Comparative performance of *Aspergillus niger* isolates of different habitats on solubilisation of tricalcium phosphate in broth and their impact on yield attributes of wheat (*Triticum aestivum* L.)

B. R. Maurya and Ajeet Kumar*

Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, VARANASI (U. P.) INDIA

ABSTRACT

Eleven strains of *Aspergillus niger* isolated from rhizospheric soils of cereals, oil seeds, pulses and vegetables crops were tested for their ability to form halozone on Pikovskaya's agar media, lowering of pH, and solubilization of tricalcium phosphate in broth at one, two and three week of incubation. Their inoculation effect on growth, yield attributes, P contents in produce and acquisition of P by straw, grain, and by whole wheat crop were also studied in sterilized pot soil experiment. *Aspergillus niger* isolated from sugarcane rhizosphere gave maximum and significant grater size of halozone and acidity of broth compared to same isolates of other rhizospheric soils. All isolates increased acidity of broth with increase in incubation period but magnitude of increase was grater up to second week of incubation. Solubilisation of tricalcium phosphate had direct correlection with acidity of the broth. *Aspergillus niger* of sugarcane rhizosphere solubilized maximum quantity of tricalcium phosphate. All strain of *Aspergillus niger* showed their significant superiority over uninoculated control in increasing growth, yield attributes, P contents in produce and acquisition of P by straw, grain, and by whole wheat crop. Response of *Aspergillus niger* strain, varied with their source of isolation. *Aspergillus niger* isolated from the sugarcane rhizospheric soil appeared to be an efficient phosphate solubilizers.

Key words: *Aspergillus niger*, Tricalcium phosphate, Yield, Uptake, pH

INTRODUCTION

The phosphate solubilising ability of various soil microorganisms is well documented in literature. Soil fungi play a vital role in solubilisation of tricalcium phosphate (Singh et al.1994). This character in respect of eleven *Aspergillus niger* strains isolated from the rhizospheric soils of cereals, oil seeds, pulses and vegetables crops of Varanasi region, was studied using Pikovskaya's medium with tricalcium phosphate (TCP) as the substrate. The phosphate solubilising action of *Aspergillus niger* was observed both in solid (agar media) as well as liquid culture (broth). Very scarce informations available on the efficacy potential of *Aspergillus niger* isolated from different habitats. In the present experiment various phosphate solubilising fungal strain were isolated like *Aspergillus niger, Aspergillus flavus, Aspergillus sulphurious, Rhizopus stolonifer, Penicillium* but *Aspergillus niger* dominated in alluvium of all rhizospheric soils of Varanasi. Therefore present experiment was designed to study the efficacy of *Aspergillus niger* strain isolated from different habitats for their potential in solubilisation of tricalcium phosphate, change in acidity and their impact on growth, yield, P content and P uptake by wheat under sterilized soil pot experiment.

MATERIALS AND METHODS

Isolation and identification of phosphate solubilizing fungi:

Eleven soil samples collected from different crop rhizosphere were kept in sterile container separately and maintained at 4 ^{o}C until analysis**.** Phosphate solubilising fungi present in rhizospheric soils of different crops of different localities were cultured on Pikovskaysa's modified medium (Sundra Rao and Sinha, 1963). Fungal colonies showing clear zone of phosphate solubilisation were selected and transferred to agar slants and maintained at 4 ^{o}C.

Halozone diameter of TCP solubilisation:

Sterilized Pikovskaya's agar medium was poured into sterilized petriplates. After solidification of the medium, a pinpoint inoculation of *A. niger* strain was made on the plates aseptically and incubated for seven days at 28 ± 2 ^{o}C. The intensity of TCP solubilisation was measured as the diameter of the halozone formed around the colonies.

Measurement of pH and solubilisation of TCP in liquid medium:

Pikovskaysa's liquid medium (100 mL; pH 7 ±0.2) was dispensed in 250 mL Erlenmeyer conical flask and 300 mg P_2O_5 of TCP was added to the flask. The sterilized media were inoculated in triplicate with 0.5mL suspension of *Aspergillus niger* strain separately, keeping uninoculated control. The entire inoculated flask were incubated in BOD for 7,14 and 21 days at 30 ± 2°C. Sterilized distilled water was added to make the volume 100 mL and the contents were filtered through what man No.42 filter paper to remove the fungal mats. The pH and water soluble phosphorus content of the filtrate were recorded for 21 days of incubation at 7 days interval by pocket pH meter and molybdophosphoric acid blue colour method described by Jackson (1973).

