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Indigenous arbuscular mycorrhiza is more important for early growth period of groundnut (*Arachis hypogea L.*) for P influx in an Oxisol

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ABSTRACT

The contribution of indigenous arbuscular mycorrhiza (AM) on phosphorus (P) uptake by groundnut was examined in a low P field soil. The fungicide benomyl was applied to eradicate mycorrhizal infection. The treatments consisted of three P levels viz. 0, 50 and 400 mg P kg^{-1} soil, with and without benomyl application. Groundnut as test crop was sown two weeks after the application of benomyl and was harvested four times covering the whole growth period. At each harvest, the shoot yield, shoot P concentration, root length, soil solution P (C_{Li}) and per cent root infection by AM was determined for benomyl treated and untreated soil at all P levels. Benomyl showed no effect on soil solution P concentration. When P was limiting, application of benomyl did reduce early groundnut growth by 40-50% at P-0, and by 25-30% at P-50. At high P supply (P-400), benomyl had little or no effect on dry matter production. Thus, indicate that the effect of benomyl on plant growth was by its influence on P uptake from soil. Phosphorus supply affected percentage of root infected by AM which was 40% of the roots at P-0, and decreased to around 30% and 10% at P-50 and P-400. In the early growing season, the P influx of maize was dependent on P in soil solution and the effect of AM was rather large. At high P supply, the contribution of AM to P influx showed a decrease. Without or low AM infection and at low P level, the P influx was 62% of that with AM. During early growth period groundnut showed a similar behaviour as maize at middle growth stage and without AM reduction of P influx, which was to an extent of 67%. In absolute terms AM is more important at maximum growth in the early growth season for groundnut. It is evident from the present investigation that AM may make an significant contribution by about 35 % to the P nutrition of groundnut, but other factors, like P solubilization by root exudates, may be even more important.

Key words: Oxisol, benomyl, arbuscular mycorrhiza, groundnut, solution concentration, P uptake, root length.

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IZVLEČEK

SPONTANA ARBUSKULARNA MIKORIZA JE POMEMBNA ZA OSKRBO RASTLIN ARAŠIDA (*Arachis hypogea* L.) S FOSFORJEM

Proučevan je bil vpliv samonikle arbuskularna mikorize (AM) na sprejem fosforja (P) pri rastlinah arašida. Fungicid benomyl je bil uporabljen za zagotovitev kontrolnih rastlin brez mikorize. Gnojenje s P je potekalo na treh nivojih in sicer 0, 50 in 400 mg P na kg tal, z oziroma brez uporabe benomyla. Arašidi so bili posajeni dva tedna po tretiranju tal s fungicidom, vzorce so jemali štirikrat tekom obdobja rasti. Pri vsakem jemanju vzorcev je bila določena teža poganjkov, koncentracija P v poganjkih, dolžina korenin, topen P v tleh in odstotek okužbe z mikorizno glivo, na obeh nivojih tretiranja s fungicidom in na vseh nivojih dodatka P. Fungicid ni vplival na vsebnost topnega P v tleh. Ko je bil P omejujoč dejavnik je fungicid zmanjšal zgodnjo rast arašidov za 40 do 50% pri P-0 in za 25 do 30% pri P-50. Pri visokem dodatku P (P-400) je imel fungicid majhen vpliv ali sploh ni imel vpliva na pridelek sušine. Tako kaže, da je fungicid vplival na rast rastlin z vplivom na sprejem P iz tal. Oskrba s P je vplivala na AM, ki je bila 40% pri koreninah skupine rastlin P-0 in se je znižala na okoli 30% in 10% pri P-50 in P-400. Pri visokih odmerkih P se je zmanjšal pomen AM za oskrbo rastlin s P. Brez AM in pri nizkem nivoju P je bil dotok P v rastline samo 62% tistega, ki je bil pri rastlinah z AM. AM je najpomembnejša za zagotovitev maksimalne rasti v začetku rastne sezone arašidov. Raziskava je pokazala da je AM značilno prispevala z okoli 35% deležem oskrbe rastlin arašidov s P, da pa so lahko celo bolj pomembni tudi drugi vplivi, kot na primer, da izločki korenin prispevajo k povečanju topnega P v tleh.

