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Interaction effects of AM fungi with Rhizobacteria and Phosphate solubilizing bacteria on the growth and nutrient uptake of chilli

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ABSTRACT

The present investigation was aimed at determining the reaction of AM fungi (Glomus intraradices) with Rhizobacteria (Psudomonas fluorescens) and Phosphate solubilizing bacteria (Bacillus megaterium) on the growth and nutrient uptake of chilli var PLR – 1. Single [T_1 - G.intraradices (Gi), T_2 - P.fluorescens (Pf), T_3 - B.megaterium (Bm)], dual [T_4 - G.intraradices + P.fluorescens (Gi + Pf), T_5 - G.intraradices + B.megaterium (Gi + Bm)], combined [T_6 - G.intraradices + P.fluorescens+ B.megaterium (Gi + Pf + Bm)] inoculums were respectively used in this experiment. Forty days old seedlings were transplanted in cement pots. The population of AM Fungi and Bacterial populations were enumerated at 45, 90 and 120 days, likewise the plant growth parameters (Number of leaves, shoot length, whole plant weight and dry weight) as well as Nutrient content (N, P. K. Na) were examined.

The maximum AM fungi colonization was recorded in single inoculation, likewise the bacterial population as well. While comparing with combined the dual treatments also produced good population of fungi and bacteria. Among these treatments, combined treatments shows superior plant growth and nutrient uptake level in chilli. When measure up with control, all other treatments created better results in plant growth and nutrient uptake. The mixed microbial inoculants were promoting the plant growth by producing such growth hormone, mineral compounds and humanizing soil conditions. In case of the single or dual treatments produced particular compounds by microbes. However, overall obviously the combined treatment was better for chilli.

Keywords: Chilli, AMF, Rhizobacteria, phosphate solubilizing bacteria, growth parameters, Nutrient content.

INTRODUCTION

Mycorrhizal fungi play a critical role in nutrient cycling and ecosystem function. They improve plant growth and survival through a mutualistic relationship in which photosynthates are exchanged for increased access to water and nutrients. Because the benefits realized are not equal among different plant–fungal species combinations, mycorrhizal fungi may help govern plant community structure and successional trajectories. In fact, both plant productivity and plant diversity have been shown to increase with increasing diversity of mycorrhizal fungi [1].Some groups of mycorrhizal fungi may also mediate plant competition through the formation of mycelial linkages, through which carbon is shared among different plant species [2]. In sustainable agriculture, arbuscular mycorrhizal (AM) fungal inoculation in

agronomical management might be very important [3]. Nitrogen-fixing bacteria and arbuscular mycorrhizal fungi were found to enhance the growth and production of various fruit plants significantly [4],besides improving the microbiological activity in the rhizosphere [5].Inoculation with plant-growth promoting rhizobacteria (PGPR) has been attributed to the production of plant growth regulators at the root interface, which stimulate root development and result in better absorption of water and nutrients from the soil [6]. Phosphorus is second only to nitrogen in mineral nutrients most commonly limiting the growth of crop. Phosphorus is an essential element for plant development and growth making up about 0.2 % of plant dry weight. Plants acquire phosphorus from soil solution as phosphate anions [7].Improvement of phosphorus (P) nutrition is one of the factors involved in plant growth promotion by PGPR. These bacteria may improve plant P acquisition by solubilizing organic and inorganic phosphate sources through phosphatase synthesis or by lowering the pH of the soil [8].

MATERIALS AND METHODS

All chemicals used were of AR grade. The PLR -1 variety chilli seeds used were obtained from the Vegetable Research Station, Palur, Caddalore. The chilli seeds were immersed in to 0.05 % sodium hypo chloride (30 min) for surface sterilization. The treated seeds were sown in cement pots.

AM fungi and Bacterial inoculation: The *Glomus intraradices,* and *Psudomonas fluorescens, Bacillus megaterium* (pure strain) were procured from Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University. AM fungal spores (*Glomus intraradices*) were propagated on maize grown in 30cm diameter pots containing sterilized sand: soil mix (1:1 v/v). The colonized maize roots were used as an inoculum, 10g of inoculum consisting of roots and soil contained 100-150 spores as a single inoculation. Besides the bacterial suspension (3 ml) containing 10^9 cells ml^{-1}. The treatments details,

