# INFLUENCE OF TWO GLOMUS SPECIES ON THE FERTILIZER EFFICIENCY OF SOKOTO PHOSPHATE ROCK IN SOYBEAN PRODUCTION AND THE RESIDUAL SOIL

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**ABSTRACT**
*A screenhouse experiment was carried out at the University of Agriculture, Abeokuta, to investigate the influence of two species of Glomus (G. mosseae and G. deserticola) on the fertilizer efficiency of Sokoto phosphate rock in promoting soybean production and the impact on the residual soil. The experiment was arranged in randomized block design with four replicates. The treatments consisted of sole inoculations of G. mosseae and G. deserticola and their combinations with phosphate rock (PR), sole application of PR, SSP and a no-inoculation, no-fertilizer control. Results obtained revealed that the percent colonization rate of arbuscular mycorrhiza fungi (AMF) was significantly higher in treatments with combinations of PR and Glomus than the sole AMF treatments and least in non mycorrhizal fungi treatments at 3 and 9 Weeks after sowing (WAS). Also, sole inoculation of G. deserticola caused significantly higher P uptake than treatments with combination of PR and Glomus as well as SSP application and least in control, N uptake was however similar in all the treatments. Consequently, growth parameters such as, plant height, numbers of leaves and branches, canopy diameter as well as leaf area significantly increased between 2-6 WAS due to inoculation of G. mosseae or G. deserticola and their combinations with PR. Furthermore, shoot biomass significantly increased at 3, 6 and 9 WAS with G mosseae and PR combination similarly, root biomass significantly increased at 3 WAS due to application of PR and at 6 and 9 WAS, combination of G. deserticola and PR increased root biomass. Nodule weight increased significantly with combinations of PR with G. deserticola at 6, 8 and 9 WAS and PR with G. mosseae at 9 WAS while nodule number increased significantly with combination of PR and the two species of Glomus at 6, 8 and 9 WAS. Sole inoculation of G. deserticola and SSP caused the plants to flower significantly earlier than other treatments and yield was significantly higher in sole G. mosseae and PR treatments than in SSP and control. At the end of soybean cropping, treatments with PR/Glomus combination and sole G. mosseae produced significantly higher N in the residual soil while sole application of PR produced the least. Application of SSP gave the highest P, while PR as well as control gave the least and soil organic matter was highest with sole inoculation of G. deserticola and least in PR application.*

**KEYWORDS:** Phosphate Rock, *Glomus mosseae*, *Glomus deserticola*, soybean, Residual soil

## INTRODUCTION

Soil fertility sustenance is a major constraint to crop production in the sub-humid zone of Nigeria. This is because the soil has low contents of many of the major nutrients required by crops and due to inability of the resource-poor farmers to procure the needed fertilizers, yields remain low and at subsistence level. Phosphate rock deposits occur at various parts of Nigeria (Nehikhare, 1987, Adegoke, 1991). These PR were however reported to have low reactivity (Mokwunye and Batiano, 2000) although the $P_2O_5$ contents range between 31.5-36.3 %. The single factor hindering the fertilizer efficiencies of these PR is their low reactivity thereby resulting in low water solubility.

Several chemical, physical and biological methods have been employed in enhancing the solubility of these materials (Chien and Black, 1975, Chien and Hammond, 1978, Mba, 1997, Nyirongo *et al*, 1999). The ability of mycorrhizal fungi to solubilize phosphate rock has been severally reported (Akintokun et al 2007).

This is attributed to ability of fungal hyphea to permeate the soil and reduce fixation of dissolved P, produce hormones and facilitate their interactions, shorten the route taken by the nutrients and tightly close the circuit. It was therefore concluded that mycorrhizal fungi are important components in the cycling of nutrients especially in humid ecosystems (Mosse, 1981, Majunath *et al*, 1989, Torelli *et al*, 2000). The AM fungi contribute to P capture and supply by linking the biotic and geochemical portions of the soil ecosystem, therefore affecting P cycling rates and patterns in both agriculture and natural ecosystems (Jeffries and Barea, 2001). These reports were further highlighted in greenhouse and field studies with isotopically labelled PR, it was observed that AM mycelium tapped phosphate released by phosphate solubilizing organisms and transferred it to the plants (Barea *et al* 2002).

