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# Field evaluation of arbuscular mycorrhizal fungi (AMF) for microbial activities and yield of maize under alluvial soil

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**Abstract:** A field experiment was conducted to evaluate the response of AMF species with different phosphorus (P) levels for root colonization, microbial population under maize in an alluvial soil. Of all the species of mycorrhizae taken under consideration, *G. mosseae* along with 75% RDF of P was found to perform better in terms of root colonization, number of spores and grain yield. Application of *G. mosseae* @ 10 kg $ha^{-1}$ + 50% P + 100% NK produced significantly higher root colonization by 177.32, 55.20, 37.75 and 101.95 per cent over the treatments 100% RDF, *G. mosseae* @ 10 kg $ha^{-1}$ + 75% P + 100% NK, *G. coronatum* @ 10 kg $ha^{-1}$ + 75% P + 100% NK, *G. decipien* @ 10 kg $ha^{-1}$ + 75% P + 100% NK and control, respectively. The similar trend was observed for number of spore count. The maximum number of bacteria ($40\times10^{-5}$ cfu $g^{-1}$ soil) was found with the inoculation of *G. mosseae* @ 10 kg $ha^{-1}$ + 75% P + 100% NK at flowering stage. The maximum grain yield (7656.61 kg $ha^{-1}$) was recorded with the application of *G. mosseae* @ 10 kg $ha^{-1}$ + 75% P + 100% NK, which was 111.92 per cent significantly higher the control treatment. *G. mosseae* along with 75% RDF of phosphorus inoculation proved to be effective in modifying the soil microbe population and community structure and also in enhancing the grain yield.

**Keywords:** AMF, Grain yield, Maize, Microbial population, Root colonization, Spore

## INTRODUCTION

Mycorrhiza is a mutualistic symbiosis between certain groups of soil fungi and most plant root systems (Hata *et al*., 2010). The most publicized benefit of mycorrhiza is the improved growth rate which is mainly due to enhanced phosphorus (P) nutrition. Various mechanisms (e.g. exploration of large soil volume, faster movement of mycorrhizal hyphae and solublization of soil phosphorus) are responsible for increasing the uptake of phosphorus by mycorrhizal plants. Non-nutritional benefits to plants, such as changes in water relations, phytohormone levels, carbon assimilation, secretion of enzymes, increased microbial count in soil, etc. have also been reported, but they are difficult to interpret (Andrade *et al*., 1998).

Phosphorus is one of the major essential macronutrients which limit plant growth owing to its low bioavailability in soils (Feng *et al.,* 2004). Improving plant acquisition of P from soil is an obvious alternative for the management of those low P soils (Zhu *et al.,* 2003). It is commonly known that arbuscular mycorrhizae (AM), they act as a direct link between soil and roots, AM fungi help plants to capture water and nutrients (especially P) from the soil, and in return, the plant provides the fungus with relatively constant and direct access to carbohydrates (Smith and Read, 2008), which are translocated from their source to root tissue and on to fungal partners.

Mar Vazquez *et al.* (2000) reported mycorrhizal colonization induced qualitative changes in the microbial population and enzyme activities in the rhizosphere of maize plants. On the other hand, soil phosphatase and urease are closely related to the P and N nutrition of plants. Thus, the enhancement of soil enzyme activities is one of the physiological and biochemical mechanisms involved in a mycorrhization effect on plant mineral nutrition. Rao and Tak (2001) found that mycorrhizal fungal inoculation resulted in enhanced plant growth, total uptake of N, P and many other nutrients, activities of dehydrogenase, phosphatase and nitrogenase in the rhizosphere in gypsum mine spoil. Owing to the energy and cost-intensive manufacture of chemical fertilizers, use of microbial inoculants to supplement a part of phosphorus requirement has attained immense importance (Bagyaraj *et al.,* 2015). To get maximum agricultural benefit, inoculation of the soil with suitable type of AM fungi is necessary. Mycorrhizal colonization modifies the microbial community structure and ecology and it is established that variations in the parameters related to microbes are very dynamic and fluctuates across the various phenological stages in crops (Rakshit *et al.,* 2016). In view of the above-mentioned possibilities, a field experiment was conducted with AMF species along with various doses of

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phosphorus with respect to their effect on the microbial population, root colonization at flowering and harvesting stage of maize crop and its impact on yield. An understanding of these effect as part of ecosystem processes is essential for obtaining the maximum benefit for plant yield in the context of soil-plant system sustainability.