T_1 - *G.intraradices* (Gi)
T_2 - *P.fluorescens* (Pf)
T_3 - *B.megaterium* (Bm)
T_4 - *G.intraradices* + *P.fluorescens* (Gi + Pf)
T_5 - *G.intraradices* + *B.megaterium* (Gi + Bm)
T_6 - *G.intraradices* + *P.fluorescens*+ *B.megaterium* (Gi + Pf + Bm)
T_7 - Control (without bio-inoculant)

Experimental Setup: In this experiment we evaluated the effectiveness of different treatment (AM fungi with Rhizobacteria and Phosphate solubilizing bacteria) on bacterial populations and AM fungi colonization moreover plant growth parameter and nutrient content. A completely randomized experiment was designed with seven treatments and five replications. The cement pots (50×45×30cm) amended with 15 kg sterilized soil-sand-manure mix was used for this experiments. Before transplanting of chilli seedlings, a thin layer of inoculum was placed in the pot at 5cm depth. Immediately forty days old seedlings were transplanted into pots (three seedlings per pot).

Enumeration of population dynamics of bacteria and AM fungi: Samples of 10 g of rhizospheric soil per plant were collected at 45, 90, and 120 days after inoculation to determine the bacteria and AM fungi population. Bacteria were identified considering cellular and colony morphology, and by Gram staining [9].

Root samples were washed well with 10% KOH solution and stained with 0.1% Trypan blue before estimation of mycorrhizal colonization. Arbuscular mycorrhizal colonization was estimated using a modified line intersect method [10] where a minimum of 100 line intersections per root sample, replicated three times, were scored for the presence of AM structures. These observations were made using the light microscopy to rate the degree of root infection by AMF in one plant per replicate (three plants per treatment). The percentage of AM infection was calculated from the following equation:

$$\text{Percentage of AM infection} = \frac{\text{Root length infected}}{\text{Root length observed}} \times 100$$

Growth parameter and Nutrient Analysis: The plants were deracinated periodically after 45, 90, and 120 days inoculation. The no. of leaves, shoot length, whole plant fresh weight and plant dry weight were

recorded. Three randomly selected plants per replicate were divided into leaves, stems and root and dried in an oven at 70±8°C for 48 hours to determine dry weights and elemental concentrations. N was determined in samples of 0.1 g dry weight using the Kjeldahl method. Samples were dry-ashed at 50±8°C for 6 h, and mixed with 2M hot HCl filtered then brought to a final volume of 50 ml with distilled water. Sodium and potassium were determined in these sample solutions. Phosphorous was analyzed by a vanadate molybdate method using a UV/Visible spectrophotometer (Shimadzu UV, 1601) [11].

Statistical Analysis: Results were then subjected to a one way ANOVA and means were compared by Duncan's Multiple Range Test ($p<0.05$) (DMRT). All analyses were performed using STATISTIC version 17.0 Software.

RESULTS AND DISCUSSION

AM fungi and Bacterial Population: AM fungi with bacteria such as *Bacillus pabuili* have the ability to enhance AMF root colonization [12]. The populations of AM fungi and Rhizobacteria, Phosphate solubilizing bacteria (after 45, 90, and 120 days) were mentioned in Table 1, 2, 3. According to our results, the population of AM fungi produced superior performance in single inoculation (T_1-*G.intraradices*) at 45 and 90 days. Similar result was observed [13]. Rhizosphere soils from control plants also contained some AM infection because of some root colonization with the native AM fungi. While match up to combined inoculation (T_6 - Gi + Pf + Bm) with double inoculation (T_2 – Gi+Pf, T_3 – Gi+Bm) AM fungi population was low in T_6. After 90 days inoculation, the AM fungi population was increased because it was adopted their new environment and exploit that nutrient. Soil microorganisms influence AM fungal development [14] and the establishment of symbiosis but no clear pattern of response has been found. Negative impacts upon the AM fungi include a reduction in spore germination and hyphal length in the extrametrical stage, decreased root colonization and a decline in the metabolic activity of the internal mycelium. After 120 days inoculation, the population was decreased in the view of the fact that inadequate nutrient to consuming. Rhizobacteria population also increased in the treatment of single inoculation (T_2 - *P.fluorescens*). After 45 days inoculation superior number of colony forming units (cfu's) for *P.fluorescens* was found in the rhizosphere of chilli plant ranged from 1.1×10^7 to 7.9×10^7 (Table. 1) and Phosphate solubilizing bacteria (*B.megaterium*) was significantly increased (2.1×107 to 8.1×107). Simultaneously double and combined inoculations were reducing the bacterial population of both bacteria (*P.fluorescens, B.megaterium*). The decrease in the population of bacteria after inoculation may be related to difficulties in adapting to their new environment[15]. However, the root exudates play a significant role in the growth of microorganisms [16]. This exudation is reduced after 60 d past planting and much of the plant's energy reserves are channeled towards fruit/seed formation, thus causing an exponential decline in the survival of introduced bacteria [17]. According to our results after 120 days inoculation the bacterial populations were diminished. Maximum high result was recorded in 90 days inoculation.