The present study was designed to investigate the influence of two species of Glomus on the fertilizer efficiency of Sokoto phosphate rock and the residual soil.

## MATERIALS AND METHODS

The experiment which was conducted in the screenhouse of University of Agriculture, Abeokuta ($7^0$ 15′ N, $3^0$ 25′ E) had the pots arranged in randomized complete block design (RCBD) with 4 replicates. The seven treatments consisted of *G. mossea*, *G. deserticola*, Sokoto phosphate rock, *G. mossea* /PR combination, *G. deserticola/PR* combination, single super phosphate (SSP) and a no inoculation or fertilizer control.

### Culturing of Mycorrhizal Spores

The spore inoculants of *G. mosseae* and *G. deserticola* containing about 70 and 60 spores per 100 g soil respectively were inoculated into potted sterile soil and planted to maize. Initially the maize seedlings were watered daily and allowed to grow for 3 months; the inflorescences were removed just before tasselling. Watering was stopped 10 days before the termination of culturing, thereafter maize roots were cut into tiny pieces (0.05cm), mixed with the potted soil and this was used as the mycorrhizal spore inoculant (Khalil, *et al*, 1994). At the end of culturing the spore densities were 60 and 40 spores per 100 g soil for *G. mosseae* and *G. deserticola* respectively.

### Pot Experiments

The soil used was loamy sand with pH 5.9, organic carbon 0.35 %, available P 9.0 mg/kg total N 0.2 %, CEC 1.9 and spore count 34 spores/100 g soil. Ten kilogram of soil was weighed into plastic pots, PR was applied at the recommended rate of 40 $kg^{-1}$ for soybean and 1.5 kg to 50g soil inoculant of *G. mossea* or *G. deserticola* were applied into the potted soils

### Measurements

The data which were collected on soybean weekly from the second week after sowing (WAS) included plant height, number of leaves, number of branches, leaf area and canopy spread. The other crop data were percent mycorrhizal root infection rate, N and P uptake, shoot and root biomass, nodule number and count at 3, 6, 8 and 9 WAS. Grain yield and soil N, P, organic matter and spore count were determined at harvest.

### Determination of mycorrhizal root infection rate

Fine root samples of soybean were collected and stored in vials at 4 $^0$C for 72 hours. The root samples were washed thereafter with tap water and cut into 10-20 mm length. About 0.25 g of fresh and fine root sample was taken and cleaned in 10 % KOH in a water bath. The sample was rinsed in water and stained with 0.05 % tryphan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) at room temperature (Phillips and Hayman, 1970). Mycorrhizal infection was quantified using the magnified intersection method described by Mcgonigle *et al*, (1990). The infection was expressed as percentage root infected.

**Determination of soil spore count**

Arbuscular mycorrhizal fungi spore count was determined from soil sub sample whose moisture content was determined at 90 $^{0}$C and passed through 2 mm sieve. One kilogram of the soil sample was vigorously washed and mixed with a substantial volume of water, the heavy soil particles were allowed to settle for a few seconds, and decanted through series of sieves with different diameters. The spores captured on the finest sieve was centrifuged at 120 rpm, thereafter the supernatant was centrifuged in sucrose at 200 rpm and poured into the finest sieve and carefully washed into a petridish. The spores were counted with the aid of dissecting microscope (100 x magnifications) using forceps for selection and emositometer for counting (Mcgonigle *et al*., 1990).

**Plant and soil analysis**

The plants were sampled at flowering (6 WAP), oven-dried at 80$^{0C}$ for 24 hrs, grinded and digested in triple acid of perchloric acid, sulphoric acid and nitric acid (2:1:1) as described by Juo *et al*., (1974). The P in solution was determined in ascorbic acid (Murphy and Riley, 1962) modified by Anderson and Ingram, (1998) while N was measured by Micro-Kjeldhal method (Bremner, 1996). At the end of the experiment, surface soil sample (0-10 cm) was taken from each pot, air-dried, passed through a 2 mm sieve and analysed for organic carbon, N and P. The organic carbon was determined using the dichromate titration method (Nelson and Summers, 1996), N was measured by the Micro-Kjeldhal method (Bremner, 1996) and P was extracted by the Bray 1 extractant (Bray and Kutz, 1945), the P in solution was determined by the vanado-molybdate method.

