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Interaction of VAM Fungi with *Bradyrhizobium japonicum* and *Trichoderma viride* on Some Physiological Parameters of Soybean

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A pot experiment was performed to see the synergistic effect of co-inoculation of two indigenous arbuscular mycorrhizal (AM) fungi with other beneficial microbes on some of the physiological parameters in *Glycine max* (L.) Merrill. A prominent improvement in physiological parameters was observed after 120 days in comparison to control. Among all the physiological parameters studied, total chlorophyll content, root and shoot phosphorus, acidic and alkaline phosphatase, percentage of protein and oil content were highest in the combination of *Glomus mosseae* + *Acaulospora laevis* + *Trichoderma viride* + *Bradyrhizobium japonicum* over other treatments and control. The stomatal conductance in morning (lower and upper epidermis) and evening (lower and upper epidermis) was found to be highest with *G. mosseae* + *A. laevis* + *T. viride* treatment. *G. mosseae* alone showed best increment in arbuscular mycorrhizal (AM) spore number and percent mycorrhizal root colonization. Overall results suggest that although, all co-inoculation treatments showed beneficial effects but the treatment with *G. mosseae* + *A. laevis* + *T. viride* + *B. japonicum* executed more pronounced response in increasing physiological parameters followed by *G. mosseae* alone and *G. mosseae* + *A. laevis* + *T. viride* respectively. Thus the co-inoculation of microbes (bioagents) with AM fungi in rhizosphere of soybean has positive effect on the different physiological growth parameters.

Key words: AM fungi, interaction, *Trichoderma viride*, *Bradyrhizobium japonicum*, *Glycine max.*

Soybean is an essential component of cropping systems throughout the world, particularly in developing countries such as India. Soybean is a comparatively new leguminous oil-seed crop for the plains of India. Soybean is an important source of inexpensive and high quality content of protein and oil[31]. With an average protein content of 40% and oil content of 20%, soybean has the highest protein content of all food crops and is second only to groundnut in terms of oil content among food legumes. Compared to other protein-rich foods such as meat, fish and eggs, soybean is by far the cheapest[30]. Due to increasing population leads to the consequent increased demand for food production and food quality in the world, which requires the improved agronomic strategies avoiding the high input costs. Recently biofertilizers such as Rhizobia and Mycorrhizal fungi are steadily receiving increased attention and recognition in this regard from scientists. This could be attributed to the fact that they pose no ecological threats, usually have a long lasting effect if properly managed can out-yield recommended doses of chemical fertilizers since microbial inoculants such as Mycorrhizal fungi enhance plant productivity directly or indirectly[33, 43].

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Arbuscular Mycorrhizal fungi (AMF) are important component of the microbiota, mutualistic symbiotic soil fungi that colonize the roots of most crop plants[14, 59]. They can absorb and cycle the nutrition, have the ability to enhance host uptake of relatively immobile nutrients particularly phosphorus (P) and other trace elements have also been reported to be transported in higher amounts towards the plant growth[9]. Inoculation of AM fungi has increased the yield of numerous field-grown crops, including soybean[9], tomato, *Capsicum*[58]. Moreover, it was demonstrated that mycorrhizae can affect the seed protein and oil content of leguminous crops[11]. Potential use of AM fungi in agriculture has received much attention to reduce the use of chemical fertilizers and pesticide in the past decades[52, 21, 28, 43]. As nitrogen is a limiting factor for soybean productivity, the activity of *Bradyrhizobium* is extremely important both from economic and environmental viewpoints. So inoculation of soybean by *B. japonicum* significantly increased nodulation, yield and seed quality[41]. A synergistic beneficial effect of dual inoculation with mycorrhizal fungi as well as rhizobium was found to improve growth and protein contents of legumes[42]. Furthermore, legumes like soybean can form tripartite symbiotic association with nodule –inducing rhizobia and AM fungi simultaneously, which may benefit both P and N efficiency[32]. The application of *Trichoderma* to the soil as a biocontrol agent, in poly house or under field conditions, not only resulted in reduced disease severity but also enhanced plant growth[20, 28].

