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Interactive Effects of AM Fungi, *Rhizobium* and Fly Ash Amendment on Nutrient Uptake (NPK), Yield and Proline Content of Lentil (*Lens culinaris* Medik.)

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ABSTRACT

The effects of microbial symbiosis (*Rhizobium* and AM fungi, namely *Funneliformis caledonius* and *Glomus bagyarajii*) on growth and contents of NPK and proline (*Lens culinaris* Medik.) in leaf tissues of lentil grown in autoclaved compost mixed garden soil (S) and 25% fly ash mixed composted garden soil were investigated. With or without inoculation of *Rhizobium* and AM Fungi (*Funneliformis caledonius* and *Glomus bagyarajii*). Application of 25% fly ash (FA) with garden soil (S) and inoculation with *Rhizobium* (R) and AMF *Funneliformis caledonius* (F) enhanced yield and NPK content in lentil leaves to a significantly high level than any other treatments. Overall yield response of Lentil (*Lens culinaris* Medik.) to various microbial inoculation and fly ash amendment may be arranged in the following increasing order as S < S+R < S+F < S+R+G < S+R+F < S+A < S+A+R S+A+G < S+A+F < S+A+R+G < S+A+R+F. The trend was recorded in the relative accumulation of phosphorus and potash content in the leaf. The nitrogen content in leaves was least in the plants grown in fly ash amended soil (S+A) without any microbial organism. The stress indicating proline content was highest in this treatment (S+A). application of *Funneliformis caledonius* (F) in this combination (S+A+F) reduced the plant stress as evident from relatively lesser proline accumulation.

***Key words*:** AMF, *Funneliformis caledonius*, *Glomus bagyarajii*, *Rhizobium*, Fly ash, Lentil

Inoculation of crop specific strain of *Rhizobium* significantly increase the production and yield of pulses [1-3]. *Rhizobium* on interaction with roots of host legume plant forms root nodules, where in *Rhizobium* transforms atmospheric nitrogen (N_2) to ammonia (NH_3), which is taken up by host plants in exchange of small amounts of carbon-containing molecules [4]. In addition to *Rhizobium*, pulses are also host plants for Arbuscular Mycorrhizal (AM) Fungi [5]. AM fungi facilitate adequate availability of water and nutrients in addition to resistance of host against stress [6-10]. AMF and *Rhizobium* are potentially key determinants of plant cohabitation and grassland productivity by maintaining adequate nutrient uptake in host from nutrient limited habitats [11]. Plants acquire synergistic benefits on association with a large number of symbionts as their growth performance predictable exceeds additive effects with each of individual symbionts. Many studies of legumes, arbuscular mycorrhizal fungi (AMF), and rhizobial interactions [12-15] have demonstrated that AMF and *Rhizobium* increase plant growth by increasing the availability of a limiting nutrients like phosphorus (P) and nitrogen (N).

Fly ash benefits soils by improving their physical, chemical, and biological characteristics of soil [16]. Fly ash amendment also boost lentil yield and NPK uptake in host plants [17]. The goal of the study was to find out the effect a 25% fly ash application in soil in combination with AMF (*Funneliformis caledonius* or *Glomus bagyarajii*) and *Rhizobium* inoculation on yield, nutrient uptake, and proline content in Lentil (*Lens culinaris* Medik.).

MATERIALS AND METHODS

The garden soil was collected from the agriculture farm areas of Aligarh Muslim University, air dried composted with farm yard manure (3:1 ratio) and divided into two portions. One part of the soil was amended with 25% fly ash (3:1 ratio) and other part of composted garden soil without fly ash amendment. The soil, either with or without fly ash, was autoclaved twice with 24 hours interval at 121°C for 20 minutes each time. The fly ash used in the experiment was directly collected from the ash pan of Kasimpur Thermal Power Plant, Aligarh (UP, India), air dried, and sieved (2 mm mesh size).

