ISSN 1996-3351

Asian Journal of Biological Sciences

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Asian Journal of Biological Sciences 4 (3): 291-299, 2011
ISSN 1996-3351 / DOI: 10.3923/ajbs.2011.291.299

Plant Growth Promoting Activities of Fungi and their Effect on Chickpea Plant Growth

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ABSTRACT

Aspergillus niger strain BHUAS01, *Penicillium citrinum* strain BHUPC01 and *Trichoderma harzianum* was tested for phosphate solubilizing potential and plant hormones production (indole acetic acid). The fungal strains were tested for their *in-vitro* phosphate solubilizing potential using Pikovskaya broth containing tricalcium phosphate (TCP) as the sole phosphorus source. All the cultures were found to solubilize TCP but with varying potential. The *Aspergillus niger* showed maximum amount of soluble phosphate (328 μg mL^{-1}), followed by *P. citrinum* (301 μg mL^{-1}) and *T. harzianum* (287 μg mL^{-1}) after 6 days of incubation at 28±2°C. Indole Acetic Acid (IAA) production was estimated maximum in *A. niger* (85 μg mL^{-1}) followed by *T. harzianum* (68 μg mL^{-1}) and *P. citrinum* (52 μg mL^{-1}) after 3 days of incubation at 28+2°C. *T. harzianum* is a well known biological controlling agent against several soil borne phytopathogens. It showed antagonistic against *Fusarium oxysporum* and *Rhizoctonia solani*. Under laboratory conditions, coinoculation of *T. harzianum and A. niger* showed significant increase in chickpea (*Cicer arietinum*) growth parameters including shoot length, root length and dry weight of shoot and root followed by single inoculation of *T. harzianum* and coinoculation of *A. niger* and *P. citrinum* over control. The study explores high P-solubilizing potential of *A. niger* and *T. harzianum*, which can be exploited for the solubilization of fixed phosphates present in the soil and produce IAA, thereby enhancing soil fertility and plant growth. Coinoculation of *T. harzianum and A. niger* could be effective biofertilizer and biocontroling agent for chickpea production.

Key words: Plant growth promoting fungus, *Aspergillus niger*, *Trichoderma harzianum*, *Penicillium citrinum*, phosphate solubilization, chickpea

INTRODUCTION

Phosphorus (P) is one of the major nutrient elements limiting agricultural production in the world. Phosphorus, however, plays an important role in N_2-fixation process, as the reduction of atmospheric nitrogen requires large amount of energy. On average, soil contains 0.02 to 0.5% total P. It is added to the soil in the form of phosphate fertilizers, a part of which (1%) is utilized by plants and the rest is rapidly converted into insoluble complexes, e.g., calcium phosphate, iron phosphate and aluminum phosphate in the soil. This leads to the need of frequent application of phosphate fertilizers, but its use on a regular basis has become a costly affair and environmentally

undesirable. Therefore, the necessity to develop economical and eco-friendly technologies is steadily increasing (Reddy *et al.*, 2002; Chuang *et al.*, 2007).

Natural phosphate rocks have been recognized as a valuable alternative for P fertilizers. In recent years, the possibility of practical use of Rock Phosphates (RP) as fertilizers has received significant interest. Conventionally, RP is chemically processed by reacting with sulphuric acid or phosphoric acid into soluble phosphate fertilizer. The process increases fertilizer cost and makes the environment worse (Reddy *et al.*, 2002; Chuang *et al.*, 2007; Xiao *et al.*, 2008).

