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# INTERACTIVE EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI AND *Rhizobium* on GROWTH AND NUTRIENT CONTENT OF *Arachis hypogaea*

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## *ABSTRACT*

The present study was intended to investigate individual and interactive effects of *Funneliformis mosseae* (an arbuscular mycorrhizal fungus) and *Rhizobium* (a root nodulating bacterium) on growth and yield of groundnut growing under natural conditions. Plants growing in arbuscular mycorrhiza (AM) and/or *Rhizobium* inoculated soil exhibited superior growth, fitness and yield. Improvement in plant growth due to microbial inoculations had a significant correlation with their response to mycorrhization, relative water content, chlorophyll content, nutrient uptake, and antioxidant activity. The increase in all parameters except N acquisition and protein concentration was significantly higher on the formation of AM than rhizobial inoculation, albeit their combination displayed synergism to uplift metabolism and yield of host legume. Thus, the study indicated that synergistic behavior among microorganisms (AM and *Rhizobium*) had the most affirmative effects on the growth and harvest index of groundnut variety - TG37A and helped plants to thrive better in soils without chemical fertilizers.

**Contribution/Originality:** This study contributes to the existing literature of tolerance aptitude of groundnut variety - TG37A against existing adverse environment. This study also documents the importance of AM and *Rhizobium* in improving the growth and metabolism of this variety.

## 1. INTRODUCTION

*Arachis hypogaea*, an annual oil yielding plant of Fabaceae family, is one of the most important cultivated food legume (rank second) and edible oilseed crop (rank fourth) in world (Yol, Furat, Upadhyaya, & Uzun, 2018). However, biotic diseases and extremes of abiotic factors like temperature, drought, and soil salinity in semi-arid tropics are the severe limitations affecting both groundnut yield and excellence. In addition, impending climate change, poor agronomic practices, and inadequate inputs have further accentuated these harmful impacts (Krishna, Singh, Kim, Morya, & Ramteke, 2015; Sharma & Bhatnagar-Mathur, 2006; Thangella, Pasumarti, Pullakhandam, Geereddy, & Daggu, 2018). Nevertheless, ever-increasing consumer stipulation and the desire to amplify worldwide export of groundnut, necessitate the investigation to improve yield of groundnut cultivars. Chemical fertilizers have mammalian toxicity, phytotoxic nature and are ineffective against abiotic constraints thereby not desirable (Sathiyabama & Balasubramanian, 2018). In view of these limitations in conventional technique, the fundamental

paradigm for cost-effective and environmentally sound technology is to utilize beneficial representatives of microbiome in such a way as to improve crop health and productivity (Ruiz-Lozano, Porcel, Azcón, & Aroca, 2012).

Out of the most prevalent rhizospheric mutualisms, the arbuscular mycorrhiza (AM) and the *Rhizobium*-legume (RL) symbioses, have particular importance as 'natural mini-fertilizer factories' (Van der Putten, Klironomos, & Wardle, 2007). Both communications are totally different in terms of their host specificity, as Legume-*Rhizobium* symbiosis is very specific, permitting only particular strains of rhizobia to nodulate with accurate host legumes, while there is very little host specificity in mycorrhizal symbiosis formation and thus AM fungi (AMF) are considered as ubiquitous soil microorganisms (Gibson, Kobayashi, & Walker, 2008; Smith & Read, 2008). The RL symbiosis is a stupendous biological interface accountable for fixing a large quantity of nitrogen in terrestrial ecosystems, (Clúa, Roda, Zanetti, & Blanco, 2018). This root nodule symbiosis, require less fossil-based energy (~35–60 per cent lesser) than conventional N-fertilized crops (Jensen et al., 2012). The extensive mycelial structures (hyphae, arbuscules, vesicles, and spores) effectively explore soil substrates and obtain soil inorganic macro-nutrients nitrogen (N), phosphorus (P) and potassium (K) and micro-nutrients like copper (Cu), iron (Fe) and zinc (Zn) with some ability for acquiring organic N and P (Miransari, 2011; Parniske, 2008). Physiological benefit of both symbioses is not restricted to uptake and transfer of nutrients to the host (Evelin, Giri, & Kapoor, 2012) rather they also construct a buffer zone for host to resist biotic and abiotic stress (Lucas, García-Cristobal, Bonilla, Ramos, & Gutierrez-Manero, 2014); improve rhizospheric soil aggregation (Singh, 2012); improve host's water absorption competence by improving root hydraulic conductivity (Aroca, Porcel, & Ruiz - Lozano, 2007) produce antioxidant molecules (Patel & Saraf, 2013) improve host photosynthetic carbon fixation (Hajiboland, Aliasgharzadeh, Laiegh, & Poschenrieder, 2010) and preserve membrane structure and phytohormones synthesis (Chandrasekaran, Boughattas, Hu, Oh, & Sa, 2014) suppress pathogenic attack (Raupach & Kloepper, 1998) and stimulate systemic resistance (Geng et al., 2018). These mechanism may act in a concerted manner and lead to increased plant growth and subsequently improve productiveness of polluted soils and recuperate agriculture by improving crop's ability to nurture on nutrient-poor soils (Abdel Latef & Miransari, 2014).

If the incidence of one symbiont boosts resource accessibility in a way that releases the host from restriction or else promotes allotment to another symbiont, then constructive correlations i.e. co-colonization of multiple symbiotic species can be practical (Afkhami, Rudgers, & Stachowicz, 2014). In most of the studies, particularly when both N and P are limiting factors, rhizobia and AM have been reported to perform synergistically and forms an integral tripartite interaction to enhance legume development and reproduction more than either microsymbiont inoculating alone and also mutually enhance each other's organization (Bauer, Kleczewski, Bever, Clay, & Reynolds, 2012; Gould & Lister, 2005; Xie et al., 1995). Both symbionts are not only potent competitors for carbon requirements and/or nutrient complementarity, but also share resemblance in the downstream signal transduction pathways (i.e. common SYM genes) as well as hormonal regulation that arbitrate the perseverance and quantity of colonization events reviewed by Mukherjee and Ané (2011); Sakamoto, Ogiwara, and Kaji (2013) signifying the evolutionarily coupling of these two symbioses (Senoo et al., 2000; Takeda, Tsuzuki, Suzaki, Parniske, & Kawaguchi, 2013). Legume - mycorrhizal symbiosis is a vital link for effectual P nutrition, leading to improved $N_2$ fixation that advocate a synergistic tripartite relationship (Sakamoto et al., 2013).