## MATERIALS AND METHODS

The present study was undertaken to evaluate the response of AM fungi for maize crop under field condition during the *Rabi* season of 2014-15 with a promising var. DHM-117, at the research farm of Bihar Agricultural University, Sabour, Bhagalpur, India. Inoculums of the three AM species viz., *Glomus mosseae*, *Glomus coronatum* and *Gigaspora decipiens* were commercial products of The Energy Resource Institute (TERI), New Delhi, India. The products consisted of fragments of colonized roots and spores of AM fungi in a vermiculite substrate. The spores of mycorrhiza were multiply with sudan grass in net house. The substrate used for the multiplication consisted of soil from the Bihar Agricultural University, research farm and river bed sand of the Ganges (w/w, 3:1). The soil was collected from the surface (0–15 cm) and passed through a 2.00 mm aperture sieve to remove roots and debris. The river bed sand was thoroughly washed with tap water to remove salt. The substrate mixture was completely sterilized by autoclaving over 1 hr with stepwise increase in temperature till the centre reached 120$^{o}$C (kept for 30 minutes). The substrate used for the pot experiment was loamy sand in texture, having a pH of 7.2 and EC of 0.22 dS m$^{-1}$. The organic carbon content of the substrate was 0.56%, and the available nitrogen, phosphorus and potassium content was found 180.77, 25.89 and 220.66 kg ha$^{-1}$, respectively.

Seeds were surface-sterilized by treatment with a 1:1 mixture of $H_2O_2$ and absolute ethanol for 2 minutes followed by a treatment with 0.05% $HgCl_2$ for 1 minute. The sterilizing agents were drained aseptically, and the seeds were washed for 10-12 times in sterile distilled water to remove all traces of the chemicals. Earthen pots of 15 cm height and 30 cm diameter were filled with 10 kg of sterilized substrate. All AM species were maintained in the pots with five replications each. After six month the inoculumn containg spores, hyphae and root segments were harvested and after processing packed in poly begs. Seal packed begs sotored at 4$^{o}$C in store room. The The experimental soil was well drained Acquic Hapludoll silty clay loam having pH 7.2, organic carbon 0.77 per cent, available phosphorus 16.80 kg ha$^{-1}$ , available potassium 220.00 kg ha$^{-1}$ and nitrogen 238.00 kg ha$^{-1}$. The experiment included eight treatments, viz. $T_1$- RDF (120:40:60), $T_2$- *G. mosseae* + 75% P + 100% NK, $T_3$- *G. coronatum* + 75% P + 100% NK, $T_4$- *G. decipiens* + 75% P + 100% NK, $T_5$- *G. mosseae* + 50% P + 100% NK, $T_6$- *G. coronatum* + 50% P + 100% NK, $T_7$- *G. decipiens* + 50% P + 100% NK and $T_8$- Control (without AMF and fertilizers). About 10 kg ha$^{-1}$ of the AM inoculum source (containing and 10-12 lakh IP) were applied below the 2.0 cm of seed (R×R=60cm and P×P=25 cm). The fertilizer has been apply as per treatments and full dose of phosphorous and potassium were applied at the time of sowing and half dose of nitrogen was applied at the knee height stage and remaining half dose of nitrogen was applied at the time of silking stage. The irrigation has applied as per requirement of the crop.

Samples of roots of the plants with adhering soil were collected at flowering and harvesting stage. They were washed repeatedly with sterilized distilled water and fragmented into small segments of 1 cm. The root segments were cleared in 10% KOH, washed with water than 1% HCL and stained with 0.05% Trypane blue by the method given by Phillips and Hayman (1970). The stained bits were examined and the arbuscular mycorrhizal colonization in the roots was recorded in terms of per cent root segments showing mycorrhiza formation. The population of AM spores in the rhizospheric soil was estimated by extracting the spores from the root by the washing-sieving-decanting method of Gerdemann and Nicolson (1963). They were examined stereo microscopically and population was computed in terms of number per 25 g of dry soil. Rhizospheric soil samples were obtained by collecting the soil adhering to the roots. The 10 g of soil samples were placed in an Erlenmeyer flask containing 90 ml of sterilized distilled water, and shaken for 30 min. Ten-fold series dilutions were prepared, and appropriate dilutions were plated in specific media. For the isolation of bacteria, fungi and actinomycetes, the Plate Count Agar, Czapek-Dox Agar (Thom and Raper, 1945) and Kenknight and Munaier's Medium, respectively were used. The numbers of colony forming cells were determined in each plot by serial dilution pour plate method (Subba, 1986). The obtained field experiment data were analyzed by using standard procedure for Randomized Block Design (RBD) with the help of a computer applying analysis of variance (ANOVA) technique (Snedecor and Cochron, 1971). The differences among treatments were compared by applying "F" test of significance at 5 per cent of probability and P values was used to examine differences among treatment means.