Table 1: Population dynamics of AM fungi and Rhizobacteria, Phosphate solubilizing bacteria colonization in chilli (PLR - 1) at 45 days inoculation

Treatments	Mycorrhizal colonization (%)	Bacterial Population (cfu g soil^{-1})	
T_1 - *G.intraradices*	60.3 ^{a}	-	-
T_2 - *P.fluorescens*	-	$7.9\times10^{7\,a}$	-
T_3 - *B.megaterium*	-	-	$8.1\times10^{7\,a}$
T_4 - Gi + Pf	40.3 ^{b}	$6.5\times10^{7\,b}$	-
T_5 - Gi + Bm	42.8 ^{b}	-	$6.9\times10^{7\,b}$

T_6 - Gi + Pf + Bm	38.7 ^{c}	$4.2\times10^{7\ bc}$	$5.0\times10^{7\ bc}$
T_7 - Control	10.3 ^{d}	$1.1\times10^{7\ d}$	$2.1\times10^{7\ cd}$

Mean values followed by the same letter are not significantly different based on Duncan's multiple range test ($p<0.05$), a>b>c. cfu colony forming units.

Table 2: Population dynamics of AM fungi and Rhizobacteria, Phosphate solubilizing bacteria colonization in chilli (PLR - 1) at 90 days inoculation

Treatments	Mycorrhizal colonization (%)	Bacterial Population (cfu g soil^{-1})	
T_1 - *G.intraradices*	72.5 ^{a}	-	-
T_2 - *P.fluorescens*	-	$10.8\times10^{7\ a}$	-
T_3 - *B.megaterium*	-	-	$12.3\times10^{7\ a}$
T_4 - Gi + Pf	51.3 ^{b}	$9.2\times10^{7\ ab}$	-
T_5 - Gi + Bm	53.2 ^{b}	-	$10.3\times10^{7\ bc}$
T_6 - Gi + Pf + Bm	44.8 ^{c}	$7.8\times10^{7\ bc}$	$8.2\times10^{7\ bc}$
T_7 - Control	18.5 ^{d}	$3.2\times10^{7\ c}$	$3.9\times10^{7\ d}$

Mean values followed by the same letter are not significantly different based on Duncan's multiple range test ($p<0.05$), a>b>c. cfu colony forming units.

Table 3: Population dynamics of AM fungi and Rhizobacteria, Phosphate solubilizing bacteria colonization in chilli (PLR - 1) at 120 days inoculation

Treatments	Mycorrhizal colonization (%)	Bacterial Population (cfu g soil^{-1})	
T_1 - *G.intraradices*	57.4 ^{a}	-	-
T_2 - *P.fluorescens*	-	$5.3\times10^{7\ a}$	-
T_3 - *B.megaterium*	-	-	$6.1\times10^{7\ a}$
T_4 - Gi + Pf	34.7 ^{b}	$3.9\times10^{7\ b}$	-
T_5 - Gi + Bm	33.1 ^{b}	-	$4.4\times10^{7\ b}$
T_6 - Gi + Pf + Bm	28.7 ^{c}	$2.8\times10^{7\ bc}$	$3.2\times10^{7\ bc}$
T_7 - Control	7.2 ^{d}	$0.8\times10^{7\ d}$	$1.0\times10^{7\ d}$

Mean values followed by the same letter are not significantly different based on Duncan's multiple range test ($p<0.05$), a>b>c. cfu colony forming units

Effect of Microbial inoculants on the growth parameters of chilli: The effect of biofertilizer treatments on vegetative growth of chilli was significantly higher in single, dual or combined inoculation than control

plants. At 45 days inoculation (Fig. 1) the number of leaves were increased by the treatment T_5 - Gi + Bm, while comparing to combined (T_6 - Gi + Pf + Bm) inoculation, T_6 was low. Significant increase in plant height, number of leaves and number of branches, fresh weight and dry weight in *Ocimum kilimandscharicum* on inoculation with *Glomus fasciculatum* compared to nonmycorrhizal plants[18]. The combined inoculation (T6 - Gi + Pf + Bm) showed superior result in Number of leaves, Shoot length, Plant fresh weight and dry weight. Additionally T_1-*G.intraradices* points up that the higher in plant fresh weight when compared to other single inoculations. The shoot length was higher on treatment T_3 - *B.megaterium*.