High yielding varieties in agriculture augment production by increasing per acre yield, consequently improving self-sufficiency and decrease dependency on imports. However, cultivation of high-yielding varieties is a chemical-intensive program with substantial requirement of both chemical fertilizers and pesticides (Samal & Rout, 2018). In recent years, more consideration has been given to the use of biofertilizers and their integration, to not only have high plant yields but also to cut the usage of environmentally unfavorable chemical fertilizers (Akhtar, Siddiqui, & Wiemken, 2011). Therefore, it would be of paramount importance to explore how endosymbionts and their mutualistic potential can modulate physiology, metabolism, and ultimately yield of a high-yielding variety of groundnut. Thus, the present study was intended to ascertain the impact of *Rhizobium*, *Funneliformis mosseae* as well

as their interaction on a) growth, b) symbiotic performance, c) nutrient acquisition, d) redox stability in a high-yielding variety of groundnut. We also measured the modulation in yield productivity of this groundnut variety to gain insights into broader aspects of biofertilizers and their integration mediated enhancement in agriculture.

## 2. EXPERIMENTAL MATERIALS, LAYOUT AND METHODOLOGY

### *2.1. Environmental Conditions and Experimental Layout*

Greenhouse experiments were carried out in the Botanical garden of Department of Botany, SGTB Khalsa College, University of Delhi (28.61°N, 77.23°E and elevation 200 - 250 m), under natural climatic conditions (minimum temp. 22–26 °C and maximum 35–44 °C; relative humidity 28–30 % and 86–100 %, in morning and afternoon respectively). Soil for the experiment was obtained from close by agricultural meadow. The soil physico-chemical properties were determined as sand and loam in 1:1 ratio by volume (with 11.5 mg P $kg^{-1}$ (Olsen & Sommers, 1982) 0.19 meq 100 $g^{-1}$ available K, 0.21 Na meq 100 $g^{-1}$, 0.86 Ca meq 100 $g^{-1}$ (Mehlich, 1953) 0.44 % total N (Nelson & Sommers, 1973) pH 7.5 (soil:water; 1:1), electrical conductivity (ECe) 0.86 $dSm^{-1}$, 0.66 % organic carbon (Walkley, 1947). The soil was filled in earthen pots with capacity of 10 kg soil. 2×2 factorial design with two mycorrhizal conditions: with or without fungal inoculum combined with two conditions of *Rhizobium* inoculation: with or without *Rhizobium*. Thus, there were four combinations of treatment (each in the multiple of six) set in a completely randomized block design.

#### *2.1.1. Biological Material*

Seeds of groundnut cultivar (TG37A: A Spanish groundnut cultivar) were procured from Directorate of Groundnut Research (DGR), Gujrat, India. This cultivar of groundnut (popular among the farmers for large scale cultivation) was released in 2004 by Bhabha Atomic Research Centre, Mumbai. This cultivar is appropriate for both kharif and rabi summer seasons all over India and is tolerant to various biotic factors causing collar rot, rust and late leaf spot.

Groundnut specific *Bradyrhizobium* culture (TAL1000) was also procured from DGR, Gujrat. It was a charcoal-based rhizobial inoculum that readily forms nodules with groundnut in a variety of soils under various agro-climatic conditions. Non-sterile spores (from monosporal culture) of mycorrhizal isolate *Funneliformis mosseae* (UTMU 128 WM1/11, T.H. Nicolson & Gerd.) C. Walker & A. Schüβler *comb. nov.* Schüβler and Walker (2010) were acquired from the Centre for Mycorrhizal Culture Collection, The Energy and Resource Institute, India. AM inoculum was bulked by inoculating *Zea Mays* L., *Sorghum bicolor* L. and *Coriandrum sativum* L. in an open-pot soil culture (Dalpé & Monreal, 2004).

#### *2.1.2. Symbiotic Microorganism Inoculation*

Healthy looking seeds of equal size were selected and consequently surface sterilized (to avoid microbial infection) using 10% hydrogen peroxide (v/v). The seeds were washed in sterilized distilled water a number of times to wipe out remaining traces of chemical soaked in water and left for overnight for imbibition. Next morning, half of these seeds were inoculated with rhizobial inoculum. Slurry of charcoal-based rhizobial inoculum was prepared and overnight soaked seeds were thoroughly stirred in this slurry. When seeds were properly coated with *Bradyrhizobium* (*R*), they were air dried at room temperature. Before sowing, half of the +R (inoculated) and half –R (non inoculated) seeds were also inoculated with 50 g of soil-based fungal inoculum [concoction of soil containing pieces of roughly 65 per cent colonized roots, approximately 23 spores per gram soil, filamentous hyphae], kept at a depth of 1.5 cm below the seed sowing plane, to aid plant root fungal colonization. Non-AM treatments were provided same weight of sterile inoculum with 10ml aliquot of an inoculum filtrate.

*2.1.3. Harvesting*

Plants were harvested for estimating various parameters at 50 days after sowing (DAS) i.e. vegetative stage and at 110 DAS i.e. at reproductive stage. Six plants of each treatment were harvested for every fresh as well as dry estimation and records calculated on per gram nodules/roots/leaves dry weight basis (full-fledged developed nodules/roots/leaves of one plant were regarded as one biological repeat). Plant materials were dried in oven at 70°C until they reached steady weight. Dried material was powdered and stored for further analyses. Yield parameters were documented daily from the commencement of flowering till seed growth and maturity stage (110 DAS). Pods/seeds number per plant was estimated by taking average of pods/seeds in six replicates per treatment at crop maturation stage. Harvested plant samples were divided into vegetative and reproductive parts and depending on their dry weights, harvest index (HI) was calculated at 110 DAS (Leport, Turner, Davies, & Siddique, 2006) as follows:

$$\text{HI} = \frac{\text{Seed dry wt.(non} - \text{aborted)}}{\text{Above ground plant biomass at harvest}}$$

*2.2. Physiological Estimations*

*2.2.1. Per Cent AM Colonization*

Cleared roots (10 % KOH) were stained with (0.05 %) trypan blue (Phillips & Hayman, 1970) and were microscopically calculated using the magnified grid-line intersection method (Habte & Osorio, 2001; McGonigle, Miller, Evans, Fairchild, & Swan, 1990). Mycorrhizal responsiveness was calculated by following formula (Zhu, Smith, Barritt, & Smith, 2001):

$$\text{MR} = \frac{\text{Dry weight of AM plants} - \text{Dry weight of non} - \text{AM plants}}{\text{Dry weight of non} - \text{AM plants}} \times 100$$

*2.2.2. Nodulation and Leghemoglobin (LHb) Protein Pigment*

The nodules were detached immediately after sampling and *Bradyrhizobium* symbiosis was estimated by counting total nodule number per root and by calculating total nodule dry weight per root system. LHb concentration was calculated by the method of Hartree (1957).

*2.2.3. Relative Water Content (RWC)*

RWC in leaves was estimated (Weatherley, 1950) and calculated as per the following formula:

$$\text{RWC} = \frac{(a-b)}{(c-b)} \times 100$$

where, 'a' is fresh wt., 'b' is dry wt., and 'c' is turgid wt. (weights taken after incubation of plant samples in 100 ml of distilled water for 4 h.)

