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Growth enhancement of medicinal plant *Withania somnifera* using phosphate solubilizing endophytic bacteria *Pseudomonas* sp. as bioinoculant

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ABSTRACT

Withania somnifera, commonly known as ashwagandha, is a wildly growing highly valuable medicinal plant, found throughout India. Ashwagandha has high medicinal value due to its antioxidant, antitumor, anti-inflammatory, immuno-modulatory, anti-ageing and rejuvenating properties. Since use of synthetic agrochemicals in the cultivation of medicinal plants is highly restricted due to their harmful effects on human health and environment, present study was aimed to explore the role of endophytic bacteria in enhancing the nutrient level in soil and growth of ashwagandha. In this study, growth promoting potential of endophytic bacterial strain PSE-1 was explored which was isolated from the roots of *W. somnifera* and identified as *Pseudomonas* sp. on the basis of 16s rRNA gene sequencing. Results obtained from the study suggest that endophytic bacteria *Pseudomonas* sp. plays important role in improving the nutrient level in soil and displayed beneficial effects on plant growth.

1. INTRODUCTION

Phosphorus (P), an important component of soil, serves as macronutrient for plants and plays key functions in their growth and development (Sharma et al., 2013). Despite its abundance in agricultural soil, P is a growth limiting factor for plant growth because of its slow diffusion and high fixation rate in soil (Miller et al., 2010). P deficiency in Indian soils is widespread as 98% of agricultural land in India is poor in soluble P (Kanwar and Grewal, 1990). Plants require approximately 30 µmol L^{-1} of phosphorus for maximum productivity, but only about 1µmol L^{-1} is available in many soils (Daniels et al., 2009). To support plant growth, P is added by farmers in the form of chemical fertilizers in soil but repeated use of fertilizers deteriorates soil quality (Tewari et al., 2004). Intensive cropping and current agricultural practices have also resulted in deficiency of P. Though various methods are being employed to manage P in agricultural lands but all are costly and practically difficult as soil pH and chemical fixation by soil minerals determines the quantity of available P. To improve mineralization and solubilization of chemically-fixed P, microbially mediated P management is an ecofriendly and cost effective approach.

Microorganisms are integral to the soil P cycle, regulating P mineralization by various mechanisms such as production of extracellular phosphatases, secretion of organic acids, production of siderophores to enhance P availability by chelating cations such as Fe, Al or Ca that are involved in the formation of insoluble phytates (Singh et al., 2011). Phosphatases have been extensively studied in soil as they catalyze the hydrolysis of ester-phosphate bonds, leading to the release of insoluble P (Tabatabai, 1994; Sharma et al., 2013). To enhance P availability in soil, present scenario is shifting towards more sustainable agriculture system by using phosphate solubilizing bacteria. Hydrolysis of organic P by microbes is highly influenced by environmental factors as well as physicochemical and biochemical properties of the molecules (Rodríguez and Fraga, 1999). Among plant-associated microorganisms, endophytes are gaining considerable importance as bioinoculants because of their ability to colonize plant tissues thus are less affected by fluctuating environmental conditions. Endophytes possess the potential to mineralize complex and insoluble forms of macro- and micronutrients by secreting extracellular hydrolytic enzymes and enhance the availability of nutrients to plants. Several reports are available on phosphate solubilizing potential of endophytic bacteria and their positive impact on plant growth (Wakelin et al., 2004; Oteino et al., 2015).

In present study, endophytic bacteria from medicinal plant *Withania somnifera* were investigated for their phosphate-solubilization potential as well as other PGP traits to investigate their role in enhancing plant growth and soil fertility. *W. somnifera* is a popular and traditional Indian medicinal herb, belonging to family Solanaceae and is also known as Ashwagandha or Indian ginseng or winter cherry. It possesses high medicinal value due to its antitumor, anti-inflammatory, immunomodulatory, anti-ageing and rejuvenating properties. Ashwagandha has high demand worldwide for its alkaloids and other phytochemicals thus overexploited from natural habitats whereas cultivation is comparatively low, because of which it has reached near extinction (Sivanesan and Jeong, 2007). *W. somnifera* is known to acclimatize easily to degraded lands therefore its cultivation on waste lands using suitable bioinoculants could meet the increased demand of phytochemicals as well as rehabilitation of the medicinal plant.