**Data analysis**

Data were subjected to analysis of variance using the SAS/GLM procedure (SAS, 1989) and the means were separated using least significant difference (LSD) at 5 % probability.

**RESULTS**

Table 1 shows that the AMF colonization rate of soybean roots was significantly higher in treatments with sole AMF inoculation than treatments without AMF at 3 and 9 WAS. However, treatments with AMF/PR combination caused significantly higher infection than those with sole AMF at the 2 sampling periods. Although N uptake was not significantly affected by treatment, inoculation of *G. deserticola* gave the highest numerical value. Uptake of P on the other hand was significantly affected by treatment. *G. deserticola* caused the highest uptake followed by treatments with AMF/PR combination and SSP, then sole *G. mossea*e and PR, while the least P uptake was recorded in control (Table 1).

**Table 1: Effects of AMF and PR on percent mycorrhizal infection rates of soybean at 3 and 9 WAS and N and P uptakes at 6 WAS.**

| Treatments | % infection rates | | Uptakes (mg/kg) | |
|---|---|---|---|---|
| | 3 WAS | 9 WAS | N | P |
| G. mosseae | 37.3 | 61.0 | 0.26 | 2.72 |
| G. deserticola | 37.3 | 61.3 | 0.31 | 3.85 |
| PR | 24.3 | 36.7 | 0.24 | 2.36 |
| Mosseae/PR | 46.0 | 72.3 | 0.23 | 3.35 |
| Deserticola/PR | 47.3 | 74.3 | 0.26 | 3.40 |
| SSP | 26.3 | 39.7 | 0.25 | 3.35 |
| Control | 22.0 | 31.7 | 0.21 | 0.75 |
| LSD (0.05) | 7.23 | 7.16 | 0.15 | 0.13 |