Ključne besede: Oxisol, benomyl, arbuskularna mikoriza, arašidi, koncentracija topnega P, privzem P, dolžina korenin.

1 INTRODUCTION

In the tropics and subtropics (Oxislos and Alfisols) strong weathering is associated with an increase in the amount of sesquioxides, which exhibit high P sorption properties (Torrent, 1997). When P fertilizers are applied to replenish soil fertility major portion of is bound in oxisols by Fe/Al oxides as P compounds of variable adsorption strength, reducing the effectiveness of P fertilization and results in low P soil. Due to these reasons, phosphorus is often limiting crop growth in these soils of low P supplying capacity. Crop species differ in their ability to grow well in low phosphorus supplying soils (Föhse *et al.*, 1988). A persistently low level of available phosphorus in the soil solution has led to numerous morphological, physiological, and biochemical adaptations by plants to survive in the nature. The results of field experiment on wheat, maize and groundnut (Bhadoria *et al*., 2001) carried out on a low P soil have shown that maize had an increased P influx during the middle growing season even though influx was very low in the early stage, groundnut showed a high P influx right from the beginning and wheat had an average to low P influx during the whole growing season. Possible reasons for this large variation of influx among the crops during the growing season at limiting P supply may have been due to the contribution of arbuscular mycorrhizae (AM) (Marschner, 1995), phosphatase activity near root (Elliott and Lauchli, 1986) and rate of root exudation (Gerke *et al.,* 1994). The present investigation was undertaken to study the significance of AM on P influx of groundnut under field conditions.

2 MATERIALS AND METHODS

Field experiments were carried out on a P fixing soil of eastern India at the Agricultural and Food Engineering Department experimental farm of Indian Institute of Technology Kharagpur, India. The soil with $pH_{(H2O)}$ 5.3, organic C 3.5g kg^{-1}, contained 16% clay, 24% silt and 6.2 ppm P (Bray.1). Treatments consisted of three P levels, P-0 (unfertilised, without P), P-50 (50 mg P kg^{-1} soil) and P-400 (400 mg P kg^{-1} soil) as single superphosphate and treatment with and without benomyl Treatments were arranged in a factorial randomised block design with four replication. Commercial formulated grades of the fungicides, benomyl was mixed thoroughly to 15 cm soil depth at 500 kg ha^{-1}. Nitrogen was applied at the rate of 20 kg ha^{-1} and potassium at the rate of 50 kg ha^{-1}. Besides that Ca and Mg and micronutrients including Zn, Fe, Cu, B, Mn and Mo were applied at the recommended doses for this soil. After levelling the field plots, seeds of groundnut (*cv. A K 12/24*) were sown in rows keeping row to row spacing of 30cm and plant to plant distance was 20cm.

The crop was harvested four times to obtain shoot yield, root length shoot P concentration, Soil solution P concentration (Adams, 1974) and per cent root infection by mycorrhizae during the growing season. Dry mass of shoots were recorded after drying in a hot air oven at 60°C to a constant weight and grinding to a fine powder. The P content was determined after tri-acid digestion, using the vanado-molybdophosphoric yellow color method
Phosphorus influx is calculated as follows:

$$In = 2(U_2-U_1) / ((t_2-t_1) (RL_2+RL_1)$$

where,
In = P influx (mol $cm^{-1}s^{-1}$),
U = P uptake (mol m^{-2}),
RL = root length (cm m^{-2}),
t = time (seconds)
subscripts 1 and 2 refers to current and previous harvests

Percentage root infection:
Root infection was assessed on a representative root sample taken from each plot in fixed positions evenly distributed at each harvest to a depth of 15 cm were taken. The roots from each plot sample were separated, washed free of soil, cut into 1-1.5 cm lengths. Root samples were stained with tryptan blue (Philips and Hayman, 1970). AM infection of each plant was determined by estimating the percent root colonization as described by Bierman and Linderman (1981).