Eighteen pots filled each with sterilized autoclaves garden soil and fly ash amended soil. The pure culture of 2

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species of AM fungi (*F. caledonius* and *G. bagyarajii*) were procured from CNBRCD's Bangalore and multiplied. Seeds of lentil (*Lens culinaris* Medik.) were surface sterilized with 0.5% sodium hypochlorite (NaClO) for 15 minutes and washed with double distilled water. The lentil seeds were treated with jaggery and *Rhizobium* culture and dried in the shade. 50 gm multiplied inoculum of each AMF species was separately inoculate in small pit in the pots. All treatments had three replicates.

Treatments

i). Composted garden Soil (S)
ii). S + *Rhizobium* (S+R)
iii). S+ *Glomus bagyarajii* (S+G)
iv). S+ *Funneliformis caledonius* (S+F)
v). S+R+ G
vi). S+R+ F
vii). S with fly ash (S+A)
viii). S+A + *Rhizobium* (S+A+R)
ix). S+A+ *Glomus bagyarajii* (S+A+G)
x). S+FA+ *Funneliformis caledonius* (S+A+F)
xi). S+A+R+ G
xii). S+A+R+ F

All 12 treatments were (i) soil (S), (ii) soil+*Rhizobium* (S+R), (iii) soil+*Glomus bagyarajii* (S+G), (iv) S+*Funneliformis caledonius* (S+F), (v) S+R+ G, (vi) S+R+ F, (vii) S with fly ash (S+A), (viii) S+A+*Rhizobium* (S+A+R), (ix) S+A+*Glomus bagyarajii* (S+A+G), (x) S+FA+*Funneliformis caledonius* (S+A+F), (xi) S+A+R+ G, (xii) S+A+R+ F.

Each treatment was carried out three times. The plants were irrigated as when required. Nutrient uptake (NPK) and proline accumulation was recorded in 90 DAS and yield in 120 DAS old plant. The proline content was determined following the conventional method of Bates *et al*. [18]. Nitrogen content in dry leaves of plants was estimated following the protocols of Linder [19], phosphorus content following the protocols of Fiske and Subba Row [20], respectively, and the potassium content was determined by flame photometer that is the method of [21]. The statistical analysis and Duncan's multiple range test were carried out using SPSS software version 20 and a one-way ANOVA (SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

Effect of tripartite symbiosis on yield, nutrient uptake and proline content

The yield responses of lentil varied on inoculation with *Rhizobium*, AM fungi (*Funneliformis caledonius* and *Glomus bagyarajii*) when applied alone or in combination and with or without fly ash amended soil. The tripartite symbiosis between lentil, *Rhizobium*, and the selected AM fungus increased the yield and nutrient uptake in host plants and reduced the stress as proline content decreased on microbial association (Fig 4). The overall effects of microbial inoculation on nutrient uptake in host plant was in the increasing order as- S < S+R < S+G < S+F < S+R+G < S+R+F < S+F < S+A+R < S+A+G < S+A+F < S+A+R+G < S+A+R+F.

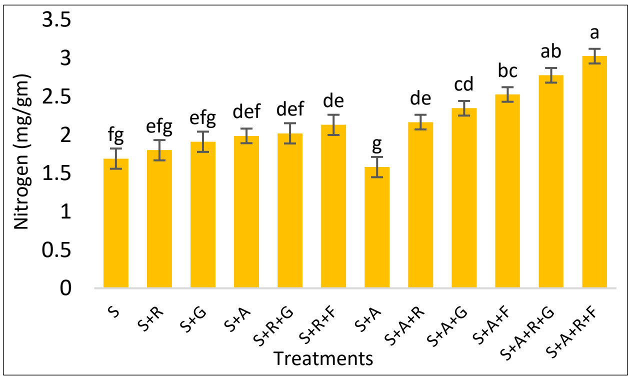

Fig 1 Interactive effects of Mycorrhiza and *Rhizobium* with 25% fly ash on nitrogen (mg/gm) content of Lentil (*Lens culinaris* Medik.) at 90 days after sowing (S= soil, F= *Funneliformis caledonius* and G= *Glomus bagyarajii*, R= *Rhizobium* and A= Fly ash)