2. MATERIALS AND METHODS

2.1. Sample Collection

Healthy plants of *W. somnifera* were collected from the Babasaheb Bhimrao Ambedkar University campus and adjoining areas of Lucknow district (26° 45' N, 81° 0' E) to explore the diversity of endophytic microorganisms. Ashwagandha plant were brought to laboratory in sterile bags and processed within a few hours for sample collection.

2.2. Isolation of endophytes

The collected plant material used for the isolation of endophytes was first cleaned by washing under running tap water to remove the adhering soil particles. Surface sterilization was performed according to the method of Santos et al. (2003). Leaves, stems and roots of ashwagandha were cut into small segments using sterilized razor blade and then rinsed with 70% ethanol for 30 seconds, followed by 0.01% mercuric chloride for 5 minutes and 0.5% sodium hypochlorite for 2 minutes. After surface sterilization, plant material was rinsed several times with sterilized distilled water under aseptic conditions. Sterility test was also performed to assure the complete sterilization of plant material for which 0.1 ml of aliquots of water from the last rinsing were spreaded on nutrient agar media (McInroy and Kloepper, 1994). No microbial growth after 24 h incubation on nutrient agar plates confirmed the surface sterilization. Surface sterilized small, thin sections of roots, stems and leaves of ashwagandha were placed on nutrient agar media. For the isolation of endophytic bacteria plant segments were placed on nutrient agar (NA) media. All the plates were incubated at 28°C and monitored regularly for any microbial growth. After 4 to 5 days of incubation, bacterial colonies appeared on nutrient agar plates which were streaked and maintained as pure cultures at 4°C.

2.3. Screening of isolated endophytes to determine phosphate solubilization potential

Phosphate solubilization test was conducted qualitatively by inoculating the isolated endophytes on Pikovskaya's agar media. Plates were incubated at 28°C for 5 days. After incubation, inoculated plates were observed for clear zone around bacterial colonies. Phosphate solubilisation index (PSI) was evaluated according to the ratio of the total diameter (colony diameter + halo zone) and the colony diameter (Edi-Premono et al., 1996).

2.4. Alkaline phosphatase activity of phosphate-solubilizing endophytes

Alkaline phosphatases play key role in solubilizing organic P at neutral and alkaline soils. Since pot study was conducted in alkaline soil (pH 8.2), alkaline phosphatase activity of isolated endophytes was also investigated.

To determine alkaline phosphatase activity, phosphate solubilizing endophytes were inoculated in Pikovskaya's broth containing 5g/L of tri-calcium phosphate as a phosphorus source and incubated for 5-6 days. Phosphatase activity of endophytes were assayed according to the method of Tabatabai and Brammer (1969) in which *p*NP linked substrate and enzymatic activity is determined from colorimetric measurement of *p*NP released in buffered substrate solution during incubation and results were reported in units of µmol *p*NP released in reaction mixture. The cultures were filtered after centrifugation at 8000 rpm for 10 minutes and supernatant was separated for phosphatase assay. To 3 ml of the supernatant, 1 ml of Tris–HCl buffer (pH 10.0) was added, followed by addition of 100 µl of *p*NPP solution. Reaction mixtures were incubated for 20 min at 37^0C. Reaction was then terminated by addition of 2 ml of 1M NaOH solution. Uninoculated broth was used as control. Release of *p*NP was measured spectrophotometrically at wavelength 410 nm (Verchot and Borelli, 2005).