An alternative has been the use of microorganisms with the capability to solubilize RP and release soluble P through the production of organic acids, chelating oxo acids from sugars, reduction of pH and production of enzymes. Several reports have indicated that some microorganisms are capable of solubilizing insoluble RP and releasing soluble P. However, few reported microorganisms represent a high potential to release soluble P from RP and this seriously restrains the biosolubilization of RP and its use as biofertilizer, hence, isolation and application of new and potential phosphate solubilizing microorganisms are significant and necessary (Achala *et al.*, 2007; Xiao *et al.*, 2008). Fungi have been reported to possess greater ability to solubilize rock-phosphate than bacteria. The fungi and probably all living organisms, synthesize a number of phosphatases which are necessary to scavenge phosphates (Pi) from medium containing bound phosphorus. Both acid and alkaline phosphatases exist in soil and are distinguished on the basis of pH ranges at which they are active. These are secreted in response to signals of the absence of Pi (Peleg *et al.*, 1996). Seed or soil inoculation with PSMs is known to improve solubilization of fixed soil P and applied phosphates, resulting in higher crop yields (Abd-Alla, 1994; El-Komy, 2005; Illmer and Schinner, 1995; Rudresh *et al.*, 2005). Filamentous fungi are widely used as producers of organic acids, particularly black *Aspergillus* and some species of *Penicillium*, these species have been tested for solubilization of RP and have been reported for various properties of biotechnological importance, such as, biocontrol, biodegradation, phosphate solubilization and P fertilizer (Richa *et al.*, 2007; Pandey *et al.*, 2008). It has been observed by many investigators that a high proportion of P solubilizing microorganisms (PSMs) especially bacteria, fungi and actinomycetes reside in the rhizosphere of plants and play an important role in solubilization of bound phosphates, making them available to plants (Gaur, 1990).

Chickpea is the third most important legume crop in the world and act as rich supplement to cereal diet in developing countries. It has high nutritive value, grown principally for its protein rich edible seeds, this crop can be used for both seed and forage production. The aim of the present study was to isolate Phosphate-Solubilizing Fungi (PSF) from rhizosphere and examine their effect on the plant growth of chickpea (*Cicer arietinum* L. cv. Radhey) under laboratory conditions.

MATERIALS AND METHODS

Collection and isolation of plant growth promoting fungi: Rhizospheric soils of chickpea were collected from Varanasi and Mirzapur district of Eastern Uttar Pradesh at year 2006. Ten grams of rhizospheric soil was transferred to a 250 mL Erlenmeyer flask containing 90 mL sterile distilled water and shaken (120 rpm) for 30 min. Serial dilutions were made in 0.85% saline sterilized distilled water and 0.1 mL aliquots (10^{3}-10^{4}) were spread on plates containing their Potato Dextrose Broth (PDA) media, Czaper-Dox media and Pikovaskaya media. PDA media containing (g L^{-1}): Potato, 200 g; Dextrose, 20 g; Agar, 15.0 g per 1000 mL distilled water; pH 5.6±0.2, Czaper-Dox media containing (g L^{1}): Sucrose, 30.0 g; $NaNO_3$, 3.0 g; K_2HPO_4, 1.0 g; $MgSO_4.7H_2O$,

0.5 g; $FeSO_4.7H_2O$, 0.01; KCl, 0.5; Agar, 15.0 per 1000 mL distilled water; pH 5.8. The plates were incubated for 2-5 days at 30°C and morphologically different colonies appearing on the medium were isolated and sub-cultured for further analysis. The fungal colonies producing distinct zones of phospate solubilization on Pikovaskaya media were raised into pure cultures maintained on Potato Dextrose Agar (PDA) slants. The fungus culture were identified as *Penicillium citrinum* BHUPC01, *Aspergillus niger* BHUAS01 from Agharkar Research Institute, Pune, Maharastra, on the basis of phenotypic characters. The pure culture of *Trichoderma harzianum*, *Fuasarium oxysporum* and *Rhizoctonia solani* were obtained from Department of Plant Pathology and Mycology, Institute of Agricultural Sciences, Banaras Hindu University, Vranasi, Uttar Padesh, India. The fungal cultures were maintained on potato dextrose agar media. The cultures were maintained by periodic transfer and stored in the refrigerator for further studies. This experiment has been conducted in Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India at 2006 and 2007.