*2.2.4. Chlorophyll (A, B, Total) Concentration and Chlorophyll A/B in Leaves*

Dimethyl sulphoxide (DMSO) based extraction of chlorophyll (Chl.) in leaves was done by the method of Hiscox and Israelstam (1979). The amount of Chl. was calculated from the extinction values following the equation of Arnon (1949).

*2.2.5. Nutrient Uptake*

Dried tissue of roots, nodules and shoots were analyzed for their nutrient composition. Concentration of Nitrogen (N) was estimated by using Nessler's reagent in the colorimetric method of Lindner (1944) and the standard curve was prepared using graded concentrations of $(NH_4)_2SO_4$. Phosphorus (P) concentration was measured by vanado-molybdophosphoric method of Jackson (1973) by taking absorbance at 420 nm. Appropriate standards of $KH_2PO_4$ were run simultaneously and against the calibration curve, P concentration was calculated in mg $g^{-1}$ D.W. Magnesium (Mg) and Potassium ($K^+$) concentration in acid based digest were measured with atomic

absorption spectrophotometry (Allen, Grimshaw, & Rowl, 1984). Blank was run without plant samples and against the calibration curve, concentration was calculated in mg $g^{-1}$ D.W.

### *2.2.6. Total Protein Concentration*

The total proteins were estimated in fresh roots, nodules and shoots by method of Bradford (1976) where spectrophotometeric O.D. was taken at 595 nm and protein concentration was calculated in mg $g^{-1}$ F.W.

**Table-1.** Result of ANOVA for independent variables including rhizobial inoculation (R), arbuscular mycorrhiza (AM) inoculation and interactions among them.

| Section I | | R | | | AM | | | R × AM | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | P ≤ 0.05 | P ≤ 0.01 | P ≤ 0.001 | P ≤ 0.05 | P ≤ 0.01 | P ≤ 0.001 | P ≤ 0.05 | P ≤0.01 | P ≤ 0.001 |
| RDW (75 DAS) | | * | ** | *** | * | ** | *** | * | ** | *** |
| RDW (75 DAS) | | * | ** | *** | * | ** | *** | * | ** | *** |
| RSR (75 DAS) | | * | ns | ns | * | ns | ns | * | Ns | Ns |
| RWC (75 DAS) | | * | ** | ns | * | ** | ns | * | ** | Ns |
| Chl. a | | * | ** | *** | * | ** | *** | * | ** | *** |
| Chl. b | | * | ** | ns | * | ** | ns | * | ** | Ns |
| Chl. a/b | | * | ns | ns | * | ns | ns | * | Ns | Ns |
| Total Chl. | | * | ** | ns | * | ** | * | * | ** | Ns |
| NN (75 DAS) | | * | ** | *** | * | ** | ns | * | ** | Ns |
| NDW (75 DAS) | | * | ** | ns | * | ns | ns | * | Ns | Ns |
| LHb concentration (75 DAS) | | * | ** | ns | * | ** | ns | * | Ns | Ns |
| MC (75 DAS) | | * | ** | ns | - | - | - | - | - | - |
| MR (75 DAS) | | * | ns | ns | - | - | - | - | - | - |
| Number of Flowers | | * | ** | ns | * | ** | ** | * | ** | Ns |
| Number of Pods | | * | ** | ns | * | ** | *** | * | * | Ns |
| Pod dry weight | | * | ** | ns | * | ** | ns | * | Ns | Ns |
| Number of Seeds | | * | ** | *** | * | ** | *** | * | ** | Ns |
| Seed dry weight | | * | ns | ns | * | ** | ns | * | Ns | Ns |
| 100 seed weight | | * | ** | *** | * | ** | *** | * | Ns | Ns |
| Above ground dry weight | | * | ns | ns | * | ns | ns | * | Ns | Ns |
| HI | | * | ns | ns | * | ns | ns | * | Ns | Ns |
| N concentration | Roots | * | ** | ns | * | ** | *** | * | Ns | Ns |
| | Nodules | * | ** | *** | * | ** | *** | * | ** | *** |
| | Leaves | * | ** | ns | * | ** | *** | * | ** | *** |
| P concentration | Roots | * | ** | *** | * | ** | *** | * | ** | *** |
| | Nodules | * | ** | ns | * | ** | *** | * | Ns | Ns |
| | Leaves | * | ** | ns | * | ** | *** | * | ** | *** |
| $Mg^{2+}$ concentration | Roots | * | ** | *** | * | ** | *** | * | ** | *** |
| | Nodules | * | ** | ns | * | ** | *** | * | Ns | Ns |
| | Leaves | * | ** | *** | * | ** | ns | * | ** | *** |
| $K^{+}$ concentration | Roots | * | ** | ns | * | ** | *** | * | Ns | Ns |
| | Nodules | * | ** | ns | * | ** | *** | * | ** | *** |
| | Leaves | * | ** | *** | * | ** | ns | * | ** | *** |
| Protein concentration | Roots | * | ** | *** | * | ** | *** | * | ** | *** |
| | Nodules | * | ** | *** | * | ** | *** | * | ** | *** |
| | Leaves | * | ** | *** | * | ** | *** | * | Ns | Ns |
| | Seeds | * | ** | *** | * | ** | ns | * | Ns | Ns |
| SOD activity | Roots | * | ** | ns | * | ** | *** | * | Ns | Ns |
| | Nodules | * | ** | *** | * | ** | *** | * | Ns | Ns |
| | Leaves | * | ** | *** | * | ** | *** | * | Ns | Ns |
| CAT activity | Roots | * | ** | *** | * | ** | *** | * | Ns | Ns |
| | Nodules | * | ** | *** | * | ** | ns | * | Ns | Ns |
| | Leaves | * | ** | ns | * | ** | ns | * | Ns | Ns |
| POX activity | Roots | * | ** | *** | * | ** | *** | * | Ns | Ns |
| | Nodules | * | ** | *** | * | ** | *** | * | Ns | Ns |
| | Leaves | * | ** | ns | * | ** | ns | * | Ns | Ns |

**Note: ns** no significant variation. * Significant variation at 95%, ** Significant variation at 99% and *** Significant variation at 99.9%.

### *2.2.7. Antioxidant Enzyme Activity*

Superoxide dismutase (SOD, EC 1.15. 1.1) activity was assayed in fresh roots, nodules and shoot by the method of Dhindsa, Plumb-Dhindsa, and Thorpe (1981) and was expressed as µkat (microkatals) per mg protein (1 katal = 1 mol $s^{-1}$catalytic activity). Catalase (CAT, 1.11.1.6) activity was assayed by method of Aebi (1984) and was expressed as nkat (nanokatals) per mg protein. Peroxidase (EC number 1.11.1.x) activity was assayed by method of Castillo, Penel, and Greppin (1984) and was expressed as nkat tetraguaiacol formed per mg protein.