2.5. Plant growth promoting activities of endophytic bacteria

Most effective phosphate solubilizer strain PSE-1 was also screened for other plant growth promoting traits including indole acetic acid (IAA), ammonia and siderophore production.

2.6. Indole acetic acid production

For IAA production, endophyte PSE-1 was grown in liquid medium containing glucose 5.0 gm, yeast extract 0.025 gm, L-tryptophan 0.204 gm per litre of solution. Uninoculated tubes were kept as control. Tubes were incubated in dark for 72 h at 27° C. After incubation, tubes were centrifuged at 10,000 rpm for 10 minutes. To 1 ml. of culture filtrate from each tube 4 ml of Salwoski reagent was added and tubes were kept for 30 minutes. After incubation, tubes were observed for the development of pink color which indicates presence of IAA (Sarwar and Kremer, 1995).

2.7. Siderophore Production

Siderophore production by PSE-1 was assayed on the Chrome azurol S (CAS) agar medium as described by Schwyn and Neilands (1987). CAS agar plates were inoculated with test organism and uninoculated plates were kept as control. All plates were incubated at 28 °C for 48–72 h. Development of halo zone around the growth was considered as positive for siderophore production.

2.8. Ammonia production

Strain PSE-1 was tested for the production of ammonia in peptone water. Freshly grown culture was inoculated in 10 ml peptone water and incubated for 48–72 h at 28°C. After incubation, Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow color was a positive test for ammonia production (Cappuccino and Sherman, 1992).

2.9. Screening of isolated endophytes for other extracellular enzymatic activities

Extracellular enzymes play an important role in hydrolyzing organic and inorganic compounds to release macro and micronutrients thus isolated bacterial endopytes were also screened for the production of extracellular amylase, protease, lipase, cellulase and chitinase enzymes.

Amylase activity was assayed on starch agar media. Endophytes were inoculated on starch agar plates and incubated at 28°C for 2-5 days. After incubation, plates were flooded with iodine solution and observed for clear zone around the colonies (Aneja, 2005). For proteolytic activity, endophytes were inoculated on skimmed milk agar (SMA) media and inoculated SMA plates were incubated at 28°C for 4 days. After incubation period, SMA plates were observed for the clear zone around bacterial colonies (Aneja, 2005). For lipase activity, endophytes were inoculated on tween 20 agar media and incubated for 3 days at 28°C. After incubation, plates were observed for the zone of precipitation around colonies (Lee et al., 2015). To determine the extracellular cellulase activity, screening was done on Czapek-mineral salt agar (CMSA) media. CMSA plates were inoculated with endophytes and incubated at 28°C for 5-7 days. After incubation plates were flooded with an aqueous solution of 1% congo red solution for 4-5 minutes (Kasana et al., 2008). After removing the iodine solution, plates were observed for sharp distinct zone around the colonies. All endophytes were screened for chitinase activity on chitin agar plate in which 1% of colloidal chitin was used. Plates were incubated at 37 °C for 5-7 days and observed for clear zone around colonies (Aneja, 2005).

2.10. Pot experiment and growth observation

After screening of P mineralization potential of endophytes and other plant growth promoting traits, most promising strain PSE-1 was evaluated for its effect on plant growth of *W. somnifera* and P level in inoculated soil by pot experiment. Inoculums of endophytic bacterial strain PSE-1 was prepared by inoculating the bacteria in nutrient broth for 24 h at 210 rpm at 28°C. After incubation, bacterial suspension was centrifuged at 8,000 rpm for 15 min., supernatant was discarded and pellets containing bacterial cells were suspended in 500 ml of 100 mM phosphate buffer. Microbial count in the suspension was $2x10^8$ per ml buffer suspension.