Evaluation of plant growth promoting activities *in vitro* condition

Quantification of IAA by fungi: The quantitative analysis of indole-3-acetic acid was performed by the method suggested by Brick *et al.* (1991). Fungi were grown in synthetic Czapek-Dox broth amended with 1000 μg mL^{-1} L-tryptophan. Fungus was inoculated with 3 PDA disk covered with actively growing mycelium into 100 mL of Czapek-Dox broth media and incubated at 30°C on rotary shaker (120 rpm).

After 3 days, 5 mL of each culture was centrifuged (8,000 rpm) for 10 min. One milliliter supernatant was mixed with 2 mL of Salkowski's reagent (2% 0.5 M $FeCl_3$ in 35% perchloric acid) and left for room temperature for 20 min. After 6 days, 5 mL fungal broth culture was centrifuge at 8000 rpm for 10 min. One milliliter supernatant was mixed vigorously with 2 mL Salkowski's reagent in test tube. The mixture was incubated at room temperature for 20 min and absorbance was measured at 540 nm using a spectrophotometer. A standard curve was drawn for comparison to determine auxin production by isolates. Three replicates were used for each for treatment.

Qualitative and quantitative estimation of phosphate solubilization on solid and broth medium: For qualitative estimation of phosphate solubilization was tested on Pikovskaya medium agar medium (Pikovaskya, 1948). fungal culture (1 PDA disk covered with actively growing mycelium) were placed on Pikovskaya,s agar (PA) plates [containing insoluble tricalcium phosphate 2.5 g, glucose 13 g $(NH_4)SO_4$ 0.5 g, NaCl 0.2 g, $MgSO_4.7H_2O$ 0.1 g, KCl 0.2 g, Yeast Extract 0.5 g, $MnSO_4$ trace, $FeSO_4.7H_2O$ trace, Agar 15 g, pH adjusted to 7.2 and dissolved in 1000 mL distilled water] and the plates were incubated at 28±2°C for 3 and 6 days. The halo formation was observed after 3 and 6 days. Colonies forming a clear halo around them, indicating P solubilization, were counted and further used to determine the P- solubilization index. Solubilization index (SI) was measured using following formula (Premono *et al.*, 1996):

$$SI = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

pH change: Fungal culture (1 PDA disk covered with actively growing mycelium) were incoculated in to 100 mL Pikovskaya,s broth media in 250 mL conical flask and incubate at 30°C for 3 and 6

days on a shaker (120 rpm). Sterile uninoculated medium served as control. Initial pH and change in pH was noted after 3 days and 6 days of inoculation by digital pH meter.

Quantitative estimation of phosphate solubilization in broth medium: For the quantitative measurement of phosphorus, fungal cultures were grown in Pikovskaya broth medium for incubated 3 days and 6 days with continuous shaking (120 rpm) at 28±2°C. A 10 mL sample of each broth culture was taken in centrifugation tube and centrifuged for 15 min at 8000 rpm. Phosphorus in solution was extracted with Ammonium bicarborate diethylene triamine penta acetic acid (AB-DTPA) (Soultanpour and Workman, 1979). Supernatant was decanted and 5 mL of supernatant was added to 20 mL of AB-DTPA extracting solution. The mixture was then shaken on a reciprocating shaker for 15 min at 180 rpm in open flasks and extract was stored in plastic bottles.