### *2.3. Statistical Analysis*

Data was finalized by taking average of values derived from six biological replicates ± standard error (S.E.) per treatment. Data was statistically ensured by analysis of variance (ANOVA) using SPSS 18.0 for Windows (SPSS, Inc., Chicago, IL, USA). One-way ANOVA, Duncan's multiple-range test was carried out at $p < 0.05$ to evaluate the means of dependent variables. Two-way ANOVA was performed (at all three levels i.e. 95%, 99% and 99.9%) to study the significance of interaction between mycorrhiza and RL symbioses on various parameters.

## 3. RESULTS

Analysis of variance divulged that independent factors [rhizobial inoculation (R), arbuscular mycorrhiza (AM) inoculations], when considered independently, had significant effect on all dependent variable (at 95%). First order (R × AM) interactions too had significant effects at 95%, on all the dependent variables under investigation Table 1.

### *3.1. Growth Attributes*

Shoot and root dry weight (SDW and RDW) of plants under different treatments were analyzed at 50 DAS Figure 1. Rhizobial and/or AM inoculation improved root growth, thereby increasing root's absorptive surface area and the degree of explored soil for resources, which could also be associated with the improved above ground plant parts. Though benefits endorsed by co-inoculation of R and AM inoculation were the most significant, AM inoculation could provide higher degree of assistance in plant growth than rhizobial inoculation. Microbial colonization being more effective in improving RDW than SDW, led to higher RSR relative to non colonized plants.

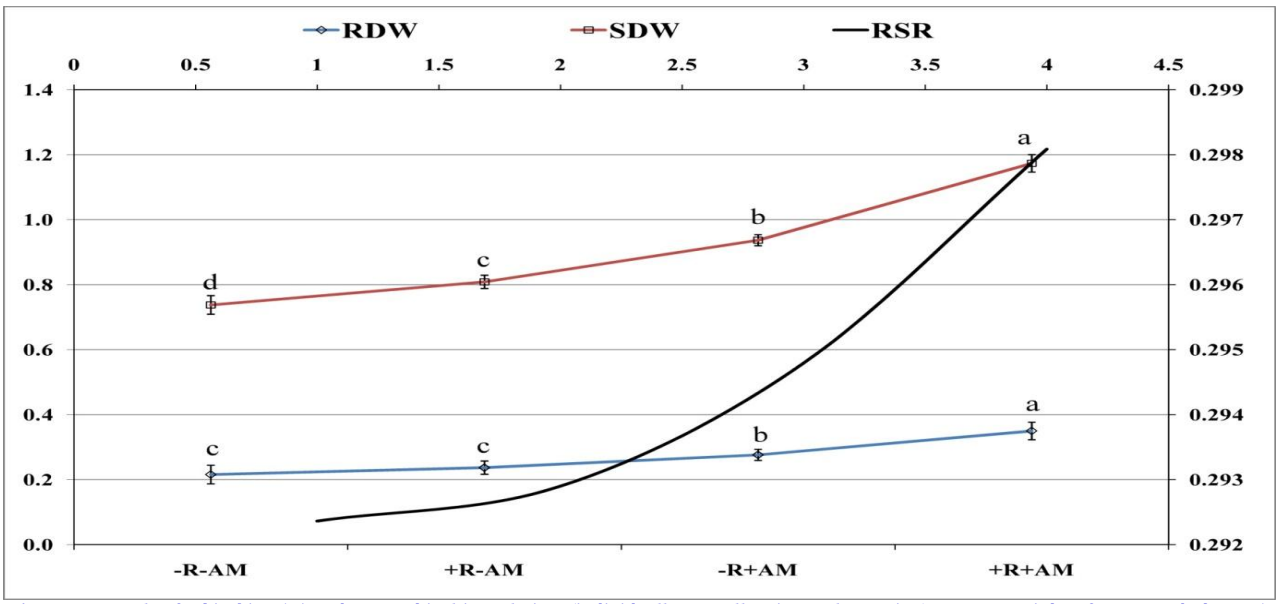


**Figure-1.** Result of *Rhizobium* (R) and mycorrhizal inoculation [individually as well as in amalgamation] on Dry weight of Roots and Shoots (g plant$^{-1}$) and Root to Shoot Ratio in groundnut variety grown in sterile and non-sterile conditions.
**Treatments:** Control (-R-AM), Rhizobial treatment (+R-AM), Mycorrhizal treatment (-R+AM) and Rhizobial and mycorrhizal treatment (+R+AM). Data represented is average of six biological replicates ± standard error (S.E.). According to Duncan's multiple-range test, values followed by the identical letter within a soil type do not vary at $p < 0.05$.

### *3.2. Symbiotic Effectiveness*

#### *3.2.1. Mycorrhizal Colonization and Responsiveness*

Microscopic assessment for calculating per cent mycorrhizal colonization (MC) in roots was carried out and the data is presented in Table 2. No sign of MC was observed in the root segments of uninoculated plants, thereby confirming the absolute absence of innate mycorrhiza. On the other hand, remarkable colonization was observed in the mycorrhizal roots at 50 DAS. Intensity of colonization by fungal endophyte in rhizobial treated plants was observed to be significantly higher than the respective –R+AM plants. Calculating plant responsiveness to mycorrhizas is a measure of mycorrhizal fungus effectiveness, thus variation in MR, with and without R application was calculated. Rhizobial application decreased plants responsiveness to mycorrhization Table 2.

**Table-2.** Result of *Rhizobium* (R) and mycorrhizal inoculation [individually as well as in amalgamation] on Mycorrhizal colonization (MC, %), Mycorrhizal responsiveness (MR, %), Nodule number (NN, plant$^{-1}$), Dry weight of nodules (NDW, g plant$^{-1}$) and Leghemoglobin (LHb) concentration (µg$^{-1}$ g F.W.) in groundnut variety.

| | ROOTS | | NODULES | | |
|---|---|---|---|---|---|
| **Treatment** | **MC** | **MR** | **NN** | **NDW** | **LHb** |
| Control | - | - | $27.54^{c}\pm0.3329$ | $0.25^{c}\pm0.0085$ | $144.275^{d}\pm4.856$ |
| +R | - | - | $33.18^{ab}\pm1.0949$ | $0.31^{ab}\pm0.0121$ | $197.451^{b}\pm3.818$ |
| +AM | $70.97\pm2.0835$ | $32.01\pm3.3021$ | $32.35^{b}\pm0.3404$ | $0.30^{b}\pm0.0060$ | $175.696^{c}\pm2.322$ |
| +R+AM | $80.24\pm3.6663$ | $28.31\pm4.5432$ | $35.90^{a}\pm0.2426$ | $0.34^{a}\pm0.0060$ | $227.706^{a}\pm0.569$ |

**Treatments:** Mycorrhizal treatment (-R+AM) and Rhizobial and mycorrhizal treatment (+R+AM). Data represented is average of six biological replicates ± standard error (S.E.). According to Duncan's multiple-range test, values followed by the identical letter within a soil type do not vary at $p < 0.05$.