Keeping the above in view, the present study was undertaken to investigate the efficacy/ effect of AM fungi (*G. mosseae* and *A. laevis*), *T. viride* and *B. japonicum* for enhancing the physiological parameters of soybean plant.

MATERIAL AND METHODS

Collection of soil sample

Composite soil sample from rhizospheric soil of soybean was collected from Botanical Garden, Kurukshetra University, Kurukshetra and kept in sterilized polythene bags at 10°C till further processing.

Isolation of dominant AM fungi from soil samples

Isolation of dominant AM spores were done by using 'Wet Sieving and Decanting Technique' of Gerdemann and Nicolson (1963). Spores were then picked by hypodermic needle under stereobinocular microscope.

Mass Multiplication of AM spores

Dominant AM spores *Glomus mosseae* (Nicol. and Gerd.) and *Acaulospora laevis* (Gerd. & Trappe) were mass multiplied by using wheat as host plant.

Mass culture of Trichoderma viride

T. viride was isolated from the soil and then further mass produced by using wheat bran and saw dust medium which was prepared by using wheat bran, saw dust and distilled water in the ratio of 3:1:4.

Mass culture of Bradyrhizobium japonicum

B. japonicum was grown on nutrient broth medium for 24 hrs for proper growth of bacteria.

Preparation of pot mixture

Surface sterilized seeds of soybean were grown in experimental earthen pots (30 x 30cms) in a sand soil mixture (300: 1500 gm). To each pot 10 % (w/w) of inoculum of each AM fungi (*G. mosseae* or *A. laevis*) was added alone and in combination with *T. viride* and *B. japonicum*. The experiment was carried out at constant temperature ($25^0 \pm 2^0$C), humidity (50-70%) and *p*H (6.8±0) in aseptic conditions of a Glass House. Observations were recorded on different growth parameters of soybean after 120 days of inoculation. Different treatments used during the present investigation were as follows:

1. Control (without any bioinoculant)
2. *Glomus mosseae* (G)
3. *Acaulospora laevis* (A)
4. *Trichoderma viride* (T)
5. *Bradyrhizobium japonicum* (B)
6. *G. mosseae* + *A. laevis* (G + A)
7. *G. mosseae* + *T. viride* (G + T)
8. *G. mosseae* + *B. japonicum* (G + B)
9. *A. laevis* + *T. viride* (A + T)
10. *A. laevis* + *B. japonicum* (A + B)
11. *T. viride* + *B. japonicum* (T + B)
12. *G. mosseae* + *A. laevis* + *T. viride* (G + A + T)
13. *G. mosseae* + *T. viride*+ *B. japonicum* (G + T + B)
14. *G. mosseae* + *A. laevis* + *B. japonicum* (G + A + B)
15. *A. laevis* + *T. viride* + *B. japonicum* (A + T + B)

16. *G. mosseae + A. laevis + T. viride + B. japonicum* (G + A + T + B)

Quantification of AM spores

It was done by Adholeya and Gaur 'Grid Line Intersect Method'[2]. Spores were counted under stereo binocular microscope by using a counter.

Identification of AM fungi

For identification of AM spores, the keys of Walker[61]; Scheneck and Perez[51]; Morton and Benny[36]; Mukerji[38] and Sharma *et al.*,[53, 54] were followed.

Mycorrhizal root colonization and growth parameters

After 120 days roots were uprooted, washed, blotted dry for determination of fresh root weight and mycorrhizal root colonization and then oven dried for root dry weight and P content estimation. Mycorrhizal root colonization was studied by 'Rapid Clearing and Staining Method' of Phillips and Hayman[47]. The percentage mycorrhizal root colonization was calculated by following formula:

$$\text{Percentage root colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number root segments studied}} \times 100$$

Estimation of total chlorophyll

The chlorophyll content was estimated by using Arnon's method[5] by using 80% acetone as solvent. Total chlorophyll (total chl), chlorophyll a (chl a) and chlorophyll b (chl b) was calculated by the standard formula.