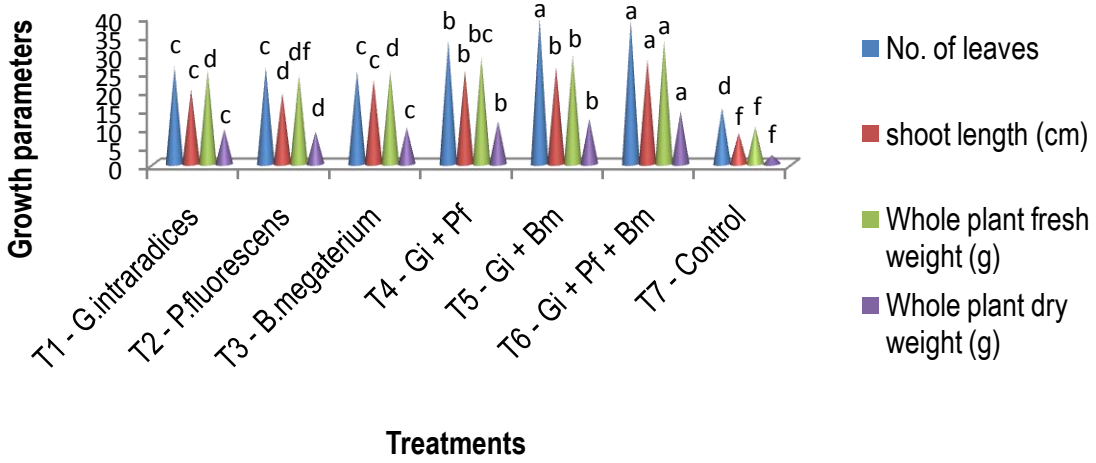

Fig 1: Showing the effectiveness of the different treatments on the growth parameters of chilli (PLR -1) plant at 45 days. Mean values followed by the same letter are not significantly different based on Duncan's multiple range test ($p<0.05$), a>b>c.

At 90 days also the combined inoculation (T_6) showed better results in growth parameter (Fig.2). The increase in plant height, number of branches, fresh weight and P updake in *Ocimum basllicum* on inoculation with *Glomus fasciculatum* + *Pesudomonas fluorescens* + *Bacillus megatherium* [19]. Muthuraj [20] recorded the highest shoot and root dry weight in *Capsicum annum* on treatment with *Pseudomonas* fluorescents + *Glomus mosseae* + *Azospirillum brasilense* compared to other treatments. In comparison between the dual inoculations, T_5 - Gi + Bm was more efficient in all growth parameters than the T_4 - Gi + Pf. *Glomus fasciculatum* and *Azotobacter chroococcum* was more efficient in all the growth parameters than the *Glomus fasciculatum* and *Pseudomonas fluorescens*[21]. The superior results were noted in single inoculations, when measure up to the control.

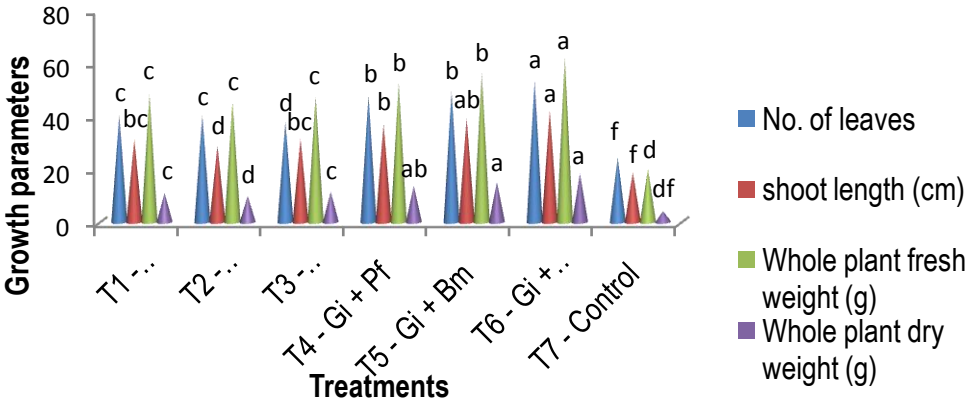

Fig 2: Showing the effectiveness of the different treatments on the growth parameters of chilli (PLR -1) plant at 90 days. Mean values followed by the same letter are not significantly different based on Duncan's multiple range test ($p<0.05$), a>b>c.