Per cent root infection was obtained as follows:

$$\%\ \text{Root infection} = \frac{(100 \times \text{Number of intersections with arbuscular infections})}{\text{Total number of intersections counted}}$$

3 RESULTS

Soil solution concentration

Table 1 shows the average soil solution concentration (C_{Li}) during the growing season at different P fertilization both in benomyl treated and untreated plots. It may be seen that C_{Li} increases exponentially with P fertilization. Application of fertilizer at the rate of 50 mg P kg^{-1} soil had negligible effect on C_{Li} which become significant only at P-400 level. Effect of benomyl on C_{Li} was non significant (Table 1) at all harvests. In general, there was decrease in C_{Li} in benomyl treated plots as compared to untreated plots and this was more at P-0 than at P-50 or P-400 levels .The results clearly show that benomyl had no major effect on P concentration in the soil solution, and consequently on the P availability in soil (Fitter and Nicholas, 1988).

Bentivenga and Hetrick (1991) also found that benomyl application could not measurably change P availability in a silty clay loam soil.

Table 1. Effect of benomyl on soil solution concentration (μM) of phosphorus of groundnut at no P (P-0), 50 mg P kg^{-1} (P-50) and 400 mg P kg^{-1} (P-400) application to the soil.

P levels (mg kg^{-1} soil)	30 DAS		50 DAS		68 DAS		112 DAS	
	Benomyl							
	-	+	-	+	-	+	-	+
0	0.90	0.62	0.85	0.52	0.73	0.45	0.71	0.45
50	2.95	2.23	2.50	2.10	2.50	2.10	2.45	2.05
400	6.39	6.25	6.30	6.20	6.25	6.20	6.05	6.00
	SEm±	LSD(0.05)						
P	1.21	3.73	1.18	3.49	1.13		1.08	3.26
B	1.06	NS	1.03	NS	3.41		0.78	NS
PxB	1.37	NS	1.33	NS	1.01	NS	1.18	NS
					1.25	NS		

(P: Phosphorus levels; B: Benomyl application; DAS: Days after sowing; SEm: Standard error of mean; LSD: Least square difference)

Root infection

The root infection by AM as effected by benomyl and P supply is shown in Table 2. In general the extent of mycorrhizal infection decreased, with increased application phosphate to the soil but the extent of this effect did not vary much between the crop species. It can be seen P supply affected percent root infected by AM, being 40% of the roots at P-0 and decreasing to around 30% and 10% at P-50 and P-400 respectively. This agrees with the results of earlier workers who have found that supply of phosphate to the plant by soil (Smith, 1982, Thompson et al., 1991) had an inhibitory effect on infection. High P application to the soil depressed AM formation and this effect has been attributed to the increase of P in the plant (Jasper, 1979, Amijee et al., 1989). There have been other experiment Mirinda et al., (1989) where it has been demonstrated that soil P levels have direct effect on AM particularly during the early stages of root colonization when the AM fungus is first developing in the soil. Benomyl was effective to suppress AM infection up to day 30. Thereafter some infection occurred, but was still less than in the untreated plot. Khaliq and Sanders (1997) found that inoculation of maize plants was effective during the early stages but response gradually disappeared during the later stages of growth. They also concluded that the inoculation response was reversed after the second harvest was because of higher root densities which might have been higher to render mycorrhizal fungus superfluous. Jakobsen (1987) and Thingstrup et al., (1998) reported that fumigation with Dazomet strongly decreased mycorrhiza formation and the effect persisted at least for 60 days after sowing.

Table 2. Effect of benomyl on root infection (%) of groundnut at no P (P-0), 50 mg P kg^{-1} (P-50) and 400 mg P kg^{-1} (P-400) application to the soil.

