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Field Response of Groundnut (*Arachis hypogea L.*) Cultivars to Mycorrhizal Inoculation and Phosphorus Fertilizer in Abeokuta, South West Nigeria

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Abstract: A field experiment was conducted in Abeokuta, south western Nigeria, to evaluate the growth and yield response of three groundnut cultivars to inoculation with mycorrhizal fungus (*Glomus mosseae*) and phosphorus (P) fertilization in 2003 and 2004 planting seasons. The design was split-split plot in Randomized Complete Block Design (RCBD) in a 3×2×2 factorial combination of groundnut cultivars (RMP_{91}, RRB and RMP_{12}), phosphorus (54 and 0 kg ha^{-1}) and mycorrhizal inoculation (inoculated and uninoculated)). Observations were made on canopy spread, leaf area, dry matter yield and grain yield. Mycorrhizal root infections, leaf P uptake and available P in the rhizosphere were also determined. Result shows that inoculation of groundnut cultivars with *G. mosseae* significantly enhanced grain yield in the 2003 planting season (54% in RMP_{91} to 66% in RMP_{12}) whereas the enhancement was lower and only significant in RRB (21%) in the 2004 planting season. Phosphorus fertilization enhanced grain yield by a range of 22% (RMP_{91}) and 40% (RMP_{12}) in the 2003 planting season while it was a range of between 20% (RMP_{91}) and 16% (RRP) in the 2004 planting season. Mycorrhizal root infection was as high as 64.1% relative to control (10.6%) in 2003 season. Inoculation with *G. mosseae* increased leaf P uptake by 30% within the two planting seasons. Phosphorus application increased the level of P in the leaf (average 32%} in the two years and rhizosphere soil P (average 1400%) only in the 2004 planting season. Percentage root colonisation ranged between 60 and 67% in all the inoculated plots in the two years. There was a marked increase in the root infection rate in the uninoculated plots (averaged 180%) in the 2004 planting season. In the 2004 planting season, available rhizosphere soil P averaged 2.06 mg kg^{-1} in the inoculated plots when compared to 2.6 mg kg^{-1} in P fertilized and 2.69 mg kg^{-1} in the P+M plots. All the treatments increased canopy spread (average 25%), leaf area (average 14%) and root dry weight (17%) over the control in the 2003 planting season, while in 2004 planting season, increase was recorded in canopy spread (average 22%), leaf area (average 40%) and shoot dry weight (average 35%) over the control. Inoculation of groundnut cultivars with the mycorrhizal fungus improved their performance in the field and compared favorably with the groundnuts fertilized with 54 kg ha^{-1} of SSP fertilizer.

Key words: Groundnut cultivars · arbuscular mycorrhizal inoculation · leaf phosphorus uptake · grain yield · transitional agro-ecological zone

INTRODUCTION

Groundnut (*Arachis hypogea* L.) is comonly grown in the Northern Guinea Savanna of Nigeria. It was a crop that accounted for large percentage of Nigeria's export produce in the 1960s. Over the years, the production has gone drastically down owing to the increasing cost of production arising from inputs such as phosphorus fertilizers, labour and unpredictable environmental factors. Attempts are currently on to effectively include this crop in the farming systems of the southern ecologies [1, 2]. Nitrogen and phosphorus are important for effective production of the crop. Groundnut is a legume that requires phosphorous for growth and development, nitrogen fixation as well as nodule formation [3, 4]. Nitrogen need of the crop is sufficiently met through nitrogen fixation in most soils while inorganic fertilizers are applied to supply the phosphorus requirement. In most tropical soils, phosphorus deficiency is a major problem [5, 6]. High costs of fertilizers and

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logistic problem in distribution have made it difficult for small scale farmers to use them as at when needed. This has reduced hectarage and yield. There is also competing demands for fertilizers from more popular crops such as maize and rice in this region. However, Arbuscular Mycorrhizal (AM) fungi are known to improve water in drought stress conditions [7] and nutrient uptakes of plants particularly P in deficient soils [8]. Plants are also protected against some root pathogens [9]. These fungi are involved in symbiotic association with the roots of with more than 95% of plants [10, 11]. There are claims that the response of different plant species to mycorrhizal fungi inoculation varies considerably, even crops within same species but different cultivars may respond differently to mycorrhizal fungi [12]. These authors noted that improved cultivars of tomato (*Lycopersicum esculentum* Mill.) in a low P soil were more responsive to AM colonization than the wild type. Ironically, significant variation was also noticed among the wild accessions and even among the improved cultivars in responsiveness to mycorrhizal fungi colonization. Studies into the use of mycorrhizal fungi in improving crop production are still relatively very few in Nigeria. Most of the available trials have been conducted under partially or wholly controlled environments. The objective of this study therefore, is to compare the effects of an AM fungus (*Glomus mosseae*) and phosphorus on the growth and yield of three groundnut cultivars under field conditions in Abeokuta, a forest savannah transition zone of south west Nigeria.