Test for available Phosphorus: Broth Phosphorus was determined by Ascorbic acid method (Watanabe and Olsen, 1965). One milLiliter of AB-DTPA extract was taken in 50 mL conical flask and 9 mL of distilled water and 2.5 mL of freshly prepared colour reagent [12 g ammonium molybdate {$(NH_4)6Mo_7.4H_2O$}+250 mL distilled water and 0.2908 mg antimony potassium tartrate {$K(SbO)C_4H_4O_6.1/2\ H_2O$} in 1000 mL of 5 N H_2SO_4(148 mL conc.$H_2SO_4L^{-1}$)]. Both the solutions were mixed and the volume was raised upto 2 L. One hundred and forty milliliter of this mixture was added to 0.74 g Ascorbic acid and stirred gently. After this colour reagent was ready for use. The optical density of the blue colour developed after 15 min was measured at 880 nm by spectrophotometer. The concentration of available phosphorus (mg mL^{-1}) was calculated against standard phosphorus KH_2PO_4 curve.

Antifungal assay: Spores of fungal culture *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* grown on Potato Dextrose Agar (PDA) medium. A small block of agar with fungal growth was cut using sterile blade and placed in the centre of a fresh PDA plate and proper growing disc of *Trichoderma harzianum* was also placed on plate and allowed to incubate at 28±2°C for 3-6 days. The zone of inhibition recorded as positive for antifungal activity (Rajendran *et al*., 2008).

Seed inoculation of chickpea with fungal strain: Seeds of chickpea (*Cicer arietinum* L.) cultivar Radhey were obtained from Indian Institute of Pulse Research (IIPR), Kalyanpur, Kanpur, Uttar Pradesh, India. Healthy seeds were surface sterilized with 0.1% $HgCl_2$ for 5 min and then 70% ethyl alcohol for 2 min with rinsed six times by sterile distilled water. *Aspergillus niger* strain BHUAS01, *Penicillium citrinum* BHUPC01 and *Trichoderma harzianum* were grown in PDA broth on shaking incubator (180 rpm) at 28±2°C for 24 h. The surface sterilized seeds of chickpea were inoculated by soaking in the spore suspension of *A. niger*, *Penicillium* and *T. harzianum* for 30 min in the presence of 16% Arabic gum as adhesive agent and air dried it. Five Seeds were sown at 5 cm depth in 250 g sterilized soil containing plastic pot. A control treatment was also maintained without inoculated seed. Pots were kept in to plant growth chamber for 21 days. Seeds treated with spore free solution are considered as control treatment. Seven treatments (6 fungal inoculations and one uninoculated control) were arranged in three replicates. All recommended agricultural practices for chickpea crop were carried out under plant growth chamber at Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University at 2006 and 2007.

Assessment of length and dry weight of shoot and root: Chickpea plants were harvested. Shoot and root length were recorded in centimeter of each plant. Then plants were dried in an oven at 65°C for 3 days. After this shoot and root dry weight were recorded in gram. The oven dried sample of roots and shoots were weighted by electronic balance. The oven dried sample roots and shoots were calculated per plant in gram.

RESULTS

***In vitro* Plant growth promoting activity of fungi:** *In vitro* plant growth promoting activities of fungi was depicted in Table 1. *In vitro*, solubilization of phosphate was determined on Pikovskaya agar media. Clear halo zone was recorded on pikovaskaya agar media after 3 and 6 days interval of incubation in BOD at 28°C. Solubilization index was recorded higher significant of *Aspergillus niger* (1.18 and 1.26) followed by *Penicillium citrinum* (1.10 and 1.20) as compare with *Trichoderma harzianum* (0.21 and 0.25) at 3 and 6 days incubation, respectively. Similarly results was recorded in solubilization of phosphate in broth culture such as *Aspergillus niger* (256 and 328 μg mL^{-1}) showed strong phosphate solubilization followed by *Penicillium citrinum* (226 and μg mL^{-1}) than *Trichoderm harzianum* (185 and 287 μg mL^{-1}) in broth cultures at 3 and 6 days incubation, respectively. pH of broth culture were measured by pH meter after 3 and 6 days of incubation. Lowest pH has recorded in *Aspergillus niger* broth culture followed by *Penicillium citrinum* and *Trichoderma harzianum* than control. Solubilization of phosphate, due to higher production of various organic acid by *Aspergillus niger* than other fungus. Plant growth promoting hormones Indole Acetic Acid (IAA) was determined by 1000 μg mL^{-1} tryptophan suplimented as precursor of IAA synthesis under laboratory condition. *Aspergillus niger* (85 μg mL^{-1}) and *Trichoderma harzianum* (68 μg mL^{-11}) was showed more significant IAA production as compare with *Penicillium citrinum* (52 μg mL^{-1}) at 3 days of incubation. *Trichoderma harzianum* was showed antagonistic against phytopathogenic fungus such as *Fusarium oxysporum* and *Rhizoctonia solani* as compare to other fungus. Over all results showed that strong phosphate solubilizing fungus *Aspergillus niger* and strong IAA producing fungus *Trichoderma harzianum* as well as antagonistic fungus were selected for further study (Table 1).