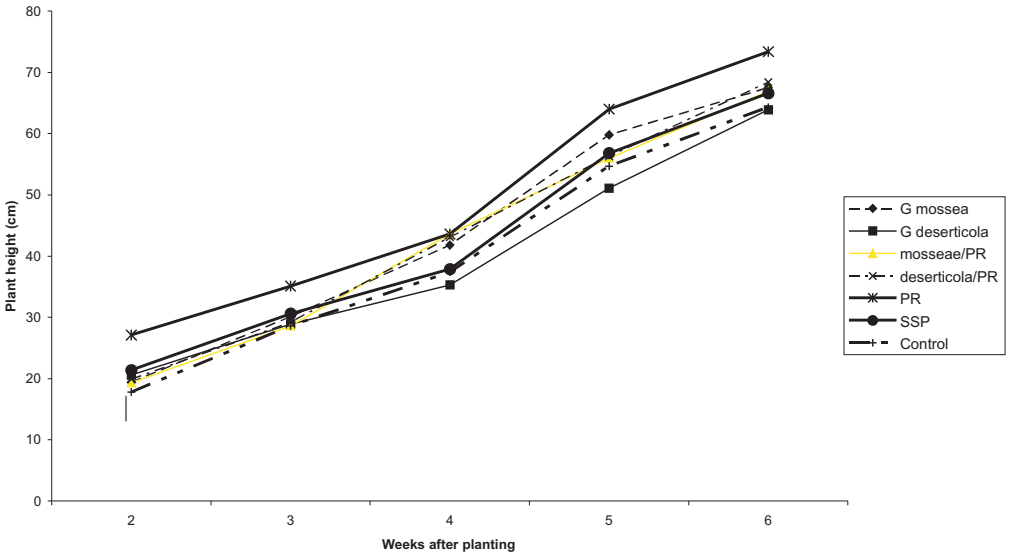


**Figure 1: Effect of AMF and PR on soybean plant height at 2-6 WAS**

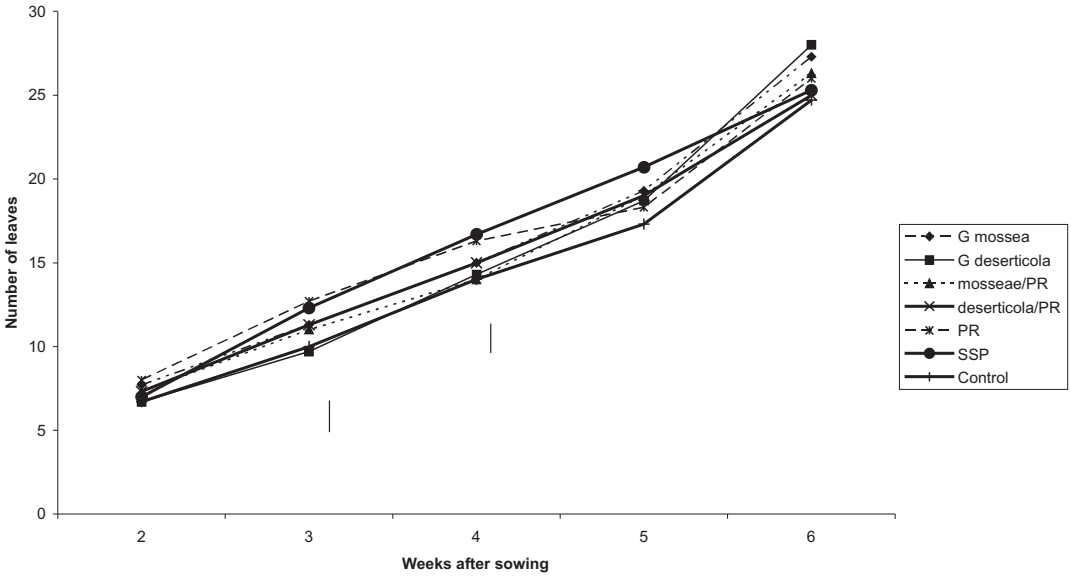


**Figure 2: Effects of AMF and PR on number of leaves of soybean at 2-6 WAS**

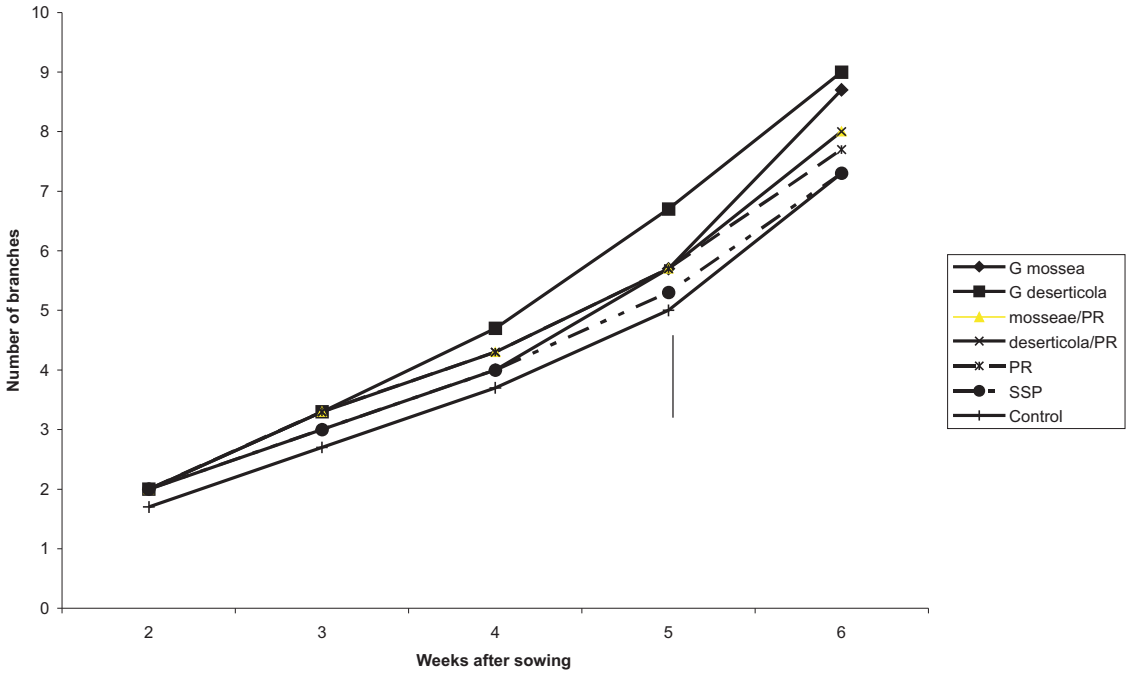


**Figure 3: Effects of AMF and PR on number of branches in soybean at 2-6 WAS**

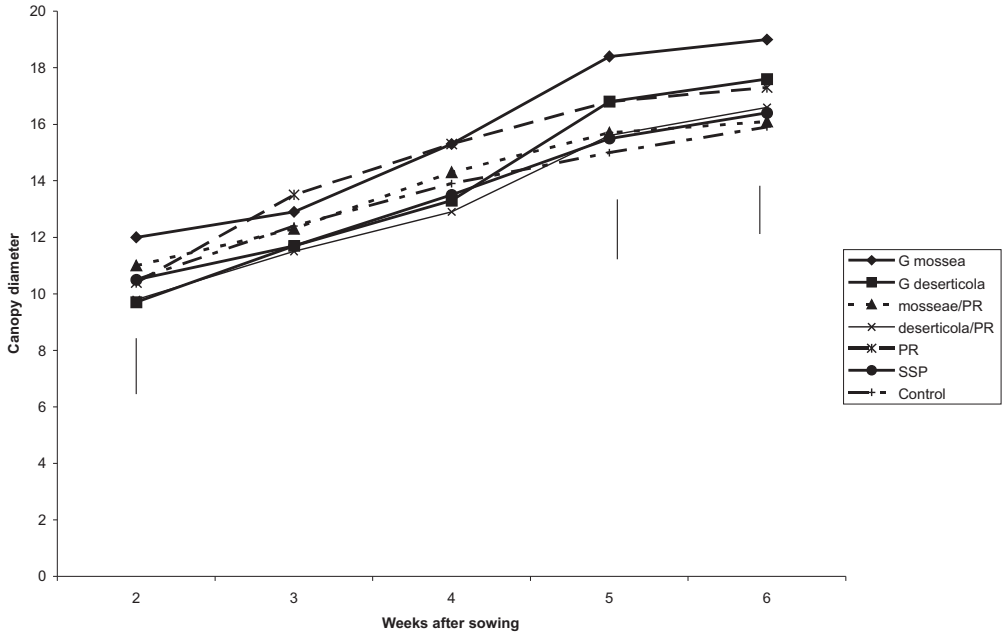


**Figure 4: Effects of AMF and PR on canopy diameter of soybean at 2-6 WAS**

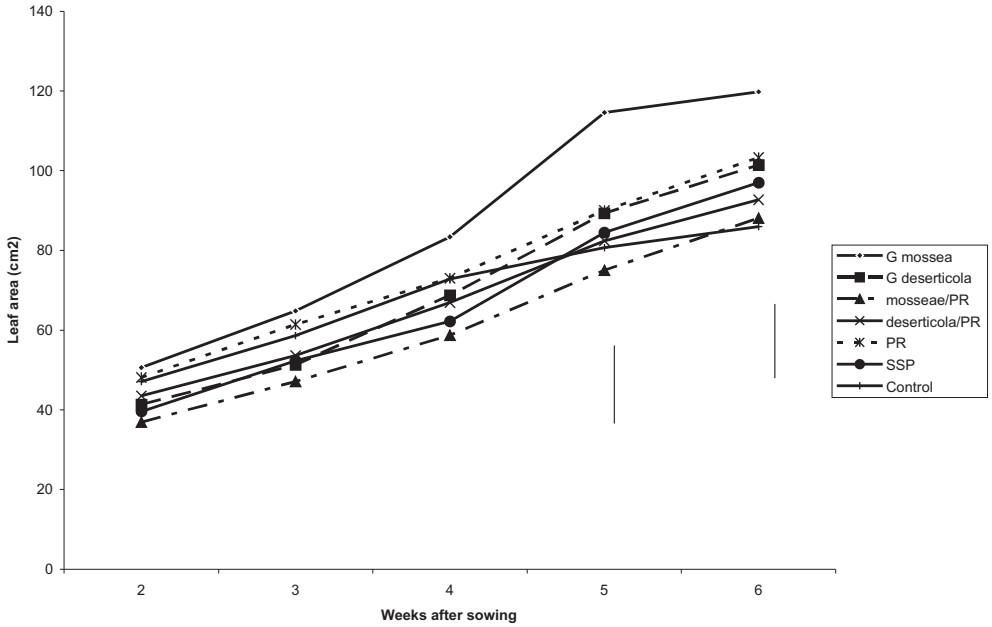


**Figure 5: Effects of AMF and PR on leaf area of soybean at 2-6 WAS**

The trend in growth and development of soybean is significantly affected by AMF and PR amendment at 2-6 WAS as shown in figures 1-5. Soil amendment with PR gave higher plants at 2 WAS and number of leaves was more in PR and SSP amended soils than in sole *G. deserticola* inoculated soil and control at 3 & 4 WAS as well as *G. mosseae*/PR combination at 4 WAS. While number of branches was higher in *G. deserticola* treated soil than in control at 5 WAS. Further more, canopy diameter was higher in *G. mosseae* treated soil than in control at 2 WAS as well as other treatments with the exception of PR and *G. mosseae* treated soils at 5 & 6 WAS. In addition, Leaf area was higher in *G. mosseae* treated soil than other treatments at 5 WAS but comparable to PR at 6 WAS.