Stomatal conductance estimation

The stomatal conductance of all experimental plants was measured by using Porometer (AP4- Delta T devices, Cambridge, UK) after 120 days of inoculation in morning and evening.

Phosphorus estimation

The phosphorus content of roots and shoots of all experimentally plants was estimated by 'Phospho-vanadomolybdate yellow colour method' [23] after 120 days.

Phosphatase estimation

Phosphatase activity was assayed by using p-nitrophenyl phosphate (PNPP) as substrate which is hydrolyzed by the enzyme to p-nitrophenol. For this ice cold sodium acetate buffer (0.05M with pH 4.8) for acid phosphatase and sodium carbonate-bicarbonate buffer (0.05M with pH 10) for alkaline phosphatase activity was used and was measured in terms of IU/g FW.

Protein estimation

Protein was estimated by the method of Bradford[12] using coomassive brilliant blue G-250 dye.

Oil extraction

Oil was extracted by petroleum ether of boiling range between 40-60^{0}C using the Soxhlet's procedure[4]. Five replicates of each treatment were taken.

Statistical analysis

Data were analyzed for significance using one-way analysis of variance (ANOVA) and the differences contrasted using a Duncan's multiple range test (DMRT) at *p* d" 0.05. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 11.5).

RESULTS AND DISCUSSION

Recognition of the complexity of interactions among microbes in the rhizosphere has led to co-inoculation of crops with both VAM fungi and other rhizosphere microorganisms.

Chlorophyll content

One of the most important indicators of physiological activity is the rate of photosynthesis, which is related to the chlorophyll content of plants. In the study, the chlorophyll content in treated plants was higher than those in non-inoculated plants (Table 1). The best results were observed in combination with *G. mosseae + A. laevis + T. viride + B. japonicum* and lowest in control. Second most effective results were observed in the plants treated with *Glomus mosseae* alone. The increase in total chlorophyll content may be due to increased uptake of phosphorus which increases the photosynthetic activity of the plants and ultimately increase the chlorophyll content in plants. Similar findings were also reported by Yadav *et al.*[65]; Rajasekaran *et al.*[48]; Karthikeyan *et al.*[27] and Arumugam[6]. Wu and Zou[63] indicated that the beneficial effect of mycorrhiza and other bioinoculants could contribute to high chlorophyll content in *Arachis hypogea*. The results are in accordance with the study of Nagarajan and Mahadevan[39] who observed that inoculation of *Helianthus annus* with AMF (*G.*

mosseae, G. deserticola, G. aggregatum and *Gigaspora*) showed significant increase in chlorophyll content in comparison to control. Furthermore, since the formation of mycorrhizae often leads to increases in the leaf area ratio and to leaf hydration, the effect of mycorrhizae on leaf morphology is also probably partly caused by the enhanced P nutrition[24].

Stomatal conductance

It is evident from Table 1 that after 120 days, morning (lower and upper epidermis) stomatal conductance and evening (lower and upper epidermis) stomatal conductance was maximum in combination *G. mosseae + A. laevis + T. viride + B. japonicum* and lowest in control. The progressive increase in stomatal conductance in AMF and other bioinoculant treated plants might be due to higher photosynthetic rate and number of stomata on lower surface of leaf. Stomatal conductance was maximum in lower surface of leaf in morning and evening in treated plants. AM fungi helping in uptaking the phosphorus and phosphorus influence stomatal conductance. The result was in close conformity with the findings of Auge[8]; Fidelibus *et al.*[18]; Nagarathna *et al.*.[40] and Kaushish *et al.*. 2011 [28].