MATERIALS AND METHODS

The experiment was conducted at the University of Agriculture Abeokuta Teaching and Research Farm, Alabata in south western Nigeria (70° 15'N, 30° 25'E). The site lies within the humid low land tropical region with two distinct seasons. The wet seasons extends from April to October while the dry season starts from November to March. The mean annual rainfall is 1113.1 mm, with a characteristic bimodal distribution which peaks in July and September with a break in August. Mean monthly temperature varies from 22°C in August to 36°C in March. The relative humidity ranges between 75% in February and 88% in July. The trial was carried out in the wet seasons of 2003 and 2004. Experimental soil characteristics is presented in Table 1 and was determined by collecting composite soil sample before planting for analysis using the standard procedures. The samples were air dried and sieved through a 2 mm mesh sieve before analysis. Particle size was determined by the pipette method, pH was measured in 0.01 M $CaCl_2$ at 1:2 soil : solution ratio, Organic carbon was determined by the Walkley-Black wet oxidation method [12]. The exchangeable cations were extracted in neutral ammonium acetate solution [14]. Ca and Mg were determined by the atomic absorption spectrophotometer and K by flame photometry. The total N was determined by the microkjeldahl method [15], available phosphorus was extracted by the Bray 1 exractant and P in solution determined by the Molybdate blue color method [16].

Inoculum preparation: Strains of *G. mosseae* was obtained from the culture stock of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria and multiplied on maize plants in green house plastic pots culture according to the procedure of Khalil *et al.* [17]. After three months, the maize plants whose inflorescence had been removed were allowed to dry to induce sporulation of the fungus. The crude inoculum which consisted of soil containing spores, hyphae and maize roots infected with the fungus contained about 500-650 spores per 100 g of soil.

Experimental procedures: The experimental design was split-split plot in RCBD in a 3×2×2 factorial combination of cultivars (RMP_{91}, RMP_{12} and RRB), Phosphorus (54 and 0 kg ha^{-1}) and Inoculation (with [M] or without [NM]) AM fungus. All treatments were replicated three times. Fifty grams of crude inoculum of *G. mosseae* was introduced into the planting holes of the inoculated plots (M) while the same quantity of attenuated inoculum was applied into the planting hole of non-inoculated plots (NM). All treatments were replicated three times. The planting space of groundnut was 75×25 cm with two seeds per hole and each plot measured 4×3 m. Single Super Phosphates (SSP) was applied basally in phosphorus fertilized plots. Measurement and observation of various parameters were taken at 100% flowering for plant height and root to shoot ratio. Leaf area was determined using the punch technique as described by Nichiporohvic [18]. Percentage root infection was determined according to the grid line intersect technique of Goivanetti and Mosse [19]. Roots and shoots weights (g $plant^{-1}$) were determined at harvest. Data were collected on shelling (%), pod number/plant, 100 seed weight (g) and grain yield (t ha^{-1}). Leaf P concentration (%) was determined according to molybdenum blue method of Murphy and Rilley [16] and

Table 1: Initial values of soil parameters at the beginning of the trials in the in 2003 and 2004 planting seasons in Abeokuta

Soil characteristics	2003	2004
Sand (%)	82.20	82.00
Silt (%)	9.30	9.40
Clay (%)	8.50	8.60
Textural class	Sandy	Sandy
CEC (cmol kg^{-1})	1.31	1.34
Organic carbon (g kg^{-1})	18.60	19.60
pH ($CaCl_2$)	4.80	5.00
Available P (mg kg^{-1})	4.92	4.95
Total N (mg kg^{-1})	1.16	1.16

leaf P uptake was subsequently calculated as the sum of its concentrations and the dry weight. Data were subjected to statistical analysis using Analysis of Variance (ANOVA) and means were separated using Least Significant Difference (L.S.D).