P levels (mg kg^{-1} soil)	30 DAS		50 DAS		68 DAS		112 DAS	
	Benomyl							
	-	+	-	+	-	+	-	+
0	60	0	32	8	28	10	25	12
50	48	0	26	10	22	12	20	14
400	0	0	6	2	12	2	14	4
	SEm	LSD(0.05)						
P	1.46	4.42	1.30	3.91	1.48	4.47	1.30	3.91
B	1.90	5.73	1.06	3.20	1.20	3.61	1.06	3.20
PxB	2.07	6.24	1.84	5.65	2.09	6.28	1.84	5.64

(P: Phosphorus levels; B:Benomyl application; DAS: Days after sowing; SEm: Standard error of mean; LSD: Least square difference)

Shoot and root growth

Table 3 gives the shoot growth as affected by P supply and Benomyl application. Phosphorus supply affected groundnut growth mainly in the later stages, early growth reduction was much smaller. Dry matter yield recorded following benomyl treatment was significantly lower over untreated plot. At high P supply (P-400), benomyl had little or no effect on dry matter production. This result indicates that benomyl has no direct effect on growth. This is in conformity with the findings of Carey et al. (1992) who concluded that Benomyl had no direct effect on the growth of wide range of plants. When P was limiting, the application of Benomyl did reduce early groundnut growth by 25 % at P-0 and by 10 % at P-50. In general, variation in dry matter production due to influence of both P levels and benomyl application has been small in the initial stage of crop growth but gradually increased up to third harvest. However the growth reduction during second harvest showed drastic decrease in benomyl treated P-0 plot than untreated plot. Groundnut had small growth rates when young, the rates increased strongly by 5-7 times in the middle for both benomyl treated and untreated plots. Reduction of growth following benomyl application is mainly attributed to eradication of AM and that might have influenced the P uptake from soil. Similarly Thingstrup et al., (1998) reported that the growth and P content of flux was lower in fumigated than in untreated plots and this effect decreases with increasing level of P application.

Table 3. Effect of benomyl on shoot growth (g m^{-2}) of groundnut at no P (P-0), 50 mg P kg^{-1} (P-50) and 400 mg P kg^{-1} (P-400) application to the soil.

P levels (mg kg^{-1} soil)	30 DAS		50 DAS		68 DAS		112 DAS	
	Benomyl							
	-	+	-	+	-	+	-	+
0	9.14	3.78	62.7	48.3	211	201	385	365
50	11.16	5.73	73.5	66.2	268	260	433	403
400	10.93	9.81	69.6	69.3	244	241	492	463
	SEm	LSD(0.05)						
P	0.31	0.92	1.93	5.82	4.73	14.3	11.9	36
B	0.25	0.78	1.57	4.73	3.89	NS	10.9	NS
PxB	0.44	1.35	2.73	NS	6.68	NS	20.1	NS

(P: Phosphorus levels; B: Benomyl application; DAS: Days after sowing; SEm: Standard error of mean; LSD: Least square difference)

Table 4. Effect of benomyl on root length (km m^{-2}) of groundnut at no P (P-0), 50 mg P kg^{-1} (P-50) and 400 mg P kg^{-1} (P-400) application to the soil.

P levels (mg kg^{-1} soil)	30 DAS		50 DAS		68 DAS		112 DAS	
	Benomyl							
	-	+	-	+	-	+	-	+
0	0.12	0.21	0.57	0.62	1.92	2.08	1.28	1.32
50	0.16	0.23	0.72	0.74	2.38	2.46	1.35	1.37
400	0.16	0.24	0.68	0.70	2.31	2.36	1.40	1.52
	SEm	LSD(0.05)						
P	0.02	0.06	0.017	0.051	0.06	0.18	0.06	0.18
B	0.02	NS	0.013	NS	0.05	NS	0.05	NS
PxB	0.03	NS	0.023	NS	0.08	NS	0.09	NS