Biomass production in soybean was significantly affected by the treatments as shown in Table 2. *G mosseae*/PR combination gave the highest shoot biomass at 3 & 6 WAS and was comparable to the maximum given by *G. deserticola* at 9 WAS and combination of *G. deserticola* and PR gave significantly higher shoot biomass than sole AMF and non-mycorrhizal fungi treatments at 3 WAS with the exception of sole *G deserticola* at 6 WAS and was comparable to the maximum at 9 WAS. Moreover, the root biomass was also significantly affected by treatments at 3, 6 and 9 WAS. At 3 WAS amendment with PR gave the maximum and higher value than sole *G. deserticola* although comparable to the other treatments. While at 6 WAS, *G. deserticola*/PR combination gave the maximum value and higher than treatments with sole *G. deserticola* and PR but comparable to other treatments and at 9 WAS it was comparable to treatments with sole *G. mosseae* and *G. deserticola*/PR combination but higher than all the others.

**Table 2: Effects of AMF and PR on soybean shoot and root biomass at 3, 6 and 9 WAS.**

| Treatments | Shoot biomass (g/plant) | | | Root biomass (g/plant) | | |
|---|---|---|---|---|---|---|
| | 3 WAS | 6 WAS | 9 WAS | 3 WAS | 6 WAS | 9 WAS |
| G mosseae | 0.23 | 2.4 | 4.3 | 0.07 | 0.31 | 0.63 |
| G deserticola | 0.43 | 2.8 | 4.6 | 0.07 | 0.23 | 0.43 |
| PR | 0.30 | 2.2 | 4.2 | 0.10 | 0.21 | 0.33 |
| Mosseae/PR | 0.70 | 3.4 | 4.0 | 0.07 | 0.26 | 0.58 |
| Deserticola/PR | 0.58 | 2.9 | 3.6 | 0.04 | 0.40 | 0.67 |
| SSP | 0.43 | 2.34 | 2.2 | 0.08 | 0.26 | 0.34 |
| Control | 0.39 | 2.6 | 2.2 | 0.06 | 0.29 | 0.40 |
| LSD (0.05) | 0.07 | 0.15 | 2.11 | 0.051 | 0.162 | 0.213 |

Soybean nodulation was significantly affected mainly by AMF/PR combination as shown in Table 3. Number of nodules was higher than in other treatments at 6 WAS and comparable to sole *G. deserticola* at 8 WAS but lowest in treatment with sole *G. mosseae* and PR. While at 9 WAS maximum value was obtained with *G. mosseae*/PR combination but comparable to other mycorrhizal fungi treatments and the least values were recorded in the non-mycorrhizal fungi treatments. The weights of nodules were also higher with *G deserticola*/PR combination than in other treatments at 6 & 8 WAS with the exception of *G. mosseae*/PR at 8 WAS. Although not significant, *G. deserticola* gave the highest value at 9 WAS.