Phosphorus content

The highest P content was found in the plants inoculated with AM fungi and other bioinoculants as compared to non-inoculated plants. In this context, the role of the AM fungi as phosphorus suppliers to the plant appears to be of great relevance. The increase in phosphorus content in shoots as well as root was found maximum in plants treated with *G. mosseae + A. laevis + T. viride + B. japonicum* for shoot and root followed by *G. mosseae* alone for shoot and root (Table 2). The increase in the phosphorus content with treated plants may be due to AMF hyphae network which help increase the P content of plants. The results of the present study coincide with reported findings of Jackobsen *et al.*[22] who reported that the fungal hyphae growing beyond the rhizospheric soil increase the absorptive

Table 1. Interaction of AMF, *T. viride* and *B. japonicum* on Chlorophyll Content and Stomatal Conductance of soybean after 120 days

Treatments	Chlorophyll Content (mg/100mg fresh wt.)			Stomatal Conductance ($mmol^{-2}s^{-2}$)			
	Chlorophyll a	Chlorophyll b	Total chlorophyll	Morning		Evening	
				Lower Surface	Upper Surface	Lower Surface	Upper Surface
Control	0.418±0.005^{f}	0.021±0.004^{f}	0.439±0.008^{f}	89.90±4.61^{f}	10.14± 1.53^{f}	25.2± 4.53^{f}	7.9±2.53^{f}
G	0.794±0.017^{c}	0.043±0.005^{ab}	0.838±0.009^{c}	271.80±5.26^{b}	24.40±1.78^{b}	64.96±3.39^{c}	16.10± 3.07^{bc}
A	0.527±0.008^{e}	0.028±0.005^{de}	0.554±0.011^{de}	184.00±5.11^{e}	17.18±2.82^{cd}	37.32±4.54^{e}	12.12±2.54^{e}
T	0.562±0.005^{de}	0.034±0.004^{cd}	0.570±0.012^{de}	210.00±8.15^{d}	22.82±2.04^{c}	40.40±3.64^{de}	13.62±1.61^{de}
B	0.491±0.006^{ef}	0.021±0.003^{ef}	0.512±0.004^{ef}	115.00±7.90^{ef}	12.08±2.26^{e}	29.24±3.38^{ef}	11.26±1.68^{ef}
G+A	0.617±0.011^{cd}	0.039±0.005^{b}	0.656±0.006^{cd}	249.00±6.54^{c}	23.38±2.42^{bc}	48.86±7.34^{de}	15.82±3.05^{c}
G+T	0.575±0.006^{de}	0.037±0.004^{c}	0.608±0.037^{d}	230.00±6.54^{cd}	22.80±2.04^{c}	41.54±1.63^{de}	13.66±2.47^{de}
G+B	0.495±0.007^{ef}	0.028±0.003^{de}	0.523±0.009^{e}	157.00±5.14^{de}	15.10±2.05^{de}	33.34±3.71^{ef}	12.94±0.38^{de}
A+T	0.558±0.008^{de}	0.034±0.005^{cd}	0.596±0.004^{d}	212.00±3.34^{d}	22.36±1.63^{c}	39.54±6.95^{e}	13.94±1.84^{d}
A+B	0.529±0.017^{e}	0.033±0.004^{d}	0.555±0.011^{de}	199.80±3.70^{e}	17.44±3.20^{cd}	38.34±2.28^{e}	12.72±0.34^{de}
T+B	0.495±0.008^{ef}	0.024±0.004^{e}	0.520±0.009^{e}	157.00±5.93^{de}	15.56±2.99^{de}	55.30±1.58^{d}	11.92±1.08^{e}
G+A+T	0.644±0.008^{b}	0.042±0.004^{ab}	0.687±0.009^{b}	361.00±4.43^{a}	27.90±2.32^{a}	89.08±2.80^{a}	18.44±0.50^{a}
G+T+B	0.607±0.005^{cd}	0.038±0.003^{bc}	0.646±0.002^{cd}	232.00±2.54^{cd}	15.90±2.40^{d}	46.48±4.32^{de}	14.02± 1.50^{cd}
G+B+A	0.587±0.009^{d}	0.038±0.003^{bc}	0.613±0.010^{d}	236.00±5.38^{cd}	23.38±2.42^{bc}	47.28±1.62^{de}	13.94±1.84^{d}
A+T+B	0.534±0.008^{e}	0.033±0.004^{d}	0.592±0.011^{d}	169.20±4.43^{de}	15.90±2.40^{d}	33.46±3.79^{ef}	11.54±0.99^{e}
G+A+T+B	0.825±0.005^{a}	0.048±0.004^{a}	0.875±0.002^{a}	355.00±8.70^{ab}	27.62±2.08^{ab}	70.22± 7.78^{b}	17.14±2.01^{b}

G- *Glomusmosseae*, A- *Acaulospora laevis*, T- *Trichoderma viride*, *B. japonicum*

Values represent mean ± standard error, n = 5.