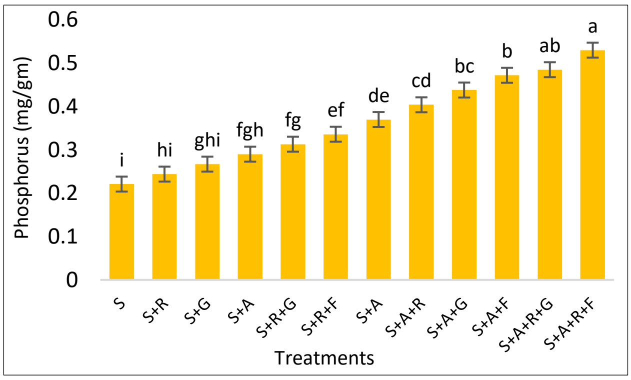

Fig 2 Interactive effects of Mycorrhiza and *Rhizobium* with 25% fly ash on Phosphorus (mg/gm) content of Lentil (*Lens culinaris* Medik.) at 90 days after sowing (S= soil, F= *Funneliformis caledonius* and G= *Glomus bagyarajii*, R= *Rhizobium* and A= Fly ash)

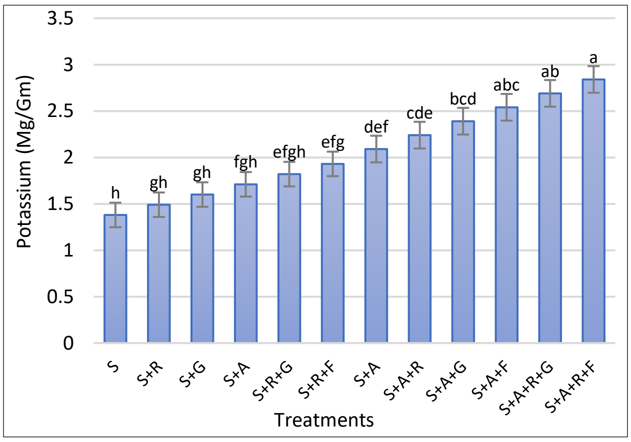

Fig 3 Interactive effects of Mycorrhiza and *Rhizobium* with 25% fly ash on Potassium (mg/gm) content of Lentil (*Lens culinaris* Medik.) at 90 days after sowing (S= soil, F= *Funneliformis caledonius* and G= *Glomus bagyarajii*, R= *Rhizobium* and A= Fly ash)

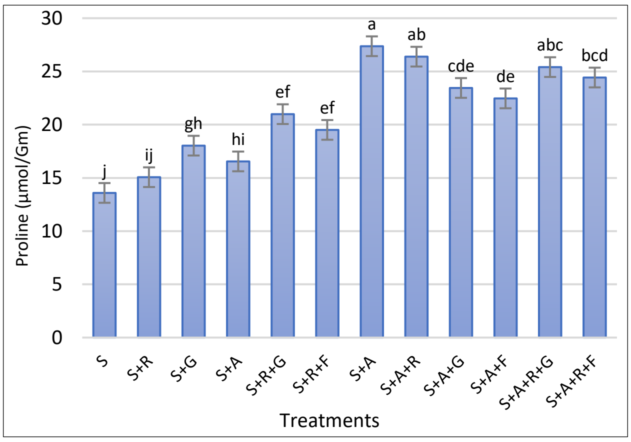

Fig 4 Interactive effects of Mycorrhiza and *Rhizobium* with 25% fly ash on Proline (µmol/gm) content of Lentil (*Lens culinaris* Medik.) at 90 days after sowing (S= soil, F= *Funneliformis caledonius* and G= *Glomus bagyarajii*, R= *Rhizobium* and A= Fly ash)

The nutrient uptake on microbial inoculation (R, F and G) was higher in 25% fly ash amended soil that is garden soil with fly ash (Fig 1-3). The pods^{-1} plant, seed weight and seed yield of lentil inoculated with microbes increased on application of 25% fly ash in garden soil as compared to soil without fly ash (Fig 5-7). The increase in number of pods^{-1} plant, seed weight and seed yield^{-1} plant increased on microbial inoculation in the order as – S < S+R < S+G < S+F < S+R+G < S+R+F < S+A < S+A+R < S+A+G < S+A+F < S+A+R+G < S+A+R+F.