**Table 3: Effects of AMF and PR on soybean nodulation at 6, 8 and 9 WAS**

| Treatments | Number of nodules | | | Weights of nodules | | |
|---|---|---|---|---|---|---|
| | 6 WAS | 8 WAS | 9 WAS | 6 WAS | 8 WAS | 9 WAS |
| G mosseae | 1.3 | 5.0 | 8.0 | 0.03 | 0.08 | 0.06 |
| G deserticola | 3.7 | 8.0 | 8.3 | 0.05 | 0.1 | 0.07 |
| PR | 3.3 | 2.0 | 1.3 | 0.04 | 0.01 | 0.02 |
| Mosseae/PR | 9.3 | 10.3 | 13.3 | 0.12 | 0.08 | 0.13 |
| Deserticola/PR | 7.7 | 10.3 | 12.0 | 0.17 | 0.19 | 0.13 |
| SSP | 4.3 | 4.7 | 4.7 | 0.05 | 0.05 | 0.05 |
| Control | 3.7 | 6.0 | 3.0 | 0.03 | 0.08 | 0.03 |
| LSD (0.05) | 2.017 | 3.68 | 8.42 | 0.103 | 0.06 | 0.107 |

The yield parameters of soybean were significantly affected by treatments as shown in Table 4. The number of days to flowering was reduced with *G deserticola*, SSP and *G mosseae*/PR combination. The yield was highest in treatments with sole *G mosseae* and PR than in SSP and control but comparable to all the mycorrhizal fungi treatments and the weight of 100 seeds was highest in treatment with PR than in other treatments with the exception of sole *G mosseae* and lowest in SSP. Furthermore, threshing percent was higher in sole *G mosseae* treatment than in SSP but comparable to other treatments.

**Table 4: Effects of AMF and PR on number of days to 1st flowering, yield and treshing percent of soybean**

| Treatment | Days to 1st flowering | Yield (g/plant) | Wt of 100 seeds (g/pot) | Treshing % |
|---|---|---|---|---|
| G mosseae | 38.7 | 14.6 | 12.8 | 62.1 |
| G deserticola | 38.0 | 12.9 | 10.9 | 64.3 |
| PR | 40.3 | 14.7 | 14.1 | 62.3 |
| Mosseae/PR | 39.3 | 12.7 | 11.5 | 59.8 |
| Deserticola/PR | 38.3 | 13.7 | 11.9 | 59.3 |
| SSP | 38.0 | 10.6 | 10.1 | 57.8 |
| Control | 39.3 | 11.7 | 11.1 | 60.9 |
| LSD (0.05) | 1.81 | 2.77 | 1.68 | 5.71 |

Some of the parameters in the residual soil were significantly affected by treatment as shown in Table 5. Soil organic matter was highest in soil treated with sole *G. deserticola* and lowest in PR treated soil and control and similar in soils inoculated with AMF and SSP applied soil. Nitrogen was highest in soils treated with *G. mosseae* with and without PR as well as in *G. deserticola*/PR treated soil and lowest in control. Phosphorus was highest in soil treated with SSP followed by soils with AMF inoculation and lowest in soil treated with PR and control. At 3 WAS and harvest, mycorrhizal spore count was higher in soils treated with AMF/PR combination than in soils treated with sole mycorrhizal fungi except in *G. deserticola* at 9 WAS and lowest in non-AMF treated soils. Spore count was however comparable in sole mycorrhizal fungi soil to PR treated soil at both sampling periods.

**Table 5: Effects of AMF and PR on soil residual N, P, organic matter and Mycorrhiza spores**

| Treatments | N (%) | P (mg/kg) | SOM (%) | Mycorrhiza spore/100 g soil. | |
|---|---|---|---|---|---|
| | | | | 3 WAS | 9 WAS |
| G mosseae | 0.82 | 8.6 | 0.42 | 38.3 | 61.0 |
| G deserticola | 0.2 | 8.8 | 0.59 | 39.3 | 70.3 |
| PR | 0.09 | 7.3 | 0.18 | 35.7 | 52.0 |
| Mosseae/PR | 0.99 | 8.3 | 0.46 | 52.0 | 83.7 |
| Deserticola/PR | 0.88 | 8.4 | 0.53 | 49.0 | 85.0 |
| SSP | 0.36 | 11.7 | 0.50 | 31.0 | 33.7 |
| Control | 0.19 | 6.9 | 0.30 | 31.7 | 44.7 |
| LSD (0.05) | 0.252 | 0.838 | 0.133 | 6.369 | 19.408 |