Pot study was conducted during 2014 in the month of October for 90 days in green house (each treatment in three replicates). Each pot was filled with 6.5 kg of sterilized soil. Soil with pH 8.2 and electrical conductivity 187.1 µs contained 90.125 kg ha^{-1} of available phosphorous. Plant height, root length, number of branches, fresh weight and dry weight for each treatment were recorded after harvesting. Total organic and available form of P in soil before and after harvesting was determined using method of Bray and Kurtz (1945) and measured by ascorbic acid method.

2.11. Identification of selected strain PSE-1 on the basis of 16s rRNA gene sequencing

Bacterial genomic DNA of isolate PSE-1 was isolated using InstaGene™ Matrix Genomic DNA isolation kit and identified on the basis of 16s rRNA gene sequencing. DNA sequence of strain PSE-1 has been submitted to gene bank NCBI and accession number was obtained.

3. RESULTS AND DISCUSSION

Total 17 endophytic bacterial strains were isolated from different plant parts of *W. somnifera,* which were screened for their phosphate solubilization potential. Isolated endophyte PSE-1 showed various PGP traits including IAA production, phosphate solubilization, siderophore

production and production of extracellular hydrolytic enzymes to enhance the availability of minerals. On the basis of 16s rRNA gene sequencing, most promising phosphate solubilizing strain PSE-1 was identified as *Pseudomonas sp.* Gene sequence has been submitted in gene bank NCBI with accession number KT761191.

Microbes mediate nutrient cycling in soils in which, extracellular enzymes e.g. protease, lipase, amylase, cellulose, phosphatases, chitinase, urease etc. play a significant role by mineralizing organic compounds (Das and Varma, 2011). Soil enzyme activity measurements have been used as indicator of soil quality and health (Bandick and Dick, 1999; Badiane et al., 2001). In present study, isolated endophytes showed various enzymatic activities among which 52.9% strains showed positive activity for amylase, 41.17% for lipase, 70.58% for protease, 29.41% for cellulase, 35.29% for phosphatase and 5.88% for chitinase. Alkaline phosphatases secreted by microorganisms have an important role in phosphate solubilization in neutral or alkaline soil (Richardson et al., 2011). Strain PSE-1, which was selected for pot study showed positive enzymatic activity for amylase, protease, cellulase and chitinase. Enzymatic activities of *Pseudomonas* have been investigated by many researchers. Significant production of amylase, protease and cellulase enzymes by endophytic fluorescent pseudomonads (FLPs) was observed by Sunkar and Nachiyar (2013) which was isolated from *Brassica oleracea*. FLPs are potent lipase and protease producers. Grbavcic et al. (2009) found *Pseudomonas* sp. as potent lipase and protease producer. Chitinase enzymes possess nematicidal potential as well as play important role in carbon cycling. Chitinase activity of FLPs and its role in biocontrol of phytopathogenic fungi (Arora et al., 2007) and nematodes was studied by Chen et al. (2015).

P solubilization by FLPs and its role in plant growth promotion is widely studied (Cattelan et al., 1999; Pandey et al., 2006). In present study, endophytic bacteria *Pseudomonas* strain PSE-1 showed significant potential for phosphate solubilization with PSI value of 3 and phosphatase activity at pH 10.0. Alkaline phosphatase activity (at pH 10.0) of PSE-1 was 300 mg L^{-1} *p*NP. PSE-1 also efficiently enhances soil P level from 90.125 kg ha^{-1} (as recorded in uncultivated soil) to 125.45 kg ha^{-1} in PSE-1 inoculated soil.