The nitrogen content of lentil plant leaves rises in the following order - S+A < S < S+R < S+G < S+F < S+R+G < S+R+F < S+A+R < S+A+G < S+A+F < S+A+R+G < S+A+R+F. Phosphorus and potassium uptake, on the other hand, were observed to be similar as yield parameters like: S< S+R < S+G < S+F < S+R+G < S+R+F < S+A < S+A+R < S+A+G < S+A+F < S+A+R+G < S+A+R+F.

The proline content was highest in lentils treated with 25% fly ash (FA) alone, indicating that application of fly ash alone stressed the lentil plant, but inoculation of *Rhizobium* and *F. caledonius* in lentil grown in soil, or *F. caledonius* in fly ash amended soil, reduced the stress as evident from reduction in proline content in respective treatments (Fig 4). Single inoculation of these microbes had no statistically significant effect on proline content compared to uninoculated lentils, showing null and void stress in the plant (Fig 4).

The NPK content of lentil plants inoculated with *Rhizobium* and *Glomus bagyarajii* or *Rhizobium* and *Funneliformis caledonius* was higher than NPK content in lentil plant grown in soil without these microbes [22-24]. Fly ash rich in phosphorus, potash and several micronutrients also promote plant growth when applied in the field. In the present study, the inoculation of *Rhizobium* and one of the two selected species of AM fungi (*F. caledonius* and *G. bagyarajii*) increased the yield and NPK content in *L. culinaris* grown in sterilized composted garden soil as compared to the plants grown without these microbes (Fig 1-3 and 5-7). Leguminous crops develop a tripartite mutual connection with AM fungi and *Rhizobium* in the natural environment [25-27]. The tripartite symbiotic association between pigeon pea, *Rhizobium* and AM fungi promoted the growth of the host plant [28-29].

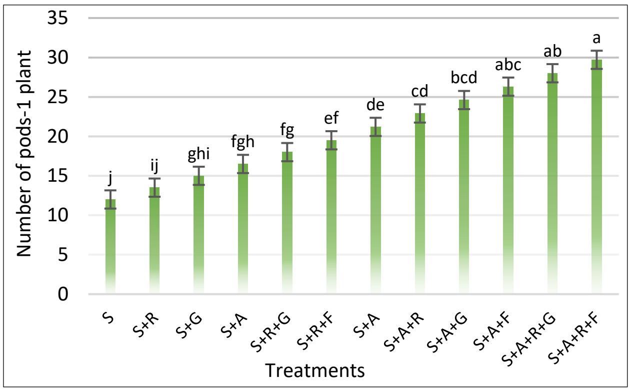

Fig 5 Interactive effects of Mycorrhiza and *Rhizobium* with 25% fly ash on number of pods^{-1} plant of Lentil (*Lens culinaris* Medik.) at 120 days after sowing (S= soil, F= *Funneliformis caledonius* and G= *Glomus bagyarajii*, R= *Rhizobium* and A= Fly ash)

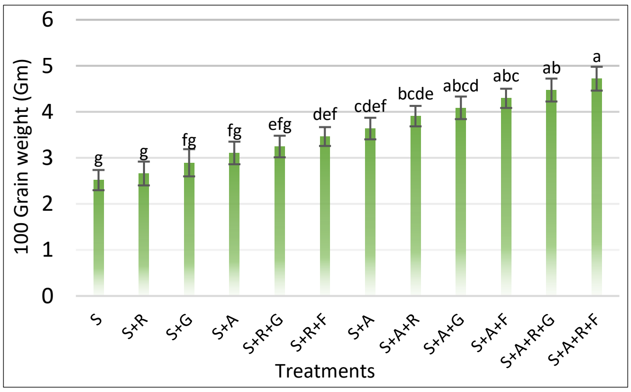

Fig 6 Interactive effects of Mycorrhiza and *Rhizobium* with 25% fly ash on hundred grain weight (gm) of Lentil (*Lens culinaris* Medik.) at 120 days after sowing (S= soil, F= *Funneliformis caledonius* and G= *Glomus bagyarajii*, R= *Rhizobium* and A= Fly ash)