## RESULTS AND DISCUSSION

**Root colonization and number of spores:** Table 1 shows the data pertaining to AM root colonization which depicts that the inoculation of different mycorrhizal fungi species significantly increased AM colonization in the root at flowering stage which were increased numerically at harvesting stage when compared with application of 100% RDF and control. The

**Table 1.** Effect of arbuscular mycorrhizal fungi on colonization and spore formation in maize rhizosphere at flowering stage and at harvesting stage. Mean values are from 24 observations (eight treatments and three replications).

| Treatment | AM infection (%) | | Number of spore ( 100 $g^{-1}$ of soil) | |
|---|---|---|---|---|
| | Flowering stage | Harvesting stage | Flowering stage | Harvesting stage |
| $T_1$ | 25.00 | 21.67 | 11.33 | 10.33 |
| $T_2$ | 44.67 | 30.00 | 27.00 | 15.00 |
| $T_3$ | 48.33 | 32.67 | 18.33 | 15.33 |
| $T_4$ | 50.33 | 36.33 | 23.67 | 14.33 |
| $T_5$ | 69.33 | 31.67 | 30.00 | 14.00 |
| $T_6$ | 67.00 | 34.67 | 14.33 | 13.33 |
| $T_7$ | 61.67 | 38.33 | 11.33 | 11.00 |
| $T_8$ | 34.33 | 26.67 | 8.33 | 7.33 |
| CD (0.05) | 12.37 | N/A | 11.67 | N/A |

**Note:** $T_1$- RDF (120:40:60), $T_2$- *G. mosseae* + 75% P + 100% NK, $T_3$- *G. coronatum* + 75% P +100% NK, $T_4$- *G. decipien* + 75% P + 100% NK, $T_5$- *G. mosseae* + 50% P + 100% NK, $T_6$- *G. coronatum* + 50% P + 100% NK, $T_7$- *G. decipien* + 50% P + 100% NK, $T_8$- Control (without AMF and fertilizers).

**Table 2.** Effect of AM fungi on soil microbial count under maize rhizosphere at flowering and harvesting Mean values are from 24 observations (eight treatments and three replications).

| | Flowering stage | | | Harvesting stage | | |
|---|---|---|---|---|---|---|
| Treatment | Bacteria (cfu×$10^{-5}$) | Fungi (cfu×$10^{-4}$) | Actinomycetes (cfu×$10^{-5}$) | Bacteria (cfu×$10^{-5}$) | Fungi (cfu×$10^{-4}$) | Actinomycetes (cfu×$10^{-5}$) |
| $T_1$ | 31.27 | 23.67 | 25.33 | 17.93 | 13.67 | 12.33 |
| $T_2$ | 40.00 | 30.00 | 31.67 | 28.33 | 11.00 | 18.00 |
| $T_3$ | 37.99 | 33.33 | 32.00 | 20.66 | 24.00 | 18.67 |
| $T_4$ | 38.00 | 31.33 | 38.32 | 27.00 | 28.55 | 22.00 |
| $T_5$ | 36.00 | 27.00 | 25.00 | 33.00 | 19.67 | 18.00 |
| $T_6$ | 37.67 | 24.00 | 26.67 | 36.67 | 13.67 | 18.67 |
| $T_7$ | 34.00 | 24.33 | 29.67 | 30.67 | 17.00 | 22.34 |
| $T_8$ | 28.33 | 23.00 | 20.33 | 14.33 | 8.67 | 11.00 |
| CD (0.05) | 7.24 | 4.43 | 4.35 | N/A | N/A | 5.62 |

**Note:** $T_1$- RDF (120:40:60), $T_2$- *G. mosseae* + 75% P + 100% NK, $T_3$- *G. coronatum* + 75% P +100% NK, $T_4$- *G. decipien* + 75% P + 100% NK, $T_5$- *G. mosseae* + 50% P + 100% NK, $T_6$- *G. coronatum* + 50% P + 100% NK, $T_7$- *G. decipien* + 50% P + 100% NK, $T_8$- Control (without AMF and fertilizers).