(P: Phosphorus levels; B: Benomyl application; DAS: Days after sowing; SEm: Standard error of mean; LSD: Least square difference)

P Influx

Benomyl application had almost no effect on P concentration in the shoot and so P uptake was closely related to dry matter production. The central question of this section is the effect of mycorrhiza on P uptake. If mycorrhizal hyphae absorb P and transport it to the root than the uptake expressed per unit of root and unit time, the influx, should be increased due to mycorrhiza. Figure 1 depict the relationship between the P influx as a function of the P concentration in soil solution. The P influx is shown for plants with full mycorrhizal infection or with no or reduced infection due to benomyl application. Furthermore is shown the P influx as calculated by the model which does not include mycorrhizal action nor the effect of root exudates on P solubility in soil, i.e., without chemical mobilization.

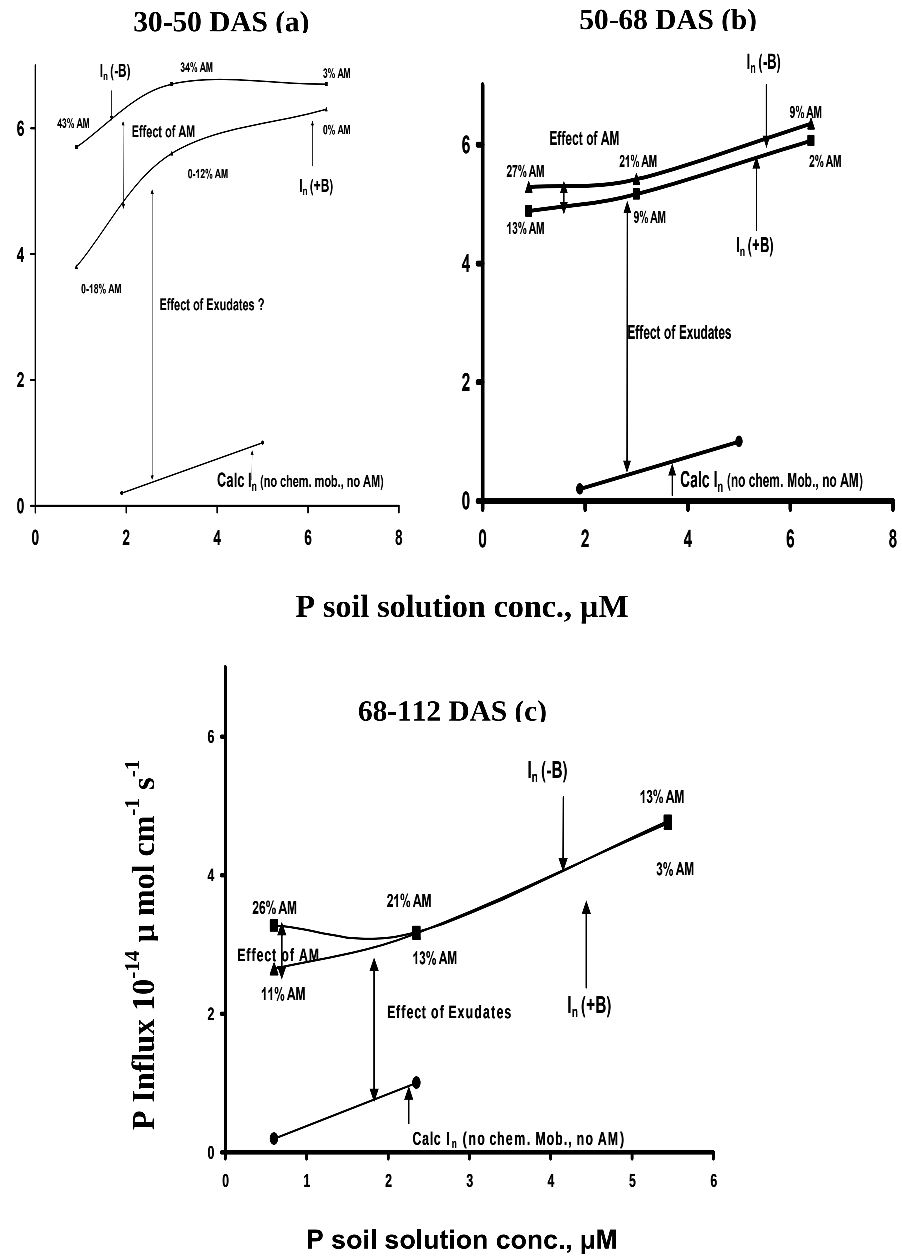

Figure 1: Phosphorus influx, (I_n), of groundnut roots as a function of P concentration in soil solution of roots not treated (-B) or treated with benomyl (+B) in early (a) and middle (b) growing season. Furthermore the mycorrhizal infection (% AM) of the roots is given and the P influx as calculated by a model without the action of arbuscular mycorrhiza nor of root exudates (DAS).

The P influx in groundnut with full mycorrhizal infection or with no or reduced infection due to benomyl application shows that in the early growing season (Figure 1) the P influx of groundnut was increased sharply and was not dependent on P supply, being around $6x10^{-14}$ mol $cm^{-1}s^{-1}$ at C_{Li} = 0.8 (P-0) as well as at C_{Li} = 6.3 µM (P-400). At this growth stage the effect of AM was largest at low P supply. Without AM infection and at low level the P influx was 67% of that with AM infection (Fig.1). In middle and end of the growth cycle, P influx was found to be related to P supply, where the degree of AM infection had no influence. Jakobsen (1986) and Smith (1982) reported two to three times increase in influx of P in to the roots of pea and clover plants respectively where mycorrhiza was not controlled. Influx of P in roots colonized by mycorrhizal fungi could be several times higher than in non-mycorrhizal roots (Smith and Read, 1997) in different crops.

4 DISCUSSION