PSE-1 also showed positive results for IAA, siderophore and ammonia production. IAA is an important phytohormone which is essential for plant growth and development. Positive impact of IAA-producing bacteria on plant growth has been widely investigated (Khare and Arora, 2010; Rana et al., 2011). Denitrification, aminization and ammonification are positive processes that make nitrogen compounds available for plant uptake via the root system (Przemieniecki et al., 2015). Ammonia production as a PGP trait was studied by many researchers (Rana et al., 2011, Przemieniecki et al., 2015). Siderophores are iron-chelating compounds which promote plant growth by facilitating iron uptake by plant roots. Siderophore production by plant-associated bacteria and their growth promoting potential has been extensively explored (Arora et al., 2001; Grobelak et al., 2015). FLPs are efficient plant growth promoting organisms even under stress conditions. Tewari and Arora (2014) investigated FLPs in growth enhancement of sunflower under saline stress. In present study, *Pseudomonas* strain PSE-1 showed multiple PGP characters *in vitro* and also exhibited promising plant growth promoting potential under field condition as observed in the form of increased plant height, fresh and dry weight and number of branches (Table 1). PSE-1 treated plants showed 51% increase in plant height, 41% increase in fresh weight and 56% increase in dry weight in comparison to control.

The role of endophytes in plant growth enhancement, nutrient availability, yield and quality of medicinal plants is demonstrated by many researchers and there are increasing interests in the use of endophytes for the cultivation of medicinal plants without using synthetic agrochemicals (Keû et al., 2015). A wide variety of endophytic bacteria and fungi have been recognized that have high significance in plant nutrient acquisition and secondary metabolite alteration of medicinal plants (Tewari et al., 2010; Qadri et al., 2013). The results described in this study show that endophytic *Pseudomonas* strain PSE-1 possesses good phosphate solubilization activity and can be used as a biofertilizer under phosphate limiting conditions.

Finding promising results using endophyte *Pseudomonas* strain PSE-1, further research is recommended to better understand the diversity and function of endophytes and their uses in the production of medicinal plants.

4. CONCLUSION

The use of endophytic microorganisms as bioinoculants provides a promising alternative to synthetic fertilizers, especially for the cultivation of medicinal plants. In present study, phosphate solubilizing endophyte *Pseudomonas* PSE-1 strain showed great potential in enhancing growth and yield of *W. somnifera* under nutrient limiting saline conditions and may be used as microbial biofertilizer for the cultivation of medicinal plants.

Table 1. Effect of endophyte *Pseudomonas* PSE-1 on growth of *W. somnifera* (values expressed are mean of three replicates)

Treatment	Plant height (cm.)	Root length (cm.)	No. of branches	Fresh weight (gm)	Dry weight (gm)
Control	46.32(± 1.1)	13.76 (± 0.60)	4.33 (± 1.52)	643.3 (± 28.3)	383.3 (± 31.4)
Strain PSE-1	70.10 (± 0.90)	16.88 (± 0.80)	5.66 (± 1.15)	910.0 (± 32.57)	596.66 (± 34.05)

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REFERENCES

Aneja KR (2005) Experiments in Microbiology, Plant Pathology and Biotechnology, 4 th revised edition, New age international publishers, Pp 102-105.

Arora NK, Kang SC, Maheshwari DK (2001). Isolation of siderophore-producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. *Curr Sci*, 81: 673-677.

Arora NK, Kang SC, Kim MJ, Maheshwari DK (2007). Role of chitinases and beta 1.3-glucanases produced by fluorescent *Pseudomonas* and in vitro inhibition of *Phytophthora capsici* and *Rhizoctonia solani*. *Can J Microbiol*, 53: 207-212.

Badiane NNY, Chotte JL, Patê E, Masse D, Rouland (2001). Use of soil enzymes activities to monitor soil quality in natural and improve fallows in semi-arid tropical regions. *Appl Soil Ecol*, 18**:** 229-238.

Bandick AK, Dick RP (1999). Field management effects on soil enzyme activities. *Soil Biol Biochem,* 31: 1471–1479.

Bray RH, Kurtz LT (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil Sci*, 59: 39-45.

Cappuccino JC, Sherman N (1992). In: Microbiology: A Laboratory Manual, third ed. Benjamin/cummings Pub. Co. New York, Pp. 125–179.

Cattelan AJ, Hartel PG, Fuhrmann JJ (1999). Screening for plant growth-promoting rhizobacteria to promote early soybean growth. *Soil Sci Soc Am J,* 63: 1670–1680.