#### *3.2.2. Nodulation (Nodule Number, Dry Weight and Leghemoglobin Concentration)*

Visual examination indicated clustered arrangement of large healthy pink coloured globose nodules in plants, however suppression of successful nodule establishment in plants growing in -R soils was clearly evidenced by lower nodule count Table 2 as compared to their +R counterparts. It was observed that absence of *Rhizobium* had comparatively higher adverse impact on nodule dry weight (NDW) accumulation and leghemoglobin (LHb) concentration than their establishment, thus although nodule formation initiated under -R condition, it's appropriate development and functioning could not be retained. Results further revealed that individually, R and AM inoculation led to remarkable enhancement in the new nodule formation and growth, which was witnessed by higher NN in +R-AM and –R+AM plants. The nodules were relatively healthier and accumulated higher biomass and LHb, when they were grown in the soils inoculated with either R or *F. mosseae*. Comparatively, rhizobial inoculation was more effective in improving NN, NDW and LHb than mycorrhization and rhizobial inoculation to mycorrhizal soils further improved development of *Bradyrhizobium*-legume association. Benefits of this tripartite association was elicited by much higher number and biomass accumulation in nodules. Higher leghemoglobin (LHb) pigment concentration in +R+AM treatments allied with higher nodular development, thereby increasing the requirement of LHb to maintain apposite oxygen tension.

### *3.3. Nutrient Content*

Higher concentration of N, K and Mg was detected in leaves as compared to roots, but P concentration was higher in roots relative to leaves. However it was observed that plants inoculated with *Bradyrhizobium* or *F. mosseae* had superior capability to maintain nutrient uptake and distribution (significant S × R and S × AM interactions), with higher effectiveness of mycorrhization as compared to R application (except for N, where rhizobial inoculation outperformed AM inoculation). Effectiveness of microbial inoculation was tissue dependent with higher augmentation provided in roots relative to shoots. Administration of R in the rooting medium along with mycorrhization, appeared to put forth a profound control on nutrients homeostasis (resulted in maximum accretion of nutrient pool), thereby further pointing towards mutual behaviour of R and AM Table 3.

### *3.4. Relative Water Content and Chlorophyll (Chl.) Content*

Higher biomass in inoculated plants was strongly associated with their water status Table 3 and superior chl. Figure 2, which might be due to increased cellular turgidity leading to cellular enlargement, nutrient acquisition and adequate capability to trap photosynthetic light, ultimately improving plant metabolic activities and biomass accumulation. Further observations recorded evidenced that microbial inoculations, especially AM, could improve chl. a concentration better than chl. b, thereby improving chl. a/b ratio. Yellowing, scorching and subsequent senescence of leaves was least in +R+AM plants. Thus, results confirmed that interactions of R and AM were preeminent to counterbalance the plant water deficit and pigment loss caused by uncontrolled factors in natural soils.

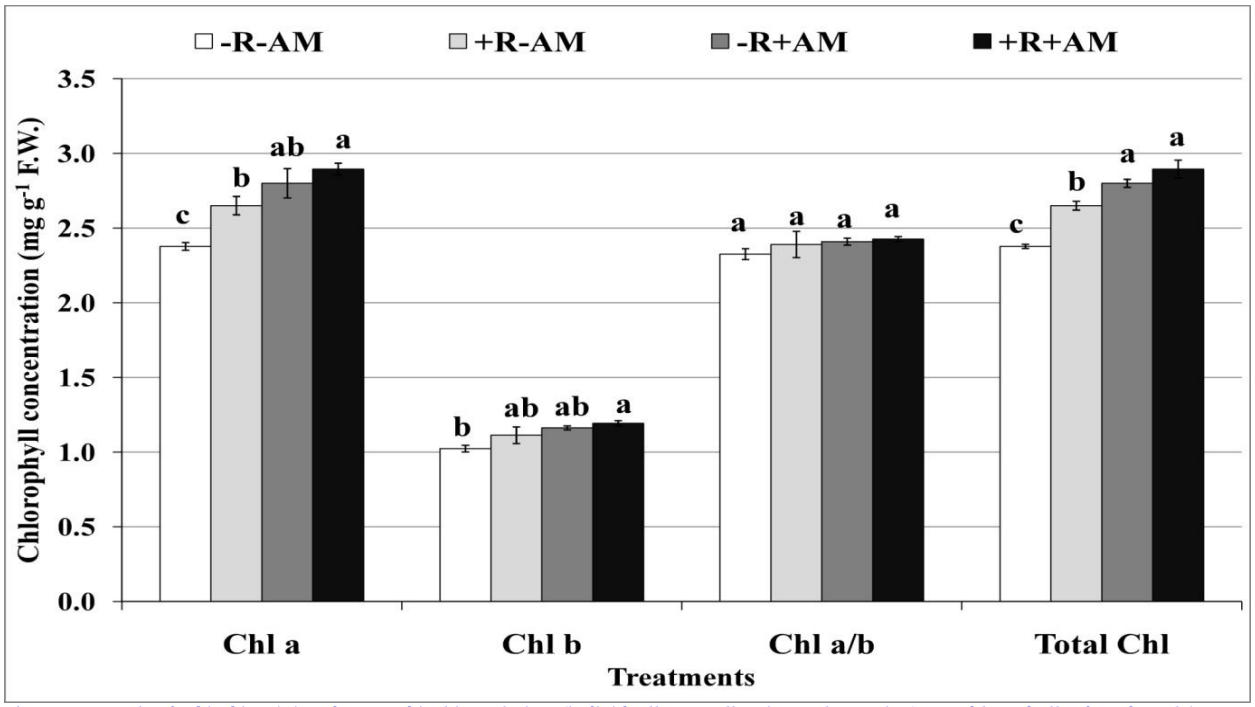


**Figure-2.** Result of *Rhizobium* (R) and mycorrhizal inoculation [individually as well as in amalgamation] on Chlorophyll a, b and total (mg $g^{-1}$ F.W.) and chlorophyll a/b ratio in the leaves of groundnut variety.
**Treatments:** Control (-R-AM), Rhizobial treatment (+R-AM), Mycorrhizal treatment (-R+AM) and Rhizobial and mycorrhizal treatment (+R+AM). Data represented is average of six biological replicates ± standard error (S.E.). According to Duncan's multiple-range test, values followed by the identical letter within a soil type do not vary at $p < 0.05$.