**Table 3.** Response of maize to AM fungi for yield and yield attributes. Mean values are from 24 observations (eight treatments and three replications).

| Treatment | Number of cob (plant$^{-1}$) | Biological yield (kg $ha^{-1}$) | Grain yield (kg $ha^{-1}$) | 100-seed weight (g) |
|---|---|---|---|---|
| $T_1$ | 0.93 | 22,589.95 | 7,595.24 | 26.50 |
| $T_2$ | 0.97 | 21,757.94 | 7,656.61 | 23.00 |
| $T_3$ | 0.96 | 20,953.70 | 7,414.79 | 23.50 |
| $T_4$ | 0.96 | 24,882.28 | 7,400.82 | 23.00 |
| $T_5$ | 0.95 | 20,527.78 | 7,174.60 | 25.07 |
| $T_6$ | 0.97 | 19,589.95 | 7,234.39 | 22.50 |
| $T_7$ | 0.94 | 22,444.44 | 7,141.54 | 22.00 |
| $T_8$ | 0.73 | 15,600.53 | 3,612.43 | 18.00 |
| CD (0.05) | 0.08 | N/A | 2,542.83 | NS |

**Note:** $T_1$- RDF (120:40:60), $T_2$- *G. mosseae* + 75% P + 100% NK, $T_3$- *G. coronatum* + 75% P +100% NK, $T_4$- *G. decipiens* + 75% P + 100% NK, $T_5$- *G. mosseae* + 50% P + 100% NK, $T_6$- *G. coronatum* + 50% P + 100% NK, $T_7$- *G. decipiens* + 50% P + 100% NK, $T_8$- Control (without AMF and fertilizers).

similar trend was observed with the spore count. Application of *G. mosseae* @ 10 kg $ha^{-1}$ + 50% P + 100% NK produced significantly higher root colonization by 177.32, 55.20, 37.75 and 101.95 per cent over the treatments 100% RDF, *G. mosseae* @ 10 kg $ha^{-1}$ + 75% P + 100% NK, *G. coronatum* @ 10 kg $ha^{-1}$ + 75% P + 100% NK, *G. decipien* @ 10 kg $ha^{-1}$ + 75% P + 100% NK and control, respectively. It was also at par with the result of *G. coronatum* @ 10 kg $ha^{-1}$ + 50% P + 100% NK, *G. decipien* @ 10 kg $ha^{-1}$ + 50% P + 100% NK. At harvesting stage the root colonization did not differ significantly among all the treatments. Likewise the spore count of AM fungi was also affected by application of different AMF species. The appli-

cation of *G. mosseae* @ 10 kg ha$^{-1}$ + 50% P + 100% NK produced significantly more spore count by 164.78, 63.66, 109.35, 164.78 and 260.14 per cent over the application of 100% RDF, *coronatum* @ 10 kg ha$^{-1}$ + 75% P + 100% NK, *G. coronatum* @ 10 kg ha$^{-1}$ + 50% P + 100% NK, *G. decipien* @ 10 kg ha$^{-1}$ + 50% P + 100% NK and control, respectively. The trend of spore count at harvesting stage was fond decrease when compared with flowering stage and was numerically increased with the application of AMF species over 100% RDF and control treatments.

Higher root colonization was significantly observed in maize after inoculation with *G. species*. Mycorrhizal fungi differ in their ability to infect and colonize roots. *Glomus* species has ability to infect and colonize plant roots faster than *Gigaspora* species, making it highly competitive (Kurle and Pfleger, 1994). The higher mycorrhizal colonization in maize could be due to strigolactones exuded by host plant roots and taken up by AMF since strigolactones stimulate fungal metabolism and branching (Parniske, 2008). Successful colonization and functional interaction between host plant and the mycobiont is based on the exchange of signaling molecules at different stages of symbiosis. The role of strigolactones as the key signaling compounds in the interaction between plants and soil-borne symbiotic AMF has been suggested recently (Soto *et al.,* 2010).