Length and dry weight of shoot and root: The plant growth promoting fungus isolates *Aspergillus niger*, *Penicillium citrinum* and *Trichoderma harzianum* were significantly enhanced the length of chickpea seedlings. Results reveal that the shoot length increased in treated plants over uninoculated control. The highest shoot length (18.21 cm $plant^{-1}$) and root length (8.56 cm $plant^{-1}$) was recorded statistically significant in combined treatment of *A. niger* and *T. harzianum* followed by single inoculation of *T. harzianum* and coinculation of *A. niger* and

Table 1: *In vitro* plant growth promoting activity of fungi

	*Solubilization index		**pH in broth		P-Solubilization (μg mL^{-1})			Growth inhibition	
Name of fungus	3 days	6 days	3 days	6 days	3 days	6 days	IAA (μg mL^{-1})	*F. oxysporum*	*R. solani*
A. niger	1.18^{c}	1.26^{c}	5.16^{a}	3.26^{a}	256^{c}	328^{c}	85^{c}	-	-
P. citrinum	1.10^{b}	1.20^{b}	5.46^{b}	4.85^{b}	226^{b}	301^{b}	52^{a}	-	-
T. harzianum	0.21^{a}	0.25^{a}	6.25^{c}	5.65^{c}	185^{a}	287^{b}	68^{b}	+	+

Values are the mean with in column sharing the same letters are not significantly different according to Dunnett T3 Test at $p \leq 0.05$
*Solubilization index = Total dimeter (Colony + halo zone)/colony diameter, **pH of broth culture 7.0 at initial. IAA at 1000 μg mL^{-1} tryptophan in broth culture suplimented

Table 2: Effect of plant growth promoting fungi on the growth of chickpea plants

Treatment	Shoot length	Root length	Shoot dry weight	Root dry weight
	----------plant^{-1} (cm)----------		---------- plant^{-1} (mg)----------	
Control	10.12±1.1^{a}	4.26±0.2^{a}	6.51±0.7^{a}	4.62±0.3^{a}
Aspergillus niger	13.61±0.8^{bc}	6.21±0.3^{b}	8.23±0.5^{b}	6.21±0.5^{b}
Penicillium citinum	12.16±0.2^{b}	5.11±0.7^{a}	7.21±1.1^{ab}	5.91±1.0^{b}
Trichoderma harzianum	16.35±0.5^{d}	7.24±0.3^{c}	9.03±1.1^{c}	7.28±1.2^{c}
A. niger+P. citrinum	14.25±1.1^{c}	6.38±0.6^{bc}	8.62±0.6^{c}	7.01±0.4^{bc}
A. niger+T. harzianum	18.21±1.0^{e}	8.56±0.8^{d}	10.25±0.7^{d}	8.29±0.6^{d}

Values are the Mean±SD, Mean values in each column with the same superscript(s) do not differ significantly by LSD ($p \leq 0.05$)