Comparing the P influx of the plots treated with and without benomyl (Figure 1) it was observed that at full AM infection, P influx could increase by factor of 3 to 4. However, without AM infection, the influx was still higher as calculated by simulation model. It is speculated that the reasons for measured influx being higher than calculated influx could be the solubilizing action of the root exudates.

According to the theory of diffusion P (Barber, 1995) transports to the root and therefore P influx should be about proportional to P concentration in soil solution, C_{Li}. This was evident in case of maize during early growing season, when influx was low, but at a later stage when P influx was high, soil solution concentration showed no effect on P influx and this could be the reason for different behavior of P influx in early and middle growing season. AM could not explain fully the variation in the measured P influx. A possible explanation, could be that in early growing season, the root functions mainly as an absorbing organ i.e., as sink for P, and in that case transport to the root is determined by the concentration in soil solution. Later, the root actively participates in P dynamics in the rhizosphere by excreting root extudates that influenced P solubility in soil.

Since in our experiments we assessed the effect of AM on the P influx we can now estimate the effect of root exudates that solubilize strongly bound soil P on P influx by knowing the difference between measured P influx without AM and calculated influx simulated by the nutrient uptake model. One has to keep in mind that the estimate of the effect of root exudates is a residual of all other determinations and calculation and all errors if committed will accumulate here. With this caution, it can be seen that chemical mobilization (action of root exudates) for groundnut was almost 50-60% of P influx in early and middle of the season and becoming less in the later part of the growth stages. The remaining 50 to 40 % of P influx not accounted for by model calculation or by AM, could be due to root exudates and other factors that solubilize strongly bound soil P. Root exudates, often as a reaction to phosphorus deficiency (Ae *et al*., 1990; Gerke *et al*., 1994;) could have made an important contribution towards P uptake, because of their influence on P solubility in soil.

The results show that AM can make an significant contribution towards P nutrition as observed in groundnut, but other factors, like P solubilization by root exudates, can be even more important.

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