In contrast, the single inoculation of *A. awamori* and *B. subtilis* significantly decreased the dry matter accumulation in roots at 45 and 80 DAS [22], while the *G. fasciculatum* inoculation reduced the root weight only at 80 DAS. Among dual inoculation treatments, the combination of *Bradyrhizobium* sp. + *B. subtilis* significantly enhanced the dry matter accumulation in the roots, shoots and dry weight of whole plant at flowering (45 DAS) and at harvest (80 DAS) relative to the control. Co-inoculation of *G. fasciculatum* + *B. subtilis* significantly enhanced the dry matter accumulation in roots and shoots at 45 and 80 DAS and total plant biomass at 80 DAS only, compared to the control. After 120 days inoculation (Fig - 3), number of leaves, whole plant fresh weight and dry weight were significantly increased in T_1 - *G.intraradices* (Gi) while comparing with other single treatments T_2 - *P.fluorescens* (Pf) and T_3 - *B.megaterium* (Bm). The dual inoculation of *Glomus fasciculatum* and *Azospirillum* sp. resulted in increase of plant growth parameters, *viz*. plant height, number of primary branches, stem girth and plant biomass [23]. Overall obvious, the combined treatment was produced highest results when compare to other treatment at 45, 90 and 120 days.

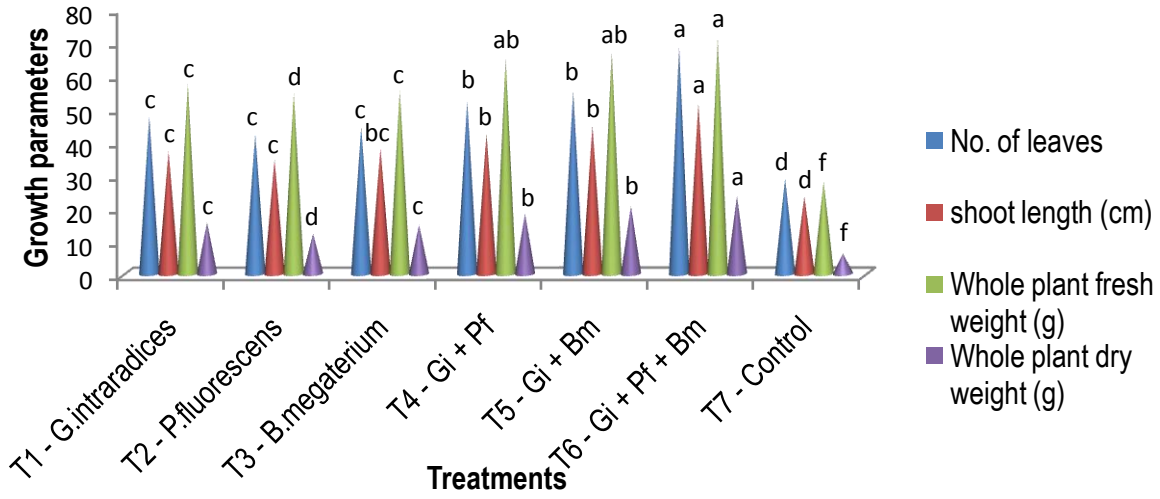

Fig 3: Showing the effectiveness of the different treatments on the growth parameters of chilli (PLR -1) plant at 120 days. Mean values followed by the same letter are not significantly different based on Duncan's multiple range test ($p<0.05$), a>b>c.

