTTGG GTGCGGTCCCCCCGGGGACCTGCCCGAAAGGCA GCGGCGACGTCCGTCTGGTCCTCGAGCGTATGG GGCTTTGTCACTCGCTCGGGAAGGACTGGCGGA GGTTGGTCACCACCAAAATTTTACCACGGGTGA CCTCGGATCAGGTAGGATTTAC



(a) (b) (c)

Figure 1: A: *Costus igneus*, B: *Talaromyces amestolkiae* and C: Microscopic observation (40X)

Construction of Phylogenetic tree

A Phylogenetic tree was constructed to compare the 18s rRNA sequence of *Talaromyces amestolkiae (*PSF 10) with the top nearest sequences databases of NCBI deposits. *T. amestolkiae* 18s rRNA sequence resembles the close relationship with the other related strains sequence database shown below in Fig 2.

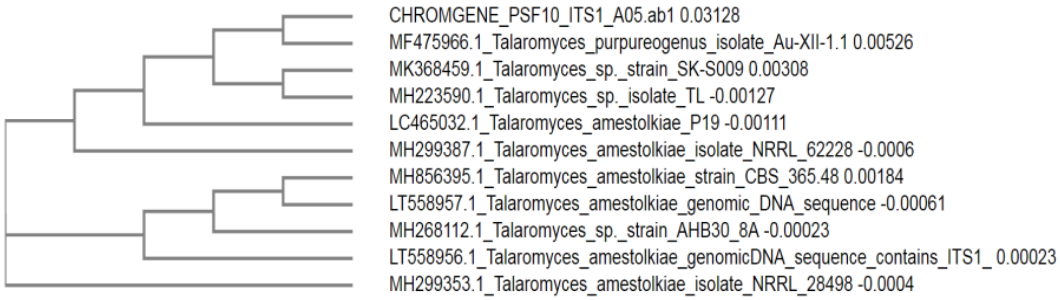


Figure 2: Phylogenetic tree constructed for *Talaromyces amestolkiae* (PSF10)

Solubilization Index (SI)

The solubilization indexes of 13 fungal cultures ranged from 1.29 to 3.21 shown in Table 2. Among these PSF 10 showed a maximum solubilization index of 3.21 (Fig 3). The results were highlighted by earlier works of Elias et al. *(*2016) isolated a total of 359 fungal isolates from 150 rhizosphere soil samples and their solubilization index (SI) was recorded ranging from 1.10 to 3.05. Verma and Ekka (2015) reported SI of 18 fungal culture strains ranged from 1.06 to 3.34.

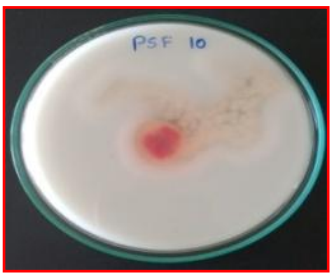


Figure 3: Solubilization Index (SI) of *Talaromyces amestolkiae*

Measurement of pH and Titrable Acidity

A total of 13 fungal cultures reduces the P^H recorded ranging from 5.4 to 3.8 and PSF 10 showed maximum decreases the P^H to 3.86 in culture broth from 6.89 (Table 2). The measure of the amount of acid present in the culture broth of 13 PSF ranged from 30.16g/L to 12.4g/L and the PSF 10 showed 30.16g/L (Table 2). The results were highlighted by Jain and Singh (2015) reported on applying the inoculation of phosphate solubilizing fungi, a decrease in P^H was observed in a liquid medium ranging from 4.0 to 3.2 from initial pH of 7.5±0.2 by the production of organic acids. Results were correlated with Khan and Gupta (2015) checked the ability of acid production and 5 isolates LAK-2, BS-1.6, CM-2, DR-1 and DR-2 showed good acid production.

Assay of qualitative acid production assay

The colour change was observed in the media during the growth of fungus, it showed colour change from blue to yellow on the agar plate when using Bromophenol blue (Fig 4a) and colour change from red to yellow in broth was observed while using Bromocresol purple (Fig 4b). The results of our work similar with earlier findings of Chadha et al. (2015) inoculated the fugal isolate with Bromophenol blue at a concentration of 0.003%, showed blue colour to yellow colouration upon incubation of 7days at 28°C. While Khan and Gupta (2015) subjected 29 acidophilic fungal isolates for qualitative assay for acid production using 0.04% of Bromocresol purple showed the formation of yellow colouration of the medium upon 5days incubation.