**Microbial population:** The data presented in the table 2 reveal that the microbial population was reduced at harvest when compared with flowering stage. The inoculation of *G. mosseae* @ 10 kg ha$^{-1}$ + 75% P + 100% NK significantly increased microbial population of bacteria, fungi and actinomycetes by 27.91, 41.19, 26.74, 30.43, 25.03 and 55.78 per cent than application of 100% RDF and control, respectively. All the applied AMF species significantly increased bacterial, fungal and Actinomycetes population when compared with control treatment. The maximum bacterial (c.f.u. 40 × 10$^{-5}$) population was found with the application of *G. mosseae* @ 10 kg ha$^{-1}$ + 75% P + 100% NK at flowering stage. At harvesting stage the increment in bacterial and fungal population was found numerical when compared with 1005 RDF and control treatments. All AMF fungi treatments gave produced significantly higher population of actinomycets at harvest in comparison to the application of 100% RDF and control treatment.

Root structure and functions change due to mycorrhizal infection. Possible AMF induced changes in root exudates and the rhizospheric microbial population, as well as possible physical barriers and chemical inhibitors from AM fungi may have practical implications in the biological control of some plant disease causing organisms. In addition to stimulating *Rhizobium*, VA mycorrhiza also influences rhizospheric bacteria beneficial to the plant. Some bacteria survive for a longer time under mycorrhizal infection than in non-mycorrhizal plants. It might be due to the AMF causing nutrient leakage from roots (quantitative changes in root exudates) or specific changes in the quality of root exudates. Similarly, after colonization on plant roots with AM fungi, the quantity of rhizospheric microbes significantly increased (John, 2011). The number of both rhizospheric bacteria and actinomycetes enhanced when plant formed mycorrhizae, while the dominant species composition also changed (Secilia and Bagyaraj, 1987). There may be two pathways for AM fungi to change microbe community structure, the first one is that the AM fungal hyphae secretion directly impacts microbe community structures; the another one is that both AM fungi in roots and on the roots alter plant physiological and biochemical processes, then directly or indirectly change the plant root secretion (Badri and Vivanco, 2009), thus alter those structures (Zhu *et al.*, 2005).

**Yield and yield attributes:** The yield and yield parameters viz. number of spore per plant, biological yield, grain yield and 100-seed weight have been increased by the application of various AMF along with chemical fertilizers over 100% RDF and control (Table 3). The maximum grain yield (7656.61 kg ha$^{-1}$) was recorded with the application of *G. mosseae* @ 10 kg ha$^{-1}$ + 75% P + 100% NK, which was 111.92 per cent significantly higher the control treatment. All the applied treatments significantly grain yield over control. The number of cob was also significantly increased with the application of AMF along with chemical fertilizers and maximum number of cob (0.97 plant$^{-1}$) were found with the application *G. mosseae* @ 10 kg ha$^{-1}$ + 75% P + 100% NK. The difference between biological yield and 100-seed weight was found non - significant among the applied treatments. The maximum biological yield (2488.28 kg ha$^{-1}$) was recorded with the application of *G. decipien* @ 10 kg ha$^{-1}$ + 75% P + 100% NK and maximum 100-seed weight (26.50 g) was found with the application of 100% RDF.

The yield and yield attributes increased significantly over control with the inoculation of AM species. Studies conducted by Sabia *et al.* (2015) also revealed a significant effect of AM inoculation on dry matter yield and quality of forage maize cultivated within a low input system. Singh *et al.,* 2015 also conducted a pot experiment for screening the AM fungi species for maize crop and found that with the application of *G. mosseae the* grain yield was found maximum (64.66 g plant$^{-1}$). This might be due to enhanced nutrient uptake by the roots. Since immobile ions in soil like phosphate lead to formation of a zone of phosphate depletion around roots in phosphate deficient soils mycorrhizal growth helps the roots to absorb phosphate ions much faster which are replenished at the root surface by diffusion. The AM hyphae attached to the roots extend beyond this depletion zone and promote nutrient translocation from the soil to the plants through the root cortex.

## Conclusion

The inoculation with AM fungus enhanced the population of soil bacteria, fungi and actinomycetes as compared to the application of 100% RDF and control. As evident from the results, the AM fungal inoculation can effectively modify the soil microbe population and community structure by increasing the soil enzymatic activities and with the application of *G. mosseae* along with 75% RDF of phosphorus, the 25 per cent phosphorus can be saved without any loss in the grain yield.

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