P. citrinum over control and single inoculation of *P. citrinum*. Single inoculation of *A. niger* and *P. citrinum* did not show significant shoot length between each other but significant increase over control. *P. citrinum* did not show significant root length over control. A significant increase in shoot dry matter of chickpea seedling was observed in response to PGPR isolates. Similarly, combined inoculation of *A. niger* and *T. harzianum* was recorded significant increase in shoot dry weight (10.25 mg plant^{-1}) followed by single inoculation of *T. harzianum* (9.03 mg plant^{-1}) and coinoculation *A. niger* and *P. citrinum* (8.62 mg plant^{-1}) over control and others. Root dry weight was varied range from 4.62 to 8.29 mg plant^{-1}. The coinoculation of *A. niger* and *T. harzianum* was also recorded significant increase in root dry (8.29 mg plant^{-1}) followed by single inoculation of *T. harzianum* and coinoculation *A. niger* and *P. citrinum* as compare to control and others. Over all results showed that the coinoculation of *A. niger* and *T. harzianum* was showed significant increased shoot length, root length and dry matter production of shoot and root of chickpea seedlings followed by single inoculation of *T. harzianum* and coinoculation *A. niger* and *P. citrinum* as compare to control and others (Table 2).

DISCUSSION

In present investigation, *Aspergillus niger* and *Penicillium citrinum* was showed more significant phosphate solubilizer followed by *Trichoderm harzianum*. The results was recorded in solubilization of phosphate in broth culture such as *Aspergillus niger* (328 μg mL^{-1}) showed strong phosphate solubilization followed by *Penicillium citrinum* (301 μg mL^{-1}) than *Trichoderm harzianum* (287 μg mL^{-1}) in broth cultures at 6 days incubation. Present finding has been supported by Mittal *et al.* (2008), phosphate solulibizing capacity of *Aspergillus* and *Penicillium* sp. was recorded range from 300 to 520 μg mL^{-1} after 6 days incubation. Lowest pH has recorded in broth culture of *Aspergillus niger* (3.26) followed by *Penicillium citrinum* (4.85) as compare to *Trichoderma harzianum* (5.65) after 6 days incubation. Similarly, pH range of *Aspergillus* sp. was varied range 3.7 to 2.1 and *Penicillium* sp. varied range 5.3 to 3.0 after 2 and 9 days incubation, respectively, in broth culture (Mittal *et al.*, 2008). Other worker has been reported that the solubilization of tricalcium phosphate varied from 111.5 to 404.07 μg mL^{-1} in the culture filtrates of various *Trichoderma* cultures (Kapri and Tewari, 2010). *A. niger* and *P. citrinum* was produce strong organic acid which caused the drop of pH value according period of incubation in broth culture of Pikovskaya media. *A. niger* and *P. citrinum* caused a marked drop in pH of liquid culture media and solubilized considerable amounts of phosphate (Alam *et al.*, 2002). *Trichoderma harzianum* was also showed phosphate solubilization in broth culture and also showed solubilization index on solid agar medium of Pikovaskaya. *Trichoderma harzianum* was found to be most effective organic phosphorus mobilizes as compare to other fungi (Tarafdar *et al.*, 2003).

In present investigation, *A. niger* (85 μg mL^{-1}) and *T. harzianum* (68 μg mL^{-1}) was showed more significant IAA production as compare with *Penicillium citrinum* (52 μg mL^{-1}) at 3 days of

incubation at 30 °C. Similar report of IAA production in *Aspergillus niger* was studied for 5-16 days and maximum production 128 to 6.8 gL^{-1} was observed in Czapek-Dox broth media with 0.1% tryptophan at 6 days of incubation (Bilkay *et al*., 2010). In similar studies, IAA production was found to be maximized at 28°C (Gunasekaran, 1978; Hasan, 2002). *Trichoderma atraviride* produced the highest level of IAA (6.2, 9.8 and 38.55 μg mL^{-1}) in presence of 200 μg mL^{-1} tryptophan, tryptamine and tryptophol, respectively (Gravel *et al*., 2007). *Trichoderma harzianum* was also showed antagonistic against phytopathogenic fungus such as *Fusarium oxysporum* and *Rhizoctonia solani* as compare to other fungus. This findings has been supported by Jaworska and D^{a}uzniewska (2007) has been demonstrated that *Trichoderma* sp. are good antagonists of fungi from the genera *Pythium*, *Fusarium*, *Rhizoctonia* and *Sclerotinia*.