Effect of Microbial inoculants on the Nutrient of chilli : The nutrients of chilli at different microbial inoculants were mentioned in Fig – 4, 5, 6. Mycorrhiza helps plants with such a shallow sparse root system to increase phosphorus uptake [24]. AM fungi are, to different degree, capable of promoting phosphorus availability by acidifying the soil and, consequently, exploiting the phosphorus in nutrient patches and by facilitating the growth and development of host plants. The mycorrhizal benefit role on nutrient uptake generally was the best in the *G. mosseae* treatment [25]. It suggests that arbuscular mycorrhizas could improve growth performance and part nutrient acquisition of peach, which were absolutely dependent on AMF species. According to our study, after 45 days inoculation the nutrient contents were increased in the combined treatments T_6 - (Gi + Pf + Bm) whereas comparing with other treatments such as single and dual inoculation. In contrast, the dual inoculation T_4 - (Gi + Pf) was increased in only on N at 45, 90 and 120 days but T_5 - (Gi + Bm) was increased in P, K. Na with the exception of N. In the case of single inoculation, the all nutrient contents were higher in T_1 - *G.intraradices* when match up to other single inoculation. But the equivalent P content was recorded in T1 - *G.intraradices* and T3 - *B.megaterium* at 45

days. Mycorrhization significantly reduced Na and Cl plant uptake, and stimulated the absorption of K and P [26]. The synergetic effects of the PGPR *Methylobacterium oryzae* and different species of AM fungi significantly affected plant growth and chlorophyll content [27]. All the treatments were produced good results when comparing with T_7 (Control). Shahram Sharafzadeh found that, the highest amount of N, P and K were achieved on *Pseudomonas* + *Azotobacter* + *Azosprillum* treatment and the lowest amount was shown on *Pseudomonas* + *Azotobacter* treatment[6]. Maximum level of Ca and Mg were obtained on *Pseudomonas* + *Azotobacter* and *Pseudomonas* + *Azosprillum* treatments which significantly differ from other treatments.

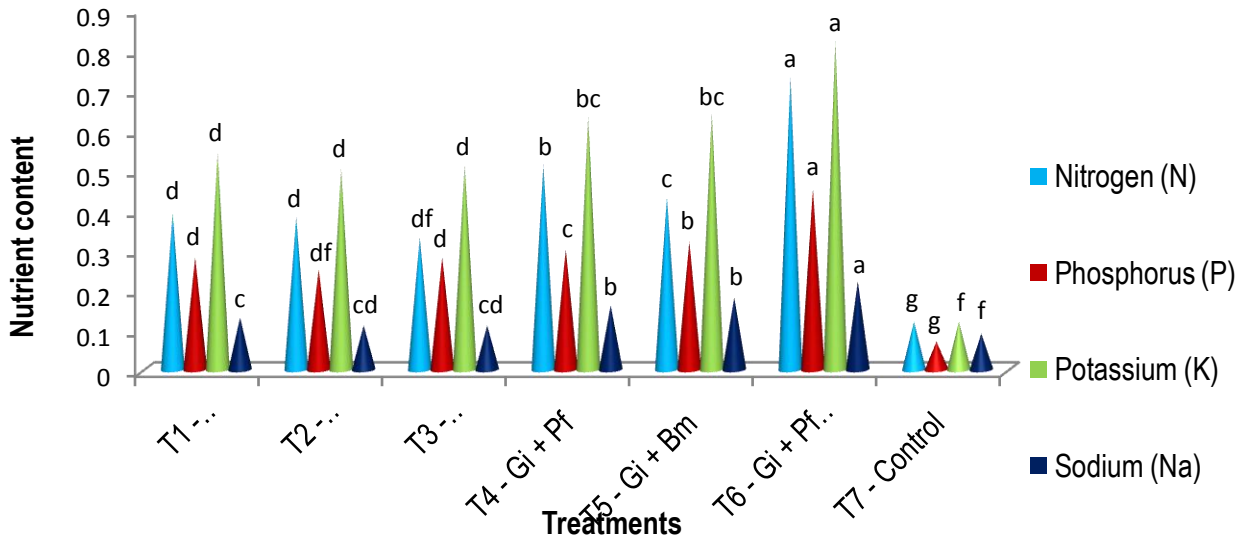

Fig 4: Reaction of different treatments on the nutrient content (%) in chilli (PLR-1) plant at 45 days inoculation. Mean values followed by the same letter are not significantly different based on Duncan's multiple range test ($p<0.05$), a>b>c.