Means were compared by using the least significant difference (LSD) test ($p \leq 0.05$). Data within each column followed by dissimilar letters differ significantly at $p \leq 0.05$.

surface area of the root, which result in a greater efficiency of nutrient absorption, especially slowly diffusing minerals ions like phosphorus[29]. VAM fungus not only increases the plant growth but also enhances phosphorus uptake[60, 55, 56].

Phosphatase activity

It is clearly evident from the data that phosphatase was greatly enhanced in inoculated plants in comparison to non-inoculated plants (Table 2). Highest increment in both acidic and alkaline phosphatse activity was observed in plants inoculated with *G. mosseae + A. laevis + T. viride + B. japonicum*. Second highest results were obtained in single inoculation with *G. mosseae* alone followed by triple inoculation *G. mosseae + A. laevis + T. viride*. Although acidic phosphatase activity was higher as compared to alkaline activity. It was found that plants with higher mycorrhizal root colonization had maximum phosphatase activity (acidic and alkaline). These enzymes help in mineralization of bound phosphorus into soluble form and make it available to the plants. This phosphorus is then absorbed by the plants through the AM colonized roots and thus absorbs maximum phosphorus from the soil. Major part of the beneficial effect of AM fungus is attributed to its role in phosphorus uptake and translocation through the involvement of phosphatase in the transport of phosphorus. This conclusion could be derived from the reports that mycorrhizal Trigonella roots have recorded greater acidic and alkaline phosphatse activities than non-inoculated roots[26]. The present findings are in agreement with the numerous other reports[1, 15].

Effect on root colonization and AM spore number

Percent mycorrhizal root colonization and AM spore number also increased in all AM treated plants over control (Table 3). After 120 days of inoculation, percent mycorrhizal root colonization and AM spore number were highest in plants treated with *G. mosseae* alone followed by *G. mosseae + A. laevis + T. viride + B. japonicum* treatment and lowest in the control. It was found that root colonization and AM spore number were greatly enhanced synergistically by interaction of AM fungi, *B. japonicum* and *T. viride*. *Trichoderma viride is* highly effective in root colonization by producing secondary metabolites and these

Table 2. Interaction of AMF, *T. viride* and *B. japonicum* on Phosphorus content and Phosphatase activity of soybean after 120 days

Treatments	% Phosphorus Content (ppm)		Phosphatase activity (IU/G FW)	
	Shoot Phosphorus	Root Phosphorus	Acidic Phosphatase	Alkaline phosphatase
Control	0.07±0.015^{f}	0.101±0.002^{f}	1.102±0.045^{f}	0.084±0.007^{f}
G	0.21±0.015^{ab}	0.271±0.002^{ab}	1.466±0.026^{ab}	0.295±0.013^{b}
A	0.13±0.027^{cd}	0.191±0.001^{de}	1.208±0.006^{cd}	0.149±0.010^{de}
T	0.12±0.015^{d}	0.181±0.001^{de}	1.197±0.029^{cd}	0.140±0.005^{de}
B	0.10±0.025^{e}	0.161±0.001^{ef}	1.171±0.022^{d}	0.126±0.010^{ef}
G+A	0.20±0.025^{b}	0.254±0.003^{b}	1.341±0.017^{bc}	0.228±0.006^{cd}
G+T	0.09±0.034^{ef}	0.151±0.004^{ef}	1.103±0.027^{e}	0.107±0.006^{ef}
G+B	0.15±0.032^{c}	0.220±0.003^{cd}	1.251±0.008^{c}	0.156±0.011^{de}
A+T	0.16±0.022^{c}	0.234±0.003^{cd}	1.305±0.014^{bc}	0.178±0.010^{d}
A+B	0.11±0.015^{de}	0.171±0.002^{e}	1.152±0.019^{de}	0.117±0.014^{ef}
T+B	0.14±0.019^{cd}	0.201±0.002^{d}	1.191±0.039^{cd}	0.135±0.013^{e}
G+A+T	0.19±0.024^{b}	0.263±0.003^{ab}	1.423±0.010^{b}	0.252±0.007^{c}
G+T+B	0.18±0.031^{bc}	0.251±0.002^{b}	1.327±0.010^{bc}	0.212±0.010^{cd}
G+B+A	0.15±0.020^{c}	0.210±0.003^{cd}	1.240±0.026^{c}	0.154±0.020^{de}
A+T+B	0.17±0.027^{bc}	0.244±0.003^{c}	1.308±0.019^{bc}	0.200±0.007^{cd}
G+A+T+B	0.22±0.020^{a}	0.280±0.003^{a}	1.487±0.018^{a}	0.319±0.024^{a}