Pot Experiment:

A sterilized pot experiment was carried out to judged the efficacy of *Aspergillus niger* strains of different habitats. Soil used in this experiment was collected from the research farm of Institute of Agricultural Sciences, BHU, Varanasi, having pH 7.5; EC 0.25 dSm^{-1}, Organic carbon 0.65 %, Available N and P_2O_5 (Olsen) were 110.5 and 18.32 mg/kg, respectively. Soil was autoclaved three times for two hour at 121 ^{o}C (15 lb/inch^2) and placed in 5 kg lots in ethanol disinfected plastic pots. There were twelve treatments, replicated three times in completely randomized designed (CRD). Ten uniform size seeds of wheat (*Triticum aestivum*) were surface disinfected with 0.5 % sodium hypocholorite solution and rinsed in sterilized water. Seeds were uniformly coated on surface by spores of *Aspergillus niger* separately and sown in sterilized pot soil. After germination, seedlings were thinned to 5 pot $^{-1}$. Plants were subsequently feed with 50 ml of nutrient solution lacking phosphate (Hogland and Arnon 1938) after 15, 35 and 50 days and watered with distilled water as required.

RESULTS AND DISCUSSION

Aspergillus niger isolated from the rhizosphere of sugarcane showed maximum and significantly greater diameter of halozone (5.9 cm) than others except strains isolated from rhizosphere of tomato and potato. Strains from mustard rhizosphere exhibits lowest diameter of halozone (3.7 cm). Strain of maize rhizosphere caused greater zone of clarity than isolates of wheat rhizosphere. All *Aspergillus niger* isolated from different habitats (different rhizospheric soils) causes the significant decrement in original value of pH (Table 1) which was according to findings of Liu et al, (2004). Decrease in pH may be due to the production of various types of organic acids by

***Author for correspondence**

Table: 1: Diameter of halozone, reduction in pH and solubilization of tricalcium phosphate by *Aspergillus niger* isolated from different rhizospheric soils.

Crop rhizosphere	Diameter of halozone (cm)	Change in acidity			P_2O_5 Liberated (ug/100 ml broth)		
		Ist Week	2nd Week	3rd Week	Ist Week	2nd Week	3rd Week
Sugarcane (F1)	5.9	4.3	2.8	2.5	136 (45.3)	212 (70.6)	221 (73.6)
Lobia (F2)	3.6	4.5	3.5	3.1	130 (43.0)	200 (68.3)	210 (70.0)
Radish (F3)	4.5	4.8	3.2	3.1	130 (43.0)	204 (68.0)	214 (71.3)
Potato (F4)	5.1	4.8	3.2	3.2	135 (45.0)	202 (67.3)	213 (71.0)
Pea (F5)	3.8	4.8	3.8	3.2	135 (45.0)	189 (63.0)	215 (71.7)
Tomato (F6)	5.2	4.4	3.7	3.3	131 (43.6)	193 (64.3)	211 (70.3)
Maize (F7)	4.7	4.7	3.9	3.8	131 (43.6)	201 (67.0)	205 (68.3)
Wheat (F8)	4.5	5.1	3.8	3.4	127 (42.3)	191 (63.7)	215 (71.7)
Pigeon pea (F9)	3.9	4.7	3.6	3.2	129 (43.0)	192 (64.0)	214 (71.3)
Mustard (F10)	3.5	4.9	3.3	3.1	127 (42.3)	204 (68.0)	214 (71.3)
Okra (F11)	4.7	5.3	3.3	3.2	131 (43.6)	205 (68.3)	215 (71.7)
LSD (0.05)	2.53	0.16	0.18	0.21	4.22	2.59	2.85

Initial pH = 7 ± 0.01; Data in parenthesis show % solubilisation of tricalcium phosphate.

Aspergillus niger during their metabolic processes, which lowered the pH of broth. This is in the conformity to the work of Omar, (1998) and Ikeda et al, (2005). At all the incubation periods maximum and significant decrease in pH was observed with *Aspergillus niger* isolated from sugarcane rhizosphere. The magnitude of decrease in pH reduced from 7 to 21 days of incubation probably due to Steen berg effect (Kpomblekou and Tabatabai, 2003). At second week of incubation, strains from radish and potato rhizosphere showed lower pH than other isolates. Solubilization of tricalcium phosphate $Ca_3(PO_4)_2$ increased with incubation period. However, the rate of solubilization was more up to second week of inoculation, thereafter it becomes almost static, due to paucity of nutrients in broth which retard the metabolic activity of *Aspergillus niger* and stops the liberation of organic acid. Maximum solubilization of tricalcium phosphate up to 15 days of incubation was also reported by Gaur & Gaind (1983). Due to Steenberg effect reduced metabolic activities perhaps diminished the organic acids and H_2CO_3 production, which ultimately decreased tricalcium phosphate solubilization, Omar (1998); and Vassilev et al (1995). Acidity of the broth had direct bearing on solubilization of tricalcium phosphate, as correlation between them was positive and significant (r = + 0.91) at 14 days of incubation. At 7 and 14 days of incubation *Aspergillus niger* isolated from the sugarcane rhizosphere solubilized maximum amount of tricalcium phosphate (136, 212 mg P_2O_5/100 mL) which were significantly higher than other strains of *Aspergillus niger*. This was worth noting that in spite of the same pH at 14 and 21 days of incubation, *Aspergillus niger* isolated from the potato rhizosphere tended to increase the solubilization of phosphorus with advancement of incubation period. This indicates that increase in incubation may increase the amount of soluble phosphate (Patel and Dave, 2000).