RESULTS

Soil characteristics: Table 1 shows the initial soil data before commencement of the experiment in the first and second planting seasons. The soil was sand and acidic in reaction with low phosphorus, nitrogen and moderate level of organic carbon.

Growth and yield response: In 2003, inoculation and P fertilization individually and in combination increased canopy cover in the groundnut cultivars except for RMP_{91}. In all the cases, the cover was similar but higher than the control (uninoculated and without phosphorus) (Table 2). Similarly, root dry weight was significantly higher and comparable in all the treatments than the control. Shelling percentage and 100 seed weight were also improved by either phosphorus fertilization, inoculation or both. In a similar manner, shoot dry weight and number of pods per plant was increased by phosphorus or AM inoculation just as the number of pods per plant was generally observed to be higher in RRB than in the other two cultivars (Table 2). In 2004 planting season, canopy spread was also increased by phosphorus fertilization and inoculation singly or in concert in all the cultivars and this was generally observed to be higher than the values obtained in 2003 (Table 2). Similarly, phosphorus fertilization and inoculation enhanced root and shoot dry weights (Table 2). Phosphorus and AM inoculation individually or in combination increased leaf area (25%), root (8%) and shoot dry weights (43%) in all the three cultivars.

Grain yield: Phosphorus fertilization and AM inoculation increased the grain yield of groundnut cultivars in 2003 and only in RRB in 2004 planting seasons (Table 2a & b). The percentage enhancement was higher in 2003 than in 2004 planting seasons. While the grain yield increase averaged 90% in 2003, it was reduced to 55% in 2004 season. However, there was about 60% increase in grain yield in the control plot in 2004 season whereas it was relatively stable in all other treatments (Table 2a & b). In 2004, grain yield of RMP_{91} and RMP_{12} were not significantly different from each other.

Table 2a: Effect of *Glomus mosseae* inoculation and phosphorus on growth and yield parameters of three groundnut varieties in 2003

Variety	Treatment	Canopy spread (cm^2)	Leaf area (m^2)	Root dry weight (t ha^{-1})	Shoot dry weight (t ha^{-1})	Shelling (%)	No. of pod $plant^{-1}$	100 seed weight/plant (g)	Grain yield t ha^{-1}
RMP_{91}	P	13.84	0.50	2.60	10.48	74.60	77.00	57.40	0.53
	M	13.73	0.42	2.35	10.62	74.58	100.00	57.02	0.64
	P+M	15.63	0.46	2.32	10.70	76.20	99.00	57.95	0.76
	Control	11.86	0.35	2.11	8.87	68.54	97.00	50.66	0.41
RRB	P	15.70	0.53	2.60	9.87	75.06	114.00	53.90	0.66
	M	13.70	0.51	2.57	10.87	72.84	124.00	51.78	0.77
	P+M	15.16	0.53	2.32	10.89	75.60	114.00	54.57	0.84
	Control	11.94	0.43	2.10	9.55	68.70	104.00	49.71	0.55
RMP_{12}	P	14.99	0.42	2.17	7.80	71.20	82.00	56.88	0.66
	M	15.29	0.44	2.27	10.24	71.22	80.00	55.70	0.66
	P+M	17.21	0.44	2.18	11.79	74.43	76.00	57.39	0.78
	Control	11.72	0.33	1.94	8.32	68.92	68.00	51.90	0.4
	$L.S.D_{0.05}$	2.09	0.05	0.10	1.38	4.57	39.00	3.82	0.11

Abbreviation: P-Phosphorus, M-Inoculated, Control-No Phosphorus and no inoculation

Table 2b: Effect of *Glomus mosseae* inoculation and phosphorus on growth and yield parameters of three groundnut varieties in 2004 planting seasons