G- *Glomusmosseae*, A- *Acaulospora laevis*, T- *Trichoderma viride*, *B. Japonicum*
Values represent mean ± standard error, n = 5.
Means were compared by using the least significant difference (LSD) test ($p \leq 0.05$). Data within each column followed by dissimilar letters differ significantly at $p \leq 0.05$.

metabolites enhance AMF growth and thus mycorrhizal spore number and colonization[34]. It was observed that inoculation of plants with AM fungi alone or in different combinations showed significant increase in mycorrhizal root colonization. According to Asimi *et al.,*[7], the mycorrhizal fungi and nodule symbiosis bacteria act synergistically, both in root colonization rate and on mineral nutrition uptake and growth of the plant. A significant promoting effect on mycorrhizal colonization density and frequency was observed in soybean plant when inoculated with Arbuscular Mycorrhizal fungi[25, 62, 44, 45]. The results of the present investigation indicate that colonization with more number of AM fungal species i.e. *G. mosseae* increase the percentage of mycorrhizal root colonization. Similarly AM inoculated plants exhibited higher percent root colonization by in Sunflower plant[57]. Mycorrhizal plants grow better than non- mycorrhizal plants due to the difference in the root colonization as arbuscules and vesicles of colonized root played an important role in P transfer.

Oil Content

As Table 3 shows that inoculating soybean with AM fungi significantly increased the oil content after 120 days of inoculation. In the present study, oil content was found to be increased in all treated plants than control. The highest value was observed in combination with G. *mosseae* + *A. laevis* + *T. viride* + *B. japonicum* followed by triple inoculation *G. mosseae* + *A. laevis* + *T. viride*. However, *G. mosseae* alone also showed better results. The increase in oil content could be due to uptake of soil phosphorus by mycorrhizal roots is attributed to extensive hyphae in the soil which absorb and translocate phosphorus to root and this availability of phosphorus may be change the lipid composition of the plants. Egberongbe *et al.*[16] found that *Glomus mosseae* and *Trichoderma harzianum* have higher values of oil content of soybean than control. The oil content was significantly increased with *Bradyrhizobium* and AMF inoculation for soybean[50], groundnut[17, 13]. According the positive effect of Mycorrhiza and other bioinoculants on oil content, it can concluded that AMF helps to absorb more of the nutrients especially away from the P depletion zone and resulted in the better increment in oil content of soybean plant in comparison to the non-mycorrhizal plants.

Protein Content

Results depicted in Table 3 showed that inoculated plants showed significant increase in

Table 3. Interaction of AMF, *T. viride* and *B. japonicum* on different parameters of soybean after 120 days