**DISCUSSION**

At the seedling and later stages of soybean growth AMF/PR combinations caused higher colonization than sole AMF and PR treatments. The increase over sole AMF inoculation ranged from 23 27 % at 3 WAS and 18 21 % at 9 WAS, whereas increase over sole PR was 93 and 103 % for *G. deserticola*/PR but 89 and 97 % for *G. mosseae* at 3 and 9 WAS respectively. Further more, sole inoculation of mycorrhiza gave more colonization than non-mycorrhiza treatments. The maximum uptake of N and P were recorded in sole *G. deserticola* treatment and the least were recorded in control, although the difference in N uptake was not significantly different. Mycorrhiza inoculation added some nutritional benefits to soybean and AMF/PR combination gave

similar N and P uptakes as in SSP while sole *G. desertcola* inoculation gave higher P uptake than SSP and other treatments. Despite the inability of sole application of PR to cause maximum N and P uptakes, it was able to increase plant height significantly at 2 WAS and subsequently although not significantly at 3-6 WAS. Furthermore, sole PR and SSP increased leaf production than treatments with sole inoculation of *G. deserticola* and control at 3 WAS and SSP produced more leaves than most treatments at 4 WAS, while sole PR treatment produced more leaves than sole inoculation of *G deserticola*, and *G. mosseae*/PR combination and control at 4 WAS. Moreover, sole inoculation of *G. mosseae* produced maximum number of branches throughout the sampling period and significantly more branches than control at 5 WAP. Sole inoculation of *G. mosseae* produced more branches than the *G. deserticola* treatments at 2 WAS and other treatments with the exception of Sole *G. deserticola* and PR treatments at 5 and 6 WAS. Furthermore, *G. mosseae* produced higher leaf area at 5 & 6 WAS than other treatments with the exception of sole PR at 6 WAS.

*Glomus mosseae*/PR treatments gave higher shoot biomass and the root biomass produced was consistently comparable to the maximum and shoot biomass produced at 3 & 6 WAS were higher than sole mycorrhiza treatments as well as non-AMF treatments. Maximum shoot biomass was produced by sole *G. deserticola* at 9 WAS and was comparable to sole PR application as well as other AMF inoculations. Maximum root biomass was produced by PR at 3 WAS, but *G. deserticola*/PR treatment gave maximum root biomass at 6 & 9 WAS. Moreover, nodulation consistently increased with AMF/PR treatments at all sampling periods although comparable to sole *G. deserticola*/PR treatment at 8 & 9 WAS and *G. mosseae* treatment at 9 WAS. The duration to flowering was reduced by SSP and all the *G. deserticola* treatments. Maximum yield was produced by sole *G. mosseae* and sole PR treatments and this was comparable to other mycorrhizal treatments but higher than SSP and control. This is consistent with the earlier observation whereby treatments with sole PR and *G. mosseae* increased soybean growth and development. Moreover, the weights of seeds were comparable in these two treatments and among most of the AMF treatments and lowest in SSP. The threshing percent was higher in sole PR treatment than in SSP but comparable to other treatments.

The maximum soil organic matter was produced by sole *G. deserticola* and this was comparable to AMF/PR as well as SSP treatments but higher than other treatments. Treatments with AMF/PR combinations as well as sole *G. mosseae* gave higher soil N than other treatments probably because of synergistic association between AMF and *Rhizobium* (Barea et al 2005) leading to higher nitrogen fixation these treatments, causing higher soil N. Soil treated with SSP had more soil P than the AMF treatments and lowest in sole PR treated soil and control because SSP contain 18% $P_2O_5$ in soluble form thereby leaving a higher level in soil than the other treatments. Inoculation of AMF seems to add P to soil although not as much as SSP treatment but applications of PR either sole or in combination with AMF seemed not to have any benefit on the P content of soil this is because although the phyto-availability of PR might have been improved by AMF, P was not released into the soil. In contrast, AMF inoculation added spore to the soil, however more spores were produced when PR was combined with AMF at all the sampling periods.

## CONCLUSION

The inoculation of plants with AMF caused more colonization by mycorrhizal fungi than non-AMF treatments but the colonization was more in the presence of PR. However, sole inoculation with *G mosseae* caused more P uptake thereby leading to higher growth and development of soybean with this treatment. Biomass production was higher with *G mosseae*/PR treatment and nodulation was more with combination of mycorrhizal fungi and PR. The duration to flowering was reduced with AMF inoculation and SSP application but yield was higher with sole applications of *G mosseae* and PR. The contents of soil organic matter, N, P and mycorrhizae spores increased with AMF inoculations but not with sole PR application. Moreover maximum P content was produced in soil treated with SSP.

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