Variety	Treatment	Canopy spread (cm^2)	Leaf area (m^2)	Root dry weight (t ha^{-1})	Shoot dry weight (t ha^{-1})	Shelling (%)	No. of pod $plant^{-1}$	100 seed weight/plant (g)	Grain yield t ha^{-1}
RMP_{91}	P	23.00	0.74	2.31	19.99	73.07	95.00	57.03	0.64
	M	21.67	0.66	2.39	16.75	72.74	90.00	55.32	0.67
	P+M	23.33	0.67	2.41	22.50	71.36	78.00	58.59	0.66
	Control	18.11	0.51	2.27	14.04	70.27	76.00	52.08	0.66
RRB	P	23.56	0.75	2.31	22.60	68.70	98.00	50.93	0.74
	M	21.71	0.8	2.41	18.99	72.52	111.00	50.07	0.74
	P+M	22.70	0.78	2.41	25.24	68.71	77.00	51.96	0.76
	Control	21.02	0.58	2.11	15.08	66.62	118.00	49.07	0.61
RMP_{12}	P	26.08	0.7	2.14	23.92	70.01	80.00	58.3	0.69
	M	23.11	0.73	2.36	18.21	70.88	74.00	52.06	0.66
	P+M	25.63	0.8	2.32	25.84	70.09	88.00	56.27	0.71
	Control	22.11	0.69	2.14	15.25	67.42	75.00	50.36	0.67
	$L.S.D_{0.05}$	2.42	0.05	0.16	0.98	6.90	29.00	5.78	0.04

Abbreviation: P-Phosphorus, M-Inoculated, Control-No Phosphorus and no inoculation

Table 3: Effect of *Glomus mosseae* inoculation and phosphorus on root to shoot ratio in 2003 and 2004 planting seasons

Variety	Treatment	Root to shoot ratio (2003)	Root to shoot ratio (2004)
RMP_{91}	P	0.25	0.22
	M	0.22	0.24
	P+M	0.22	0.21
	Control	0.24	0.25
RRB	P	0.25	0.24
	M	0.24	0.22
	P+M	0.23	0.23
	Control	0.21	0.23
RMP_{12}	P	0.28	0.21
	M	0.24	0.22
	P+M	0.2	0.22
	Control	0.22	0.21
$L.S.D_{0.05}$		0.05	0.05

Abbreviation: P-Phosphorus, M-Inoculated, Control-No Phosphorus and no inoculation

Mycorrhizal infection: In 2003, phosphorus fertilization significantly reduced root AM infection in all the groundnut cultivars (values ranged between 8-12%). Whereas development of infection in inoculated plots as well as plots fertilized with phosphorus was significantly higher (60-65%) than control plots (10-12%) (Fig. 1a). Similar observations were recorded in the two seasons of the experiment (Fig. 1a & b). There were significant differences among cultivars inoculated with mycorrhizal fungi, those fertilized with phosphorus and control in both years. Plot fertilized with phosphorus had about 14% root colonization of the inoculated plots in all cultivars. The control plots without phosphorus fertilization had average of about 11% infection in 2003 planting season with a similar trend in 2004 planting season. A major deviation was the increase in the percentage of infection recorded for control plots which was about 210% of what was obtainable in the 2003 planting season (Fig. 1a & b).

Root to shoot ratio, P uptake and phizosphere P: Root to shoot ratio was not significantly influenced by either phosphorus fertilization or mycorrhizal inoculation in all the groundnut cultivars in the two planting seasons (Table 3). Leaf phosphorus uptake was enhanced by phosphorus fertilization, mycorrhizal inoculation and both when compared to all the control plots in the two planting seasons (Fig. 2a & b). In all the cultivars, phosphorus uptake was significantly enhanced by 155% on the average. Rhizosphere available phosphorus was increased by phosphorus and mycorrhizal inoculation singly or in combination in all the groundnut cultivars only in the 2004 planting season (Fig. 3a & b). Plot with P fertilization [P] has the highest available phosphorus (1400%) among the three cultivars, followed by the plots under AM inoculation and P fertilization [P+M] (930%) and the inoculated plots [M] (300%) of the control in RMP_{91}. The range of enhancement was similar in RRB and RMP_{12} (Fig. 3a & b).