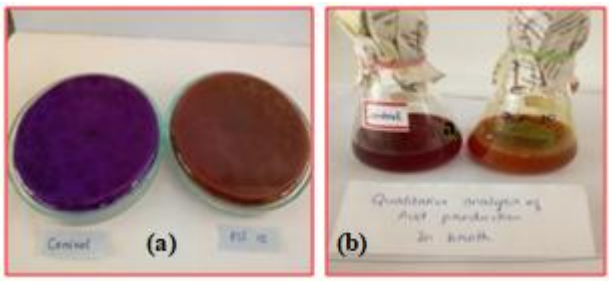


Figure 4: Qualitative acid production on agar plate (a) and in broth (b) of *Talaromyces amestolkiae*

Estimation of Phosphate

The phosphate solubilization during incubation of 13 PSF cultures of tri-calcium phosphate as a source ranged from 270µg/ml to 45µg/ml and PSF 10 showed 45µg/ml (Table 2). Verma and Ekka (2015) reported the concentration of phosphate in culture broth amended with tri-calcium phosphate inoculated by different PSF isolates gradually increases ranging from 219.16µg/ml to 59.17µg/ml.

Table 2: Phosphate solubilization activity of isolates

S. No.	Culture Code	SI	pH	TA g/L	Conc. of P (µg)
1.	PSF 1	2.47	4.8	23.2	145
2.	PSF 2	2.70	4.6	21.68	100
3.	PSF 3	2.16	5.05	15.92	185
4.	PSF 4	1.29	5.4	12.08	270
5.	PSF 5	3.15	3.9	24.72	50
6.	PSF 6	2.51	4.7	21.6	135
7.	PSF 7	3.08	4.09	24.8	60
8.	PSF 8	3.02	4.30	26.08	70
9.	PSF 9	1.60	5.1	16.8	220
10.	**PSF 10**	**3.21**	**3.86**	**30.16**	**45**
11.	PSF 11	2.71	4.6	21.04	100
12.	PSF 12	1.49	5.28	13.6	235
13.	PSF 13	1.33	5.17	12.4	260

Screening for siderophore and IAA production

Upon screening, 13 isolates were showed positive result for siderophore production by change from blue to pink (Fig 4a) and IAA production by developing pink colour in culture filtrate (Fig 5b). Ghosh et al. (2017) have reported that isolates of *Trichoderma* have shown positive results for siderophore production in CAS agar plate. Priya et al. (2013) have isolated and evaluated the IAA producing P solubilizing microbes using Salkowski reagent and the colour developed within 30min.

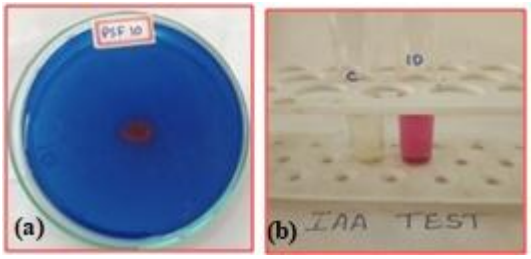


Figure 5: Siderophore and Indole Acetic Acid (IAA) production by *Talaromyces amestolkiae*

4. Conclusion

Predominantly Phosphate solubilizing biofertilizers can be used as an alternative to chemical P fertilizer because they nature friendly and non-hazardous to farmers and agriculture fields and the production of beneficial phosphate solubilizing fungi from rhizosphere soil of medicinal plants resource may improve the soil fertility, enhance the plant growth and reduce the risk of environmental pollution, nutrients amendment to the crops and avoids the entry of invading pathogenic microorganism to crop plants and diminish the accumulation of P. Hence P solubilizing microorganisms play an important role in supplementing P to the plants allowing sustainable use of P fertilizers. High P solubilization efficiency of *Talaromyces amestolkiae* and its ability for the production of siderophores and IAA production, this *Talaromyces amestolkiae* inoculums is recommended as P solubilizing biofertilizer which will be used for agriculture and horticulture crop fields.

Acknowledgment

Authors are gratefully acknowledged by the Department of Microbiology, Kuvempu University, Shivamogga, Karnataka, India, for providing the necessary facilities and support for the successful completion of this work.