**Table-3.** Result of *Rhizobium* (R) and mycorrhizal inoculation [individually as well as in amalgamation] on Relative Water Content (RWC, %) in leaves, Nitrogen (N), Phosphorus (P), Potassium ion ($K^+$) and Magnesium ion ($Mg^{2+}$) concentration (mg $g^{-1}$ D.W.) in roots and leaves of groundnut variety.

| Treatment | RWC in leaves | Roots | | | | Leaves | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | P | K | Mg | N | P | K | Mg |
| -R-AM | $70.71^{d}$ ±1.231 | $13.00^{c}$ ±0.455 | $2.77^{d}$ ±0.095 | $8.60^{d}$ ±0.185 | $9.71^{c}$ ±0.170 | $19.30^{c}$ ±0.310 | $2.18^{b}$ ±0.132 | $17.06^{c}$ ±0.485 | $12.27^{c}$ ±0.315 |
| +R-AM | $74.87^{c}$ ±0.345 | $16.51^{b}$ ±0.040 | $3.69^{c}$ ±0.122 | $10.50^{c}$ ±0.213 | $11.25^{b}$ ±0.254 | $25.03^{b}$ ±0.585 | $2.69^{ab}$ ±0.064 | $19.29^{b}$ ±0.419 | $13.76^{b}$ ±0.199 |
| -R+AM | $82.40^{b}$ ±0.761 | $15.80^{b}$ ±0.298 | $4.20^{b}$ ±0.107 | $11.68^{b}$ ±0.051 | $12.21^{ab}$ ±0.469 | $24.79^{b}$ ±0.096 | $2.98^{a}$ ±0.083 | $20.68^{ab}$ ±0.218 | $14.69^{ab}$ ±0.152 |
| +R+AM | $94.68^{a}$ ±0.462 | $18.56^{a}$ ±0.215 | $4.65^{a}$ ±0.206 | $12.90^{a}$ ±0.060 | $13.19^{a}$ ±0.346 | $27.76^{a}$ ±0.296 | $3.23^{a}$ ±0.173 | $22.11^{a}$ ±0.483 | $15.65^{a}$ ±0.257 |

**Treatments:** Control (-R-AM), Rhizobial treatment (+R-AM), Mycorrhizal treatment (-R+AM) and Rhizobial and mycorrhizal treatment (+R+AM). Data represented is average of six biological replicates ± standard error (S.E.). According to Duncan's multiple-range test, values followed by the identical letter within a soil type do not vary at $p < 0.05$.

### *3.5. Protein Concentration*

Effect of R and/or AM inoculation on total protein concentration was determined in roots and leaves and observations recorded are shown in Figure 3. As compared to roots, superior nutrient homeostasis in foliar tissue accounted for better accumulation of proteins. Improvement in status of protein pool was recorded in plants treated with R and/or AM inoculated ones and the enhancement in proteins was coupled with enhanced vigor of +R-AM, -R+AM and +R+AM plants over –R-AM plants. Although both R application and AM inoculation (when applied individually) significantly improved protein concentration, yet R mediated enhancement in proteins was considerably higher than mycorrhization mediated enrichment of protein pool. Further, rhizobial inoculation as well as mycorrhization complemented each other and significantly improved protein status in groundnut variety.

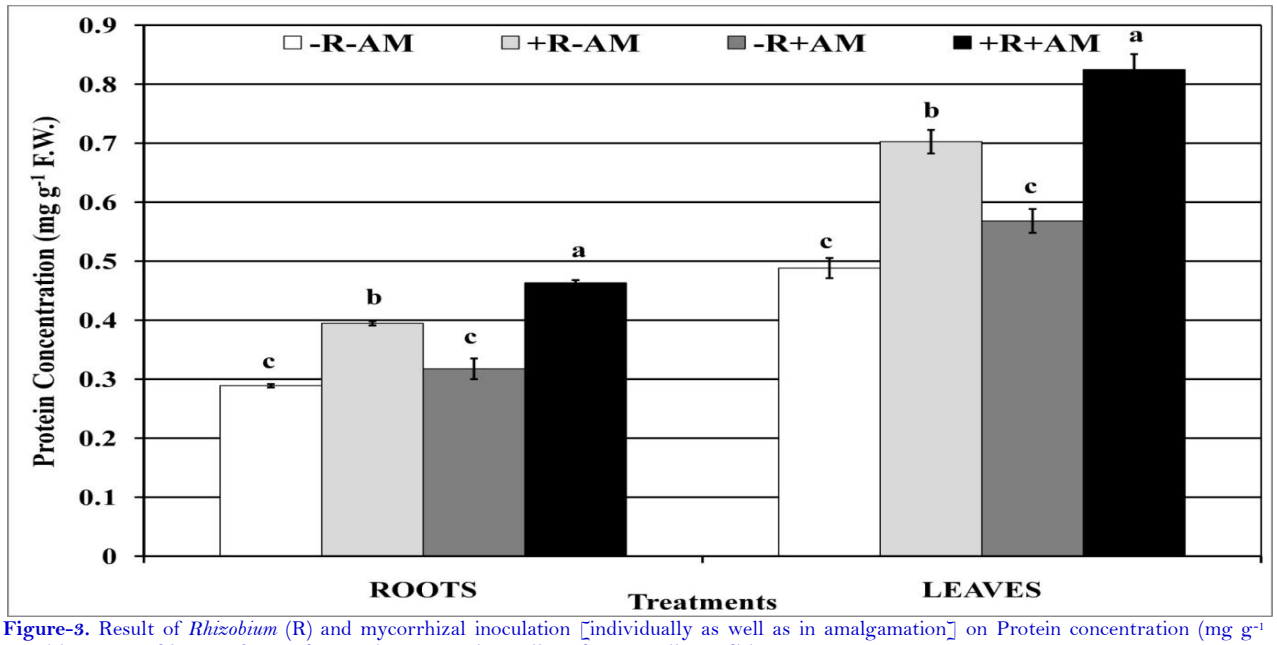


**Figure-3.** Result of *Rhizobium* (R) and mycorrhizal inoculation [individually as well as in amalgamation] on Protein concentration (mg $g^{-1}$ F.W.) in roots and leaves of groundnut variety grown in sterile and non-sterile conditions.

**Treatments:** Control (-R-AM), Rhizobial treatment (+R-AM), Mycorrhizal treatment (-R+AM) and Rhizobial and mycorrhizal treatment (+R+AM). Data represented is average of six biological replicates ± standard error (S.E.). According to Duncan's multiple-range test, values followed by the identical letter within a soil type do not vary at $p < 0.05$.

### *3.6. Enzymatic Antioxidant Activity*

Activity of superoxide dismutase (SOD), catalase (CAT) and peroxidise (POX) in groundnut variety was strongly correlated with plant tissue, where higher activity was recorded in roots Table 4. Antioxidant enzyme activity further accelerated when plants were treated with R and/or *F. mosseae* inoculum. Superior antioxidant enzyme activity under rhizobial inoculation and/or mycorrhization pointed towards competent scavenging of ROS in microbial treated plants relative to corresponding –R-AM ones. Although rhizobial inoculation and mycorrhization exhibited synchronisation (+R+AM) in up-regulating antioxidant activity, however individually mycorrhization (-R+AM) mediated upsurge in antioxidant activity was considerably higher than rhizobial inoculation (+R-AM) mediated upsurge. Improvement in root antioxidant machinery was more significant as compared to leaves, pointing towards microbial mediated better heath to below-ground plant parts, thereby imparting better adherence to soil for water and nutrient uptake.