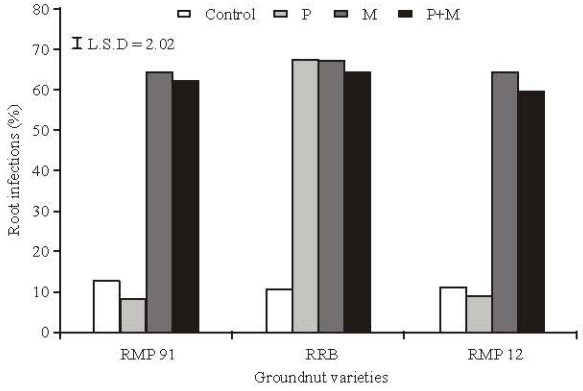

Fig. 1a: Effect of *Glomus mosseae* inoculation and phophorus on root infection in 2003 planting season

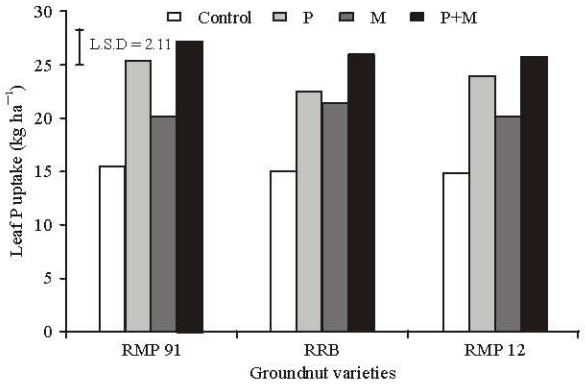

Fig. 2a: Effect of *Glomus mosseae* inoculation and phosphorus on leaf P uptake in 2003 planting season

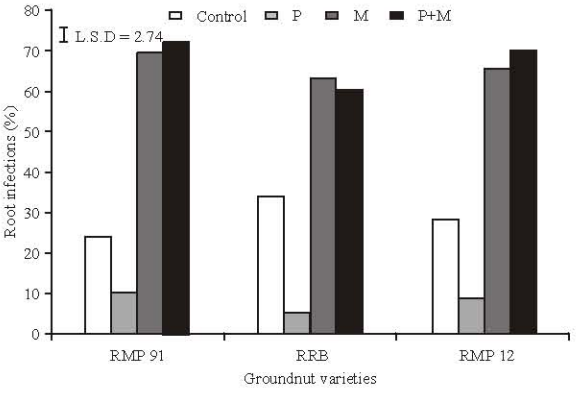

Fig. 1b: Effect of *Glomus mosseae* inoculation and phophorus on root infection in 2004 planting season

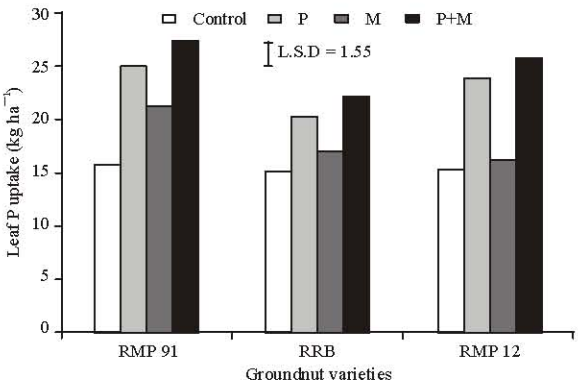

Fig. 2b: Effect of *Glomus mosseae* inoculation and phosphorus on leaf P uptake in 2004 planting season

DISCUSSION

The trial site is low in phosphorus and nitrogen but moderate in organic carbon. These are optimum conditions for establishment of mycorrhizal associations with plant roots [4]. The infection results of this experiment confirm the findings of Simpson and Daft [20] on the ability of groundnut to form mycorrhizal association particularly with *Glomus* sp. It also showed that *G. mosseae* can compete favourably among the varieties of other soil fungi, if it is present in reasonable quantity. Inoculation of groundnut with the fungus brought about enhancement in the general growth and the eventual improvement in the economic yield. This growth enhancement is due to potential of the fungus to improve water and phosphorus uptake as suggested by Harley and Smith [21] and Sieverding [22]. In addition, nitrogen fixation could have been enhanced by the fungi as reported by Simpson and Daft [19]. This is possible since the field was not nitrogen fertilized [23]. Osonubi *et al.* [24] had also reported an increase in phosphorus uptake in alley cropped cassava in a degraded alfisol after inoculation with *G. deserticola*. The behavior of the rhizosphere phosphorus as obtained in this study supports this assertion. The infection in the first year was similar to the second year in all the plots. The observation in the control plot where the infection was higher may be a result of spread of propagules from plots inoculated in the previous year. A similar observation was recently reported in an open field situation by Bhoopander and Mukerji [25]. As expected, the enhancement of growth of inoculated plants was reflected in almost all the growth parameters measured in this experiment. This may be a direct effect of the