Chen L, Jiang H, Cheng Q, Chen J, Wu G, Kumar A, Sun M, Liu Z (2015). Enhanced nematicidal potential of the chitinase pachi from *Pseudomonas aeruginosa* in association with Cry21Aa. *Scientific Reports*, DOI: 10.1038/srep14395.

Daniels C, Michan C, Ramos JL (2009). New molecular tools for enhancing methane production, explaining thermodynamically limited lifestyles and other important biotechnological issues. *Microb Biotechnol,* 2: 533–536.

Das SK, Varma A (2011). Role of Enzymes in Maintaining Soil Health. In Shukla G and Varma A (eds.), Soil Enzymology, Soil Biology 22, Springer-Verlag Berlin Heidelberg

Edi–Premono M, Moawad MA, Vleck PLG (1996). Effect of phosphate solubilizing *Pseudmonas putida* on the growth of maize and its survival in the rhizosphere. *Indonesian J Crop Sci,* 11: 13–23.

Grbavcic S, Bezbradica D, Izrael-Zivkovic L, Avramovic N, Milosavic N, Karadzic I, Knezevic-Jugovic Z (2009). Production of lipase and protease from an indigenous Pseudomonas aeruginosa strain and their evaluation as detergent additives: compatibility study with detergent ingredients and washing performance. *American-Eurasian J Agric & Environ Sci*, 5 (4): 556-563.

Grobelak A, Napora A, Kacprzak M (2015). Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. *Ecol Eng*, 84: 22–28.

Kanwar JS, Grewal JS (1990). Phosphorus fixation in Indian soils. Publications and information division, Indian Council of Agricultural Research, New Delhi, India.

Kasana RC, Salwan R, Dhar H, Dutt S, Gulati A (2008). A Rapid and Easy Method for the Detection of Microbial Cellulases on Agar Plates Using Gram's Iodine. *Curr Microbiol*, 57(5): 503-507.

Keû A, Slimene IB, Karkouch I, Rihouey C, Azaeiz S, Bejaoui M, Belaid R, Cosette P, Jouenne T, Ferid Limam (2015). Characterization of endophytic *Bacillus* strains from tomato plants (*Lycopersicon esculentum*) displaying antifungal activity against *Botrytis cinerea* Pers. *World J Microbiol Biotechnol,* 31: 1967–1976.

Khare E, Arora NK (2010). Effect of Indole-3-acetic acid (IAA) produced by *Pseudomonas aeruginosa* in suppression of charcoal rot disease of chickpea. *Curr Microbiol,* 61(1): 64-68.

Lee LP, Karbul HM, Citartan M, Gopinath SCB, Lakshmipriya T, Tang T (2015). Lipase-Secreting Bacillus Species in an Oil-Contaminated Habitat: Promising Strains to Alleviate Oil Pollution. *BioMed Res Int*, DOI: 10.1155/2015/820575.

McInroy JA, Kloepper JW (1994). Studies on indigenous endophytic bacteria of sweet corn and cotton. In: Molecular ecology of Rhizosphere microorganisms, Biotechnology and

the release of GMOs. O'Gara, Dowling DN and Boesten (eds). VCH, New York. Pp. 19-27.

Miller SH, Browne P, Prigent-Cambaret C, Combes-Meynet E, Morrissey JP, and O'Gara F (2010). Biochemical and genomic comparison of inorganic phosphate solubilisation in *Pseudomonas* species. *Environ Microbiol Rep*, 2: 403–411.

Oteino N, Lally RD, Kiwanuka S, Lloyd A, Ryan D, Germaine KJ and Dowling DN (2015). Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Front Microbiol*, 6: 745.

Pandey A, Trivedi P, Kumar B, Palni LMS (2006). Characterization of a phosphate solubilizing and antagonistic strain of *Pseudomonas putida* (B0) isolated from a sub-alpine location in the Indian central Himalaya. *Curr Microbiol,* 53: 102–107.