**Table-4.** Result of *Rhizobium* (R) and mycorrhizal inoculation [individually as well as in amalgamation] on Superoxide dismutae (SOD, µkats mg protein), Catalase (CAT, µkat $mg^{-1}$ protein) and Peroxidase (POX, nkat $mg^{-1}$ protein) activity in roots and leaves of groundnut variety.

| Treatment | ROOTS | | | LEAVES | | |
|---|---|---|---|---|---|---|
| | SOD | CAT | POX | SOD | CAT | POX |
| -R-AM | $84.59^{c}$ ±2.449 | $5.93^{c}$ ±0.348 | $448.50^{d}$ ±4.364 | $62.40^{c}$ ±1.492 | $6.21^{d}$ ±0.090 | $188.06^{c}$ ±4.562 |
| +R-AM | $98.30^{bc}$ ±2.808 | $6.75^{b}$ ±0.216 | $522.95^{c}$ ±4.572 | $69.91^{bc}$ ±2.745 | $6.85^{c}$ ±0.124 | $211.47^{b}$ ±5.603 |
| -R+AM | $106.86^{ab}$ ±1.510 | $7.26^{ab}$ ±0.165 | $569.47^{b}$ ±4.719 | $74.61^{ab}$ ±2.160 | $7.26^{b}$ ±0.082 | $226.10^{ab}$ ±2.036 |
| +R+AM | $115.63^{a}$ ±5.823 | $7.79^{a}$ ±0.175 | $617.08^{a}$ ±8.385 | $79.41^{a}$ ±2.700 | $7.67^{a}$ ±0.104 | $241.07^{a}$ ±4.936 |

**Treatments:** Control (-R-AM), Rhizobial treatment (+R-AM), Mycorrhizal treatment (-R+AM) and Rhizobial and mycorrhizal treatment (+R+AM). Data represented is average of six biological replicates ± standard error (S.E.). According to Duncan's multiple-range test, values followed by the identical letter within a soil type do not vary at $p < 0.05$.

### *3.7. Yield Parameters*

Harvest is an important trait measuring reproductive efficiency as well as source-sink balance of legumes and in multiplicative conjunction with biomass; it is a determinant of crop yield. Ultimate yield of crop plant is the function of various yield components comprising its flowering aptitude and their subsequent transition into pods enclosing harvestable seeds. Thus the effect of R and/or AM in modulating various yield components and subsequently HI of groundnut was analyzed till maturity at 110 DAS. Relative to inoculated plants, the competence of vegetative phase to progress into reproductive buds considerably reduced in non-inoculated plants and was elicited in the form of delayed as well as significantly restrained progression of flowers into pods Table 5. On the other hand fungal endophyte in the rhizosphere boosted flowering and robustness of pods and seeds more significantly than the rhizobial application. HI, an attribute portraying proportion of aboveground assimilates allocated to seeds, also improved with R and/or AM inoculation. Moreover, rhizobial inoculation and mycorrhization when applied individually had significant impact on protein accumulation in the economically important plant part i.e. seed. Rhizobial inoculation outperformed mycorrhization which could be highly correlated with better N acquisition by +R-AM plants. Moreover, when the rhizobial coated seeds were sown in mycorrhizal soils, the improvement in protein assimilation in seeds was most prominent, thereby improving the fitness of groundnut seeds in +R+AM plants.

**Table-5.** Result of *Rhizobium* (R) and mycorrhizal inoculation [individually as well as in amalgamation] **on yield components** of groundnut variety.

| Treatment | Number of pods per plant | Number of seeds per plant | Seeds dry weight per plant (g) | Above ground dry weight (g $plant^{-1}$) | HI | Protein concentration (mg $g^{-1}$ seeds) |
|---|---|---|---|---|---|---|
| -R-AM | $56^{b}$±2.309 | $42^{b}$±1.730 | $10.12^{d}$±0.530 | $23.99^{d}$±1.240 | 0.431 | $0.422^{c}$±0.029 |
| +R-AM | $74^{a}$±1.732 | $65^{a}$±2.520 | $21.34^{a}$±0.520 | $49.00^{a}$±1.580 | 0.442 | $0.618^{b}$±0.019 |
| -R+AM | $76^{a}$±2.081 | $41^{b}$±5.290 | $13.67^{c}$±1.040 | $30.77^{c}$±1.470 | 0.431 | $0.535^{b}$±0.0127 |
| +R+AM | $83^{a}$±6.082 | $58^{a}$±3.460 | $17.99^{b}$±1.090 | $39.58^{b}$±2.000 | 0.455 | $0.725^{a}$±0.008 |

**Treatments:** Control (-R-AM), Rhizobial treatment (+R-AM), Mycorrhizal treatment (-R+AM) and Rhizobial and mycorrhizal treatment (+R+AM). Data represented is average of six biological replicates ± standard error (S.E.). According to Duncan's multiple-range test, values followed by the identical letter within a soil type do not vary at $p < 0.05$.

## 4. DISCUSSION

Superior perpetuation of osmotic, photosynthetic, nutrient and redox equilibrium in +R plants directed to overall improvement in plant growth as evidenced by their improved biomass at both vegetative as well as reproductive stage. Increased nodulation and LHb concentration owing to rhizobial inoculation could be because of increased proportion of effective infections. Improvement in nodulation and functionality in +R-AM plants was evidenced by considerably higher nitrogen and protein concentration in roots and shoots of these plants as compared to –R-AM plants. Plants with rhizobial inoculation also evidenced superior P concentration which could

be the probable source of energy for improved nodulation. In view of the fact that N is a vital constituent of chlorophyll (Tucker, 2004); rhizobia inoculated enhancement in chl. concentration could be an indirect consequence of improved $N_2$-supplementation via enhanced legume–*Rhizobium* symbiosis. Superior RWC recorded in these samples could also be due to improved root density as well as vigor that upgraded water absorption aptitude of plants by widening the rhizosphere to be explored. Interestingly, activity of defense related proteins i.e. SOD, CAT and POX, also increased with higher activity recorded in roots thereby indicating the tendency of maintaining redox equilibrium in roots more significantly. Superior perpetuation of osmotic, photosynthetic, nutrient and redox equilibrium in +R plants directed to overall improvement in plant growth as evidenced by their improved biomass at both vegetative as well as reproductive stage.