References

[1] Aneja K R 2007. Experiments in Microbiology, Plant Pathology and Biotechnology. *New age International Publishers*, New Dehli.4**:** 157-162.
[2] Booth C 1971. Illustrated the genus *Fusarium*. Common Wealth Mycological Institute.1-237.
[3] Chandha N Prasad R and Varma A 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.

[4] 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.4***8:** 267-273.

[5] 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.

[6] Ghosh S Banerjee S and Sengupta C 2017. Bioassay, characterization and estimation of siderophores from some important antagonistic fungi. *Journal of Biopesticides.1***0 (2):** 105-112.

[7] Gomes E A Silva U De C Marriel I E Oliveira C A De Lana U G De P 2014. Rock Phosphate Solubilizing Microorganisms Isolated from Maize Rhizosphere soil. *Revista Brasileira de Milho e Sorgo.1***3 (1):** 69-81.

[8] Gulati A Sharma N Vyas P Sood S Rahi P Pathania V and Prasad R 2010. Organic acid production and plant growth promotion as a function of phosphate solubilization by *Acinetobacter rhizosphaerae* strain BIHB 723 isolated from the cold deserts of the trans-Himalayas. *Archives of Microbiology.1***92:** 975-983.

[9] Hemavani C and Thippeswamy B 2011. Arbuscular mycorrhizal status of medicinal plants of Bhadra Wild Life Sanctuary, India. *Journal of Mycology and Plant Pathology.4***1 (2):** 291-295.

[10] 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.

[11] 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.

[12] Kumari P D S U Nanayakkara C M and Bandara M A U 2010. Development of a fungal inoculum for efficient phosphate solubilization in agriculture, Proceedings of the 15th International Forestry and Environment Symposium, 26-27 November 2010. Published by Department of Forestry and Environmental Science, University of Sri Jayewardenepura, Sri Lanka.223-229.

[13] Laha S and Paul S 2019. *Costus igneus*-A therapeutic anti-diabetic herb with active phytoconstitutents. *International Journal of Pharmaceutical Science and Research.*1**0 (8):** 3583-3591. DOI: 10, 13040/IJPSR.0975-8232.

[14] Lokesh S T Naveen Kumar K J and Thippeswamy B 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.

[15] Mahamuni S V Wani P V and Patil A S 2012. Isolation of Phosphate Solubilizing Fungi from Rhizosphere of Sugarcane & Sugar Beet Using Tcp & Rp Solubilization. *Asian Journal of Biochemical and Pharmaceutical Research.1* **(2):** 237-244.

[16] Mahantesh P and Patil C S 2011. Isolation and Biochemical Characterization of Phosphate Solubilizing Microbes. *International Journal of Microbiology Research.3* **(1):** 67-70.

[17] Malviya J Singh K and Joshi V 2011. Effect of phosphate solubilizing fungi on growth and nutrient uptake of Ground nut (*Arachis hypogaea*) plants. *Advanced Bioresource.2* **(2):** 110-113.

[18] Mittal V Singh O Nayyar H Kaur J and Tewari R 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.4**0:** 718-727.

[19] Nelofer R Syed Q Nadeem M Bashie F Mazhar S and Hassan A 2016. Isolation of Phosphate-Solubilizing Fungus from soil to Supplement Biofertilizer. *Arabian Journal of Science and Engineering.4*1: 2131-2138. DOI 10.1007/s13369-015-1916-2.

[20] Nisha K Padma Devi S N Vasandha S and Sunitha Kumari 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.

[21] Pant G and Agrawal P K 2014. Isolation and Characterisation of Indole Acetic Acid producing plant growth promoting rhizobacteria from rhizospheric soil of *Withania somnefera*. *Journal of Biological and Scientific Opinion.2* **(6):** 377-383.

[22] Pradhan N and Sukla L B 2005. Solubilization of inorganic phosphates by fungi isolated from agriculture soil. *African Journal of Biotechnology.5* **(10):** 850-854.

[23] Sahoo H R and Gupta N 2014 Evaluation of Phosphate Solubilising Potential of Some Endophytic Fungi under Solid and Liquid State. *BMR Microbiology.1* **(1):** 1-6.

[24] Verma A and Ekka A 2015. Assessment of Phosphate Solubilising Potential of Fungal Isolates from Soils of Three Blocks of Raipur, Chhattisgarh, India. *Journal of Ravishankar University.2***8 (2):** 44-50.

[25] 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.