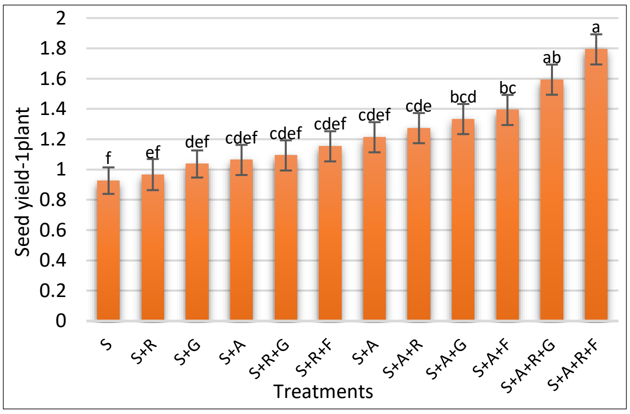

Fig 7 Interactive effects of Mycorrhiza and *Rhizobium* with 25% fly ash on seed yield^{-1} plant of Lentil (*Lens culinaris* Medik.) at 120 days after sowing (S= soil, F= *Funneliformis caledonius* and G= *Glomus bagyarajii*, R= *Rhizobium* and A= Fly ash)

Nutrient uptake in *Lens culinaris* inoculated with *Rhizobium* alone or with one of the selected AM fungi increased significantly and in turn promoted crop yield (Fig 1-3 and 5-7). *Rhizobium* associated with pulse crops fix atmospheric nitrogen in the form available to the host plant [30-32] and AM fungi in exchange of small amounts of carbon from host plant increase the uptake of water, micro and macronutrients in addition to phosphorus solubilization [33-34]. This combination of the roles of both *Rhizobium* and AM fungi had been more effective in the present experiment (Fig 1-3 and 5-7).

The extraradical mycelium of AM fungi extend from the host's root to the nearby soil volume past the rhizosphere and enhancing plant uptake of less accessible nutrients, primarily phosphorus [35-36]. In addition to phosphorus availability, AM fungi also facilitate uptake of other diffusion-limited nutrients like Cu, Zn in the host plants [37] as both these micronutrients play important role in useful enzyme activities.

Chemical fertilization, especially in large quantities, is not recommended; thus, delivering N and P naturally through AM fungi and *Rhizobium* considerably contribute to improved yield output while being agriculturally and environmentally sustainable [38-41]. Overall plant growth-promoting effects of *Rhizobium*, *F. caledonius*, and *G. bagyarajii*, each applied alone or in combination, improved plant growth in the following ascending order: Soil (S) < S+R < S+G < S+F < S+R+G < S+R+F. This demonstrates that a tripartite symbiosis between *L. culinaris*, *Rhizobium*, and *F. caledonius* increased yield and nutrient uptake of plant more than with other symbionts were used singly or in combination (Fig 1-3 and 5-7).

These findings clearly show that the combined inoculation of nitrogen-fixing *Rhizobium* bacteria and AM fungi namely *G. bagyarajii* or *F. caledonius* enhanced uptake of nutrients in host plant without any antagonizing effect of interaction between these microorganisms and host plant.

Similar beneficial effects of tripartite symbiotic partnerships have been reported in other leguminous crops [42-43]. In this experiment, the accumulation of proline is indicated stress in the decreasing order as- S+A > S+A+R > S+A+R+G > S+A+R+F > S+A+G > S+A+F > S+R+G > S+R+F > S+F > S+G > S+R > S. This statistical categorization of proline built up in lentil suggested that plants were under stress without presence of *Rhizobium* or AM fungi.

The application of fly ash alone increased the proline concentration in our studies, indicating stress (Fig 4) this could be due to the presence of heavy metals in the fly ash as reported earlier [44-45]. The increase in proline concentration in plants caused either by fly ash or drought increases to the extent of the defensive capacity of the host plant [46]. As is also evident with the finding of present experiment.

CONCLUSION

Our findings show that adding Mycorrhiza and *Rhizobium* along with FA 25% helpful in promoted growth and economic yield of lentil (*Lens culinaris* Medik.) than growing only in FA amended soils. Our findings on inoculation of the *Rhizobium* AM fungi (*Funneliformis caledonius* and *Glomus bagyarajii*) in pulse crops in context of climate change the and environment friendly sustainable use of FA.

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