Treatments	% Root colonization	AM spore number per 10 g of soil	Protein content	Percent of Oil
Control	39.93±5.11^{f}	13.6±4.21^{f}	34.02±0.286^{f}	17.88±0.303^{f}
G	93.26±3.96a	95.2±3.19^{a}	42.48±0.349^{ab}	24.10±0.158^{ab}
A	60.65±4.15^{d}	56.0±5.43^{d}	35.18±0.349^{ef}	19.48±0.349^{ef}
T	77.35±1.79^{c}	71.4±4.27^{c}	35.62±0.414^{ef}	22.06±0.304^{de}
B	44.42±2.92^{e}	23.0±3.03^{ef}	36.60±0.316^{e}	19.08±0.383^{ef}
G+A	87.44±2.61^{b}	82.4±2.40^{b}	39.52±0.303^{cd}	23.80±0.158^{c}
G+T	78.64±5.18^{c}	72.2±2.68^{c}	38.36±0.320^{d}	23.00±0.158^{cd}
G+B	45.61±6.96^{e}	38.2±4.96^{e}	37.16±0.694^{de}	22.80±0.316^{d}
A+T	76.35±2.84^{c}	68.2±5.76^{cd}	37.69±0.255^{de}	21.70±0.158^{e}
A+B	68.29±4.07^{cd}	57.0±4.69^{d}	36.20±0.158^{e}	21.50±0.158^{e}
T+B	48.42±4.20^{de}	42.0±5.91^{de}	35.78±0.414^{ef}	21.30±0.158^{e}
G+A+T	89.97±6.24^{b}	83.8±4.43^{b}	41.08±0.708^{b}	24.00±0.158^{b}
G+T+B	84.62±3.34^{bc}	78.2±4.14^{bc}	40.20±0.308^{c}	23.50±0.316^{c}
G+B+A	50.30±3.92^{de}	48.4±5.02^{de}	39.20±0.158^{cd}	22.60±0.316^{d}
A+T+B	53.22±5.03^{de}	52.4±5.22^{d}	36.96±0.676^{e}	22.00±0.158^{de}
G+A+T+B	90.90±5.52^{ab}	92.0±3.80^{ab}	42.88±0.496^{a}	24.88±0.303^{a}

G- *Glomusmosseae*, A- *Acaulospora laevis*, T- *Trichoderma viride*, *B. Japonicum*

Values represent mean ± standard error, n = 5.

Means were compared by using the least significant difference (LSD) test ($p \leq 0.05$). Data within each column followed by dissimilar letters differ significantly at $p \leq 0.05$.

protein content in comparison to the control. After 120 days, higher value of protein content was maximum in combination i.e. *G. mosseae + A. laevis + T. viride + B. japonicum* followed by single inoculation of *G. mosseae* and triple inoculation *G. mosseae + A. laevis + T. viride* respectively and lowest in control. There are many similarties between Rhizobial and VAM symbiosis, which suggest common properties in interaction with plants[64]. Both microsymbionts are surrounded in the established stage of the symbiosis by plat-derived membranes: the pribacteriod membranes in the infected nodule cells and the prehaustorial membranes around arbuscules in the mycorrhizal roots, respectively. These interfaces are characterized by symbiosis- specific proteins[46]. Applying *Rhizobium,* individually or in combination with AMF had stimulating effect on seed protein concentration. The results of the present study coincide with the reported finding of Mobasser *et al.,*[35] who reported that with the inoculation of *G. mosseae* and *T. harzianum* affect the protein content of corn. These results are in agreement with Al- Karaki and Clark[31], suggested that root colonization by AMF may affect protein composition of plant by altering P nutrition or by eliciting other metabolic responses in the host plant. Mostafavian *et al.*[37] also reported higher percentage of protein in soybean when inoculated with mycorrhiza and other biofertilizers.

CONCLUSION

On the basis of findings, it can be concluded that the co-inoculation of AM fungi with other bioinoculants is more effective in increasing total chlorophyll content, root and shoot phosphorus, acidic and alkaline phosphatase, stomatal conductance, percentage of protein and oil content of soybean plant after 120 days. This increment could be attributed to the enhanced uptake of nutrients, better water absorption, increased surface area of roots and also secretion of some enzymes by inoculated microorganisms. Inoculation of *G. mosseae + A. laevis + T. viride + B. japonicum* was the most efficient combination in most of the physiological growth parameter of soybean as compared to untreated plants after 120 days of treatment under polyhouse condition. These results could help us to gain a better insight into the effect of bioinoculants and AM fungi on soybean growth and may also used practical applications in field of soybean.

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