On basis of these results, it may be concluded that *Aspergillus niger* isolated from sugarcane rhizospheric soil was efficient solubilizers of tricalcium phosphate.

Pot experiment

Inoculation of *Aspergillus niger* isolated from the different habitat significantly increased all the growth and yield parameter of wheat when compared with the uninoculated plants (Table 2). The promotive

Table2: Influence of *Aspergillus niger* isolated from different rhizospheric soil on growth and yield attributes as well as % P and

Treatment	Plant Height (cm)	Plant fresh weight (g/plant)	Root fresh weight (g/root)	Root volume (cc/root)	Effective tillers /pot	Panicle length (cm)	Straw Yield (g/pot)	Grain Yield (g/pot)	Phosphorus (%)		Uptake of Phosphorus (mg/pot)		
									Grain	Straw	Grain	Straw	Total
Fo (Control)	40.8	4.08	0.28	0.49	5.4	6.0	6.2	2.9	0.28	0.17	8.2	10.6	18.8
F1	50.9	7.2	0.38	0.67	8.3	7.7	9.3	5.2	0.37	0.27	19.3	252	44.5
F2	48.9	5.5	0.35	0.67	7.2	7.5	7.9	4.5	0.34	0.22	15.3	17.4	32.7
F3	49.3	4.9	0.33	0.60	7.1	7.2	7.4	4.4	0.37	0.20	16.3	14.8	31.1
F4	49.2	6.3	0.36	0.64	6.7	7.8	7.9	4.2	0.39	0.24	16.4	19.0	35.4
F5	48.9	6.4	0.34	0.63	6.0	7.6	8.2	4.5	0.34	0.23	15.3	18.9	34.2
F6	50.2	6.9	0.37	0.66	7.7	7.9	8.5	4.9	0.36	0.25	17.7	21.3	39.0
F7	49.4	5.8	0.36	0.67	6.2	7.1	7.9	4.9	0.34	0.22	16.6	17.4	34.1
F8	49.3	6.4	0.35	0.55	7.5	7.9	8.2	4.1	0.33	0.20	13.6	16.4	40.0
F9	49.5	6.5	0.36	0.87	7.2	7.4	8.4	4.8	0.36	0.21	17.3	17.7	35.0
F10	49.8	6.1	0.37	0.65	7.1	7.2	7.3	4.9	0.35	0.19	17.2	13.9	31.1
F11	48.9	6.2	0.36	0.60	7.0	7.4	8.3	4.7	0.34	0.22	16.0	18.3	34.3
LSD (0.05)	0.24	0.3	0.03	0.04	0.1	0.2	0.3	0.2	0.02	0.06	0.45	3.91	4.36

effect of phosphate solubilising fungi on wheat growth in pot was demonstrated by Kumar and Narula (2001). Inoculation of sterilized pot soil with *Aspergillus niger* significantly increased phosphorus accumulation in shoot and grain of wheat. The maximum plant height (50.9 cm), fresh plant weight (7.2 g/plant) and fresh root weight (0.38 g/root) were recorded with *Aspergillus niger* of sugarcane rhizosphere followed by rhizosphere of tomato which indicates its significant superiority over other strains of *Aspergillus niger*. *Aspergillus niger* of sugarcane habitat produced 34.7 and 28.3 % higher number of effective tillers and panicle length, respectively in comparison to control. All rhizospheric *Aspergillus niger* yielded significantly higher straw and grains than control. The maximum effective tillers and panicle length with the sugarcane rhizosphere strains definitely had attributed to the greater yield. The greater yield of straw was recorded compared to grain. Increase in biomass and yield of wheat and other crop by inoculating the *Aspergillus* strains also has been reported by Tarafdar and Rao (1996). All *Aspergillus niger* exhibited higher P content and significantly greater phosphorus uptake by straw & grain than control (Table 2). Higher content of phosphorus was found in grain than straw but uptake of phosphorus tended just reverse of it. Nutrient uptake does not depend only on contents of nutrients. It varies also with the quantity of produce. Hence in spite of higher content of phosphorus in grain, greater uptake of phosphorus was caused by comparatively greater quantity of straw. Maximum value of (44.5 mg/pot) of P uptake by crop was recorded with sugarcane rhizosphere *A.niger* followed by wheat rhizosphere. The maximum P content as well as P uptake by grain (0.37 % and 19.3mg/pot) in comparison to control was recorded with *Aspergillus niger* isolated from sugarcane rhizosphere which may be due to greater solubilization of inherent unavailable soil phosphorus and its translocation to plants and seeds (McDowell et al, 2003).

It is concluded that The *Aspergillus niger* isolated from sugarcane rhizosphere perform better in soil as a inoculant which may help in availability of phosphorus for better crop stands.

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