Trichoderma sp. was showed antagonistic against *Fusarium* sp. In the natural habitats, the hytopathogenic fungi like *Pythium* and *Rhizoctonia* are unable to solubilize phosphates and can be suppressed easily by the high competitive efficiency of *T. harzianum* through P-uptake (Altomare *et al*., 1999).

Tryptophan is naturally secreted in root exudates of tomato plants and most of the auxin found in the rhizosphere is believed to come from the biosynthesis by microorganism (Kamilova *et al*., 2006). Apart from phosphate solubilization, simultaneous synthesis and release of pathogen suppressing metabolites like siderophores, phytohormones and lytic enzymes, by PSMs can prove to be useful (Vassilev *et al*., 2006).

The coinoculation of *A. niger* and *T. harzianum* was showed significant increased shoot length, root length and dry matter production of shoot and root of chickpea seedlings followed by single inoculation of *T. harzianum* and coinoculation *A. niger* and *P. citrinum* as compare to control. Similarly, Saber *et al*. (2009) also reported that the inoculation of mung bean seeds with *A. niger* and/or *Penicillium* sp. in the presence of RP or calcium superphosphate (CSP), increased significantly the growth (except for branches No. $plant^{-1}$), seed yield and P-uptake, as well as, improved the nodulation status and population of total and phosphate dissolving fungi in the rhizospheric soil of mung bean under *in vivo* conditions. Our finding has been supported by *Trichoderma* spp. has been also shown to exhibit plant growth-promoting activity on numerous cultivated plants (Altomare *et al*., 1999; Harman, 2000; Yedidia *et al*., 2001). Rudresh *et al*. (2005) also reported that increase in growth and yield parameters of chickpea grown in phosphate-deficient soil amended with insoluble rock phosphate due to *Trichoderma* inoculation, as compared to uninoculated controls under both glass house and field conditions.. Mittal *et al*. (2008) has been reported the effect of six phosphate-solubilizing fungi (PSF, two strains of *Aspergillus awamori* and four of *Penicillium citrinum*) isolated from rhizosphere of various crops, was observed on the growth and seed production of chickpea plants (*Cicer arietinum* L. cv. GPF2) in pot experiments. Plant growth of chickpea was enhanced due proper root colonization of plant growth promoting rhizoabcteria which provide plant hormones (IAA), phosphorus to plant. The data also suggest that colonization of P-solubilizers in the rhizosphere of chickpea plants is independent of the P-solubilizing activity and IAA production. The main mechanism of effect on the growth and yield of chickpea crop by P-solubilizers remains unclear as it can be due to P-solubilization activity and/or IAA production (Mittal *et al*., 2008).

CONCLUSION

The experimental results showed that the coinoculation of *A. niger* and *T. harzianum* was most effective biofertilizer for plant growth of chickpea followed by single inoculation of *T. harzianum* and coinoculation *A. niger* and *P. citrinum* as compare to control under *in vitro*. Moreover, these

combinations brought about significant increase in the growth parameters of chickpea under laboratory condition, suggesting their applicability for crop improvement. Coinoculation of *T. harzianum and A. niger* could be effective biofertilizer and biocontroling agent for chickpea production.

ACKNOWLEDGMENT

The corresponding author is thankful to Co-Supervisor Dr. Janardan Yadav, Associate Professor, Department of Soil Science and Agricultural Sciences, Institute of Agricultural Sciences, BHU, Varanasi for providing necessary facility for completing this research work.

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