Przemieniecki SW, Kurowski TP, Karwowska A (2015). Plant growth promoting potential of Pseudomonas sp. sp0113 isolated from potable water from closed water well. *Arch Biol Sci*, 67(2): 663-673.

Qadri M, Johri S, Shah BA, Khajuria A, Sidiq T, Lattoo SK, Abdin MZ, Riaz-Ul-Hussain S (2013). Identification and bioactive potential of endophytic fungi isolated from selected plants of the Western Himalayas. *Springer plus,* 2: 8.

Rana A, Saharan B, Joshi M, Prasanna R, Kumar K, Nain L (2011). Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. *Ann Microbiol,* 61: 893–900.

Richardson AE, Simpson RJ (2011). Soil microorganisms mediating phosphorus availability. *Plant Physiol,* 156: 989– 996.

Rodríguez H, and Fraga R (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv,* 17: 319–339.

Santos RMG, Rodrigues-Fo E, Rocha WC, Teixeira MFS (2003). Endophytic fungi from *Melia aedarach*. *World J Microbiol Biotechnol,* 19: 767–770.

Sarwar M, Kremer RJ (1995). Determination of bacterially derived auxins using a microplate method. *Lett Appl Microbiol*, 20(5): 282–285.

Schwyn B, Neilands JB (1987). Universal chemical assay for the detection and determination of siderophores. *Anal Biochem*, 160: 47–56.

Sharma SB, Sayyed RZ, Trivedi MH, and Gobi TA (2013). Phosphate solubilising microbes: sustainable approach for managing phosphorus deûciency in agricultural soils. *Springer plus,* 2: 587.

Singh B, Kunze G, Satyanarayana T (2011). Developments in biochemical aspects and biotechnological applications of microbial phytases. *Biotechnol Mol Bio Rev,* 6: 69–87.

Sivanesan I and Jeong BR (2007). Direct shoot regeneration from nodal explants of *Sida cordifolia In Vitro. Cell Dev Biol Plant*, 43: 436-441.

Sunkar S, Nachiyar CV (2013). Isolation and characterization of an endophytic bacterium from *Brassica Oleracea* with potential enzyme and antibacterial activity. *Asian J Pharm Clin Res*, 6(2): 183-187.

Tabatabai MA (1994). Soil enzymes In: Weaver RW, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai A, Wollum A (eds) Methods of soil analysis. Microbiological and biochemical properties, Soil Science Society of America, Madison, 2: 775–833.

Tabatabai MA, Bremner JM (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatise activity. *Soil Biol Biochem,* 1: 301–307.

Tarafdar JC, Jungk A (1987). Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. *Biol Fert Soils,* 3: 199–204.

Tewari S, Arora NK (2014). Multifunctional Exopolysaccharides from *Pseudomonas aeruginosa* PF23 Involved in Plant Growth Stimulation, Biocontrol and Stress Amelioration in Sunûower Under Saline Conditions. *Curr Microbiol,* 69(4): 484-494.

Tewari SK, Das B, Mehrotra S (2004). Cultivation of medicinal plants—tool for rural development. *J Rural Tech*, 3: 147–150.

Tiwari R, Kalra A, Darokar MP, Chandra M, Aggarwal N, Singh AK, Khanuja SPS (2010). Endophytic Bacteria from *Ocimum sanctum* and their Yield Enhancing Capabilities. *Curr Microbiol,* 60: 167–171.

Verchot LV, Borelli T (2005). Application of para-nitrophenol (pNP) enzyme assays in degraded tropical soils. *Soil Biol Biochem,* 37: 625–633.

Wakelin SA, Warren RA, Harvey PR, Ryder MH (2004). Phosphate solubilization by *Penicillium* sp. closely associated with wheat roots. *Biol Fertil Soils,* 40:36 – 43.