As compared to rhizobial inoculation, mycorrhizal colonization was resourcefully more competent in improving plant growth. Highly dense root system in mycorrhizal plants relative to non-mycorrhizal ones, could be due to mycorrhization mediated alteration in root morphology in a structural, spatial, quantitative and temporal conduct (Kapoor, Sharma, & Bhatnagar, 2008); thereby resulting in better rooting structure and superior total potential absorbing surface relative to the non mycorrhizal plants (Khalil, Eissa, El-Shazly, & Nasr, 2011; Zai et al., 2007). A higher improvement in root than shoot biomass in -R+AM and +R-AM groundnut plants (as indicated by increased RSR) pointed towards the facilitation served by rhizobia inoculation as well as mycorrhization to preferentially recover root system for superior rhizosphere exploration so as to improve capitalization of nutrient and water uptake. Higher allocation of photosynthates to the below ground plant parts and the resultant higher RDW and MC could prompt advanced trade of N and P by mycorrhiza in exchange (Fellbaum et al., 2012; Hammer, Pallon, Wallander, & Olsson, 2011) thereby ensuring superior benefits to those plants. Comparatively higher RWC in leaves of mycorrhizal plants could be due to AM induced superior root morphology (Kothari, Marschner, & George, 1990) superior root hydraulic conductivity even at low water potential (Kapoor et al., 2008) higher stomatal conductance insisting for added transpiration (Sheng et al., 2008) and higher solutes accumulation, thus improving plant osmotic equilibrium (Abdel Latef & Miransari, 2014). Higher $Mg^{2+}$ and chl. concentration in mycorrhizal plants corroborated with their higher growth, advocating that higher requirement of fixed carbon in mycorrhizal plants could have enhanced $Mg^{2+}$ uptake thereby improving chl. concentration which could have consequently improved $CO_2$ assimilation efficiency (Wu, Zou, & He, 2010) and hence plant fitness.

Although total proteins (defence as well as non defence related) increased more appreciably in +R-AM plants relative to –R+AM plants, defence related antioxidant proteins increased more significantly in –R+AM plants. Increase in antioxidants could be the plant's inherent capacity to up-regulate proteins related to stress adaptation (Ghosh & Xu, 2014). Abbaspour, Saeidi-Sar, Afshari, and Abdel-Wahhab (2012) and Cicatelli et al. (2012) suggested that an efficient approach used by AM plants to attain tolerance is to increase antioxidant enzyme activity of plant origin. In this regard, Alguacil, Hernandez, Caravaca, Portillo, and Roldan (2003) and Ruiz-Lozano et al. (2012) proposed that mycorrhiza inoculation induces host's antioxidant activity by improving its growth and assimilation of low mobility micronutrients exploited as co-factors of metallo-enzymes e.g. SOD, CAT etc.. In the present investigation mycorrhizal inoculation also improved nodulation and nitrogen fixation, which could be attributed to increased growth of root, which favours rhizobial colonization (Goss & De Varennes, 2002; Patreze & Cordeiro, 2004). Further, mycorrhization can augment surface area of roots for additional absorption of nutrients and AM nutrient uptake pathway operates more extensively than direct uptake by root transporters (Smith, Facelli, Pope, & Smith, 2010). Thus, mycorrhization can result in better assimilation of minerals especially P, which would result in more energy available for $N_2$ fixation by rhizobia (Dardanelli et al., 2008). P improvement in inoculated plants was proportionate to the improvement in nodulation and N fixation, thus authenticating the P essentiality for sufficient $N_2$ fixation (Rotaru & Sinclair, 2009). Mycorrhization-adjudicated efficient assimilation of N in the host plants could also be because of increased ammonium ($NH_4^+$) and nitrate ($NO_3^-$) uptake from the soil by extra radical mycelium (ERM) of mycorrhiza, as has been proposed by Guether et al. (2009); Tian et al. (2010) and Fellbaum et al. (2012).

In the present study, increase in N concentration was more significant in +R-AM plants relative to –R+AM plants thus proposing that, improvement in N assimilation via increase in rhizobial $N_2$-fixation was the preferred stratagem than the increase in direct uptake of inorganic N. Higher P acquisition in mycorrhizal plants, was perhaps due to higher P uptake via mycorrhiza which can even completely replace direct uptake by plant transporters (Smith, Jakobsen, Grønlund, & Smith, 2011). In fact, mycorrhizal hyphae expand beyond the depletion zones surrounding roots and obtain nutrients that are far from the root surface (Smith & Read, 2008). As compared to rhizobial inoculation, mycorrhization was considerably better in sustaining the osmotic equilibrium along with photo-assimilating aptitude, thereby leading to much higher biomass accumulation.

Individually, mycorrhiza mediated improvement in overall plant growth and performance was higher than rhizobia inoculated improvement, but +R+AM plants possessed maximum competence to grow in experimental soils. Higher performance of +R+AM plants (in terms of relative water content, chlorophyll content, nutrient and redox equilibrium) could be either due to improved root growth, thereby providing better niche for AM colonization or due to cumulative effect of their individual affirmative effects. Furthermore as compared to –R+AM plants, MR decreased in +R+AM plants which specified that rhizobia inoculation also contribute in attenuating indigenous burden and hence abridged the exclusive investment of mycorrhiza in upregulating plant metabolism. Highest accumulation of photosynthetic pigments in +R+AM plants was also owing to improved $N_2$-fixation via functional complementarity displayed by rhizobia and mycorrhiza. Nutrient accumulation (N, P, K and Mg) further increased in rhizobial inoculated mycorrhizal plants which could be due to cross-assistance provided by each microbe to another i.e. increased P uptake via mycorrhization further upregulated R-mediated improvement in $N_2$ fixation which led to enhanced growth and mycorrhization as well as improved nutrient uptake in +R+AM plants. Relative assessment of rhizobial inoculation and mycorrhization indicated that improvement in all but nitrogen metabolism and N acquisition was more significant by mycorrhization than rhizobial inoculation, albeit their amalgamation displayed synergism to uplift rhizobial symbiosis and overall functioning of host legume.

Consequently, higher biomass accumulation accounted for higher photo-assimilates being loaded in the sink during seed filling, thereby strongly linking the plant dynamism at vegetative phase with that of reproductive phase. Interestingly in the present investigation, transition of vegetative phase to reproductive phase was significantly improved in both rhizobial and mycorrhizal inoculation, with improved flowering, pod formation, seed set and eventually harvest (as evidenced by HI). Better performance of inoculated plants at reproductive stage was tightly coupled with superior RWC and chlorophyll concentration, thus suggesting that the effectual water and photosynthates movement towards sink might have accounted for superior flower initiation, better pollen germination and pollen tube growth leading to healthier pod formation and finally higher seed set and protein concentration. Improvement in seed weight and the resultant harvest index in correspondence with the improvement in aboveground dry weight of inoculated plants substantiated the importance of efficient photosynthate allocation in the direction of seed filling in up regulating the ultimate harvest. Maximum preservation of water and redox status and photosynthetic pigments in +R+AM plants subsequently routed most efficient carbon allocation at reproductive stage, leading to utmost continuance of high protein yielding peanuts as is also statistically evidenced by 2-way ANOVA.

## 5. CONCLUSIONS

Thus, 'symbiosis-promoting effect' of these microbes had most affirmative effects on growth and harvest index of groundnut variety and helped plants to thrive better in soils without any chemical fertilizers. Thus, present investigation indicates that shaping dynamic rhizosphere microbiome can enhance the productivity of this variety for sustainable agriculture in changing environment and can offer many economical and environmental benefits.

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