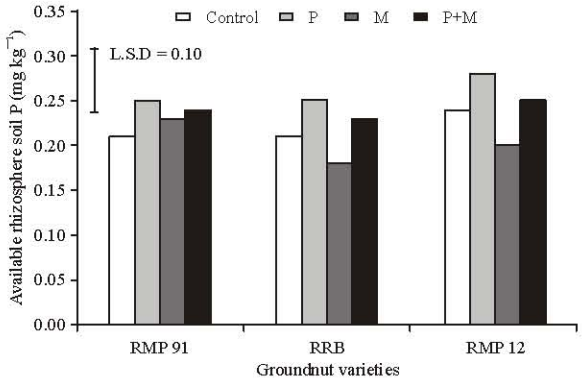

Fig. 3a: Effect of *Glomus mosseae* inoculation and phosphorus on available rhizosphere soil P in 2003 planting season

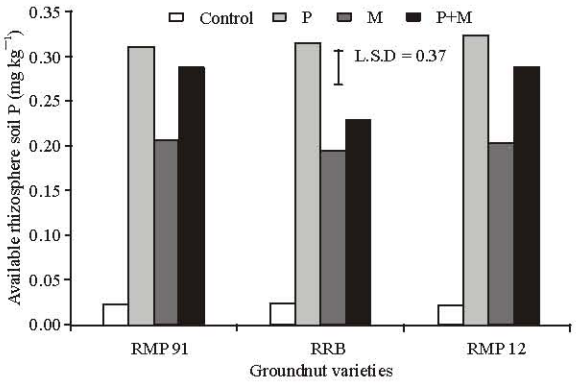

Fig. 3a: Effect of *Glomus mosseae* inoculation and phosphorus on available rhizosphere soil P in 2004 planting season

enhanced P uptake through the root as reported by Kothari *et al.* [26] and Li *et al.* [27] and may also have an inducing effect on other nutrients [28]. Similar increases in biomass production have been reported by Al-Karaki [29] and Al-Karaki *et al.* [30]. This study did not show any varietal difference all the parameters considered except the number of pods. The number of pods seemed higher in RRB but this did not translate to higher grain yield apparently due to lower shelling percentage recorded compared to the other two cultivars.

In this study, the grain yield in all the cultivars for the two seasons suggested equal effect of phosphorus fertilization at 54 kg ha^{-1} and inoculation with mycorrhizal and even compared to the plots with inoculation and phosphorus fertilization except in RMP_{12} where a marginal grain yield increase in plots fertilized and inoculated (P+M) was achieved. The increase may be linked with the phosphorus factor rather than the combined effects of phosphorus and inoculation as one may be tempted to belief. Earlier reports suggested suppression of infection and possible reduction in its effectiveness with increase availability of phosphorus in the soil [4, 31]. This claim is supported by the behavior of infections in the experiment where phosphorus fertilized plots had the lowest infection in all the groundnut cultivars in the first year and even much lower than the control in the second year. The inoculation of groundnut cultivars with the fungus contributed to its overall grain yield in the two planting seasons. The similarity in grain yield between all the treatments in RMP_{91} and RMP_{12} in the second year could be interpreted to mean that P fertilization at 54 kg ha^{-1} could produce the same effect on grain yield with inoculation. The enhanced yield in control plots in the second year could be attributed to the cross contamination of propagules from adjacent inoculated plots and resultant build-up of the fungal population and the subsequent increased root infection in the second year as observed by Bhoopander and Mukerji [24].

CONCLUSIONS

In this study, it is clear that groundnut can benefit from mycorrhizal symbiosis as a component of sustainable agriculture owing to its contribution to overall growth and grain yield. Also, for sustainable positive effect of inoculation, field inoculation may have to be annual until after the fungal population reaches a critical level. Inoculation of groundnut is not being canvassed as a substitute for phosphorus or any other soil nutrient but could allow the crop to put into use more of the soil nutrients per unit time. This will provide more time for replacement since nutrients are continuously removed during cropping and they need to be replaced. Further field studies in respect of other AM fungi for comparison, long term evaluation of field conditions as they affect the performance of mycorrhizal fungi and trials of mixture of inocula from different species of fungi deserve attention.

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