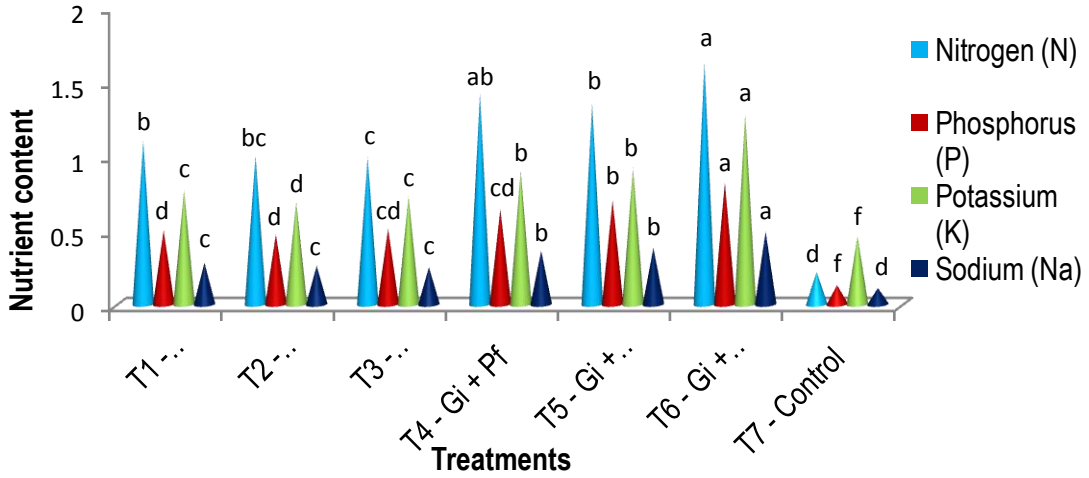

Fig 5: Reaction of different treatments on the nutrient content (%) in chilli (PLR-1) plant at 90 days inoculation. Mean values followed by the same letter are not significantly different based on Duncan's multiple range test ($p<0.05$), a>b>c.

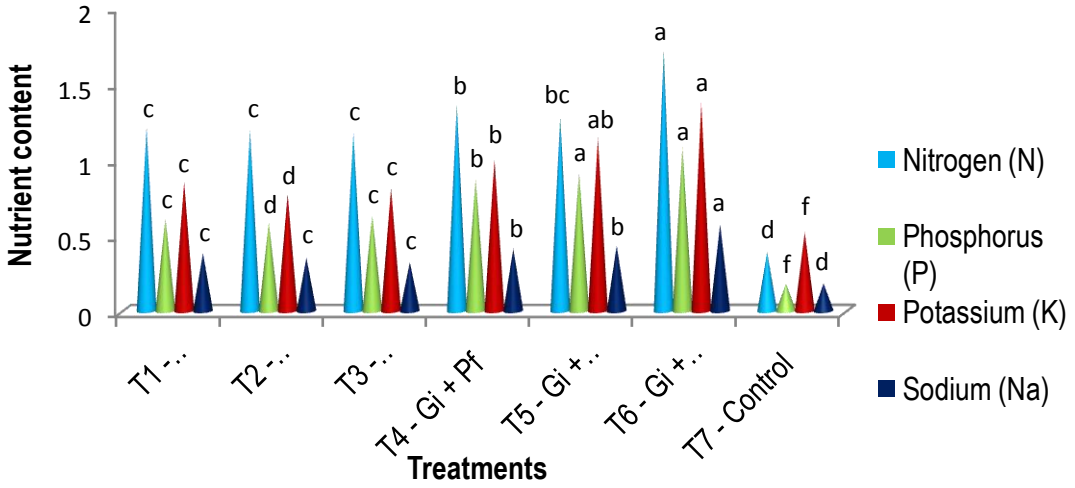

Fig 6: Reaction of different treatments on the nutrient content (%) in chilli (PLR-1) plant at 120 days inoculation. Mean values followed by the same letter are not significantly different based on Duncan's multiple range test ($p<0.05$), a>b>c

APPLICATION

This method is an alternative to hazardous agrochemicals and produce more plant growth. Also protect the agricultural ecosystem.

CONCLUSION

In Conclusion, The use of natural resources for instance AM fungi, Rhizobacteria and Phosphate solubilizing microorganisms, release up a new horizon for enhanced besides protecting the agro-ecosystems form hazards of agrochemicals. Consequently, the results in the present experiment showed that combined inoculation produced good results in plant growth and nutrient values. Besides the AM fungi and bacterial population were increased only in the single treatments. Our results obviously suggested that, all treatments such as single, dual, combined were showed superior results when comparing with control. Hence, such combination can be recommended after further testing in the field for producing better growth of chilli.

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