Bangladesh J. Bot. **54**(1): 39-47, 2025 (March) DOI: https://doi.org/10.3329/bjb.v54i1.80589

MICROBIAL INTERACTION OF ARBUSCULAR MYCORRHIZAL FUNGI AS A BIOENHANCER ON GERMINATION, GROWTH AND YIELD OF SOYBEAN

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Keywords: Arbuscular Mycorrhiza Fungi, Symbiosis, Soybean, Bioenhancer, Increase pod, Soil health, Yield

Abstract

The symbiosis between soybeans and Arbuscular Mycorrhiza Fungi (AMF) has drawn significant research interest due to its potential in sustainable agriculture and improved crop yields. Thus, the present study was carried out to evaluate the response of AMF as bioenhancers in case of BARI Soybean-6. Doses were 12 with 4 replications and the total number of pots was 48. The results indicated that combined application of AMF, vermicompost (VC), nitrogenous fertilizer (NF), and phosphorus (P) increased germination up to 100%, fresh root weight (0.831 g), dry root weight (0.219g), fresh shoot weight (4.522g), dry shoot weight (1.244 g), root length (13 cm), shoot length (59.75 cm), number of leaves (40.75), pod number (5.75), spore population (231.3 ± 12.0) and root colonization (30.0 ± 2.9). This combined effect of treatment T_{12} was found to be statistically significant in most cases. .

Introduction

Soybean (*Glycine max* L. Merrill) is a dominant oil- source of oil, protein, vitamins, minerals, and some functional elements of the human body for instance isoflavones, lecithin and polysaccharides (Islam 2019). In Bangladesh, soybean occupies 0.041 million ha of land and its production is 0.064 million tons (BBS 2020). It accounts for approximately 50% of the total production of global oilseed crops. Among the plant-based foods, it is fairly unique because the protein in soybean products is considered one of the most complete proteins. It is a bushy, free-branching annual legume. The symbiosis of Arbuscular mycorrhiza fungi (AMF) with plants had been reported 400 million years ago. Such types of links are established as a succession of biological processes, which lead to a variety of useful effects in both natural ecosystems and agricultural biotas (Bagyaraj 2014). Mycorrhizae are symbiotic with almost all types of plants (Shi *et al.* 2023) and nearly 90% of terrestrial plants are able to form a symbiosis with AMF, which is considered evolutionarily important for plants to cope with many environmental challenges (Begum *et al.* 2019). Mycorrhizae are located in the roots of vascular plants. The symbiotic association of AMF is a classic example of a mutualistic relationship, which can regulate the growth and development of plants (Pringle *et al.* 2009). The mycelial network of fungi extends under the roots of the plant and promotes nutrient uptake that is otherwise not available (Johnson *et al.* 2003). Many plants cannot absorb sufficient inorganic phosphate (Pi) for structural or metabolic use via their root system (direct pathway) and partly rely on the uptake of Pi through the AMF hyphal network (the mycorrhizal pathway) (Ferrol *et al.* 2019). Mycorrhizae infect plant roots and then produce an intensive network of hyphae. The external hyphae of mycorrhizae can absorb nutrients, thereby increasing nutrient availability for plants, mycorrhizae

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also increase plant resistance to drought (Bhardwaj *et al.* 2023), and soil salinity and have the potential to act as biological control agents against various soil-borne-pathogens (Evelin *et al.* 2019). Fungal mycelium colonizes the roots of many plants even if they belong to different species, resulting in a common mycorrhizal network (CMN). This CMN is considered a primary component of the terrestrial ecosystem with its significant effects on different plant communities, particularly on invasive plants and the fungal-mediated transport of phosphorus (P) and nitrogen (N) to plants (Goussous and Mohammad 2009). During colonization distinct structures (*i.e.* arbuscules, vesicles) are formed by the AMF within the host roots (Harrison 1999). From the symbiosis, plants gain minerals such as N, P, K, S, Mg, Fe, Mn, Cu, Zn and protein contents in exchange for carbon. The plant makes sugars by photosynthesis and supplies them to the fungus, and the fungus supplies the plant water and mineral nutrients, such as phosphorus, and nitrogen taken from the soil (Smith and Smith 2008). AMFs are used as bio-inoculants, and researchers encourage their use as prominent bio-fertilizers in sustainable crop productivity and plant growth (Bender *et al.* 2015). Therefore, the purpose of this study was to evaluate the impact of AMF on soybean seedling's growth and yield production of soybean.

Materials and Methods

The study was conducted in the net house of the Botanical Garden and Research Laboratory of the Botany Department, Jagannath University, Dhaka-1100. Geographically, the experimental site is located at 23°42’37” N latitude and 90°24’40” E longitude. The experiment was conducted from September 2020 to November 2021, during the cold season. BARI Soybean-6 was used as a plant material for the experiment. The experiment consists of 12 treatments. The experiment was laid out in a Completely Randomized Design (CRD) with 4 replications and the total number of pots was 48. Doses decided based on the symbiotic association by AMF provided on inoculum form (500-600 mycorrhizas spore/5 kg soil) per pot requirement by recommended doses on soybean. The treatments are *viz.* T_1: Control, T_2: Vermicompost (VC) (5g), T_3: Arbuscular mycorrhiza (AM) (25g), T_4: Phosphorus (P 100%) (2g), T_5: Nitrogenous fertilizer (NF 100%) (3g), T_6: AM+100% P (AM 25g and 2g TSP), T_7: AM+50% P (AM 25g and 1g TSP), T_8: AM+NF (AM 25g and Urea 3g), T_9: AM+VC (AM 25g and 5g), T_{10}: AM+VM+P (AM 25g, VC 5g and 0.5g TSP), T_{11}: AM+ VM+NF (AM 25g, VC 5g and Urea1g), T_{12}: AM+VM+P+NF (AM 25g, VC 5g, Urea 1g and TSP 0.5g). Treatment of AMF is applied before and after transplanting. The root slide technique estimated the percentage of AM infection (Read 1991). The presence or absence of infection in the root pieces was recorded and the percent infection was calculated as follows:

$$\text{Root infection (\%)} = \frac{\text{Number of AM Positive segments}}{\text{Total members of segments spore}} \times 100$$

The data of the different treatment's fresh shoot weight, root weight, shoot length, number of pods, and spore population were subjected to statistical analysis using analysis of variance. The result was compared using the Least Significant Difference (LSD). This statistical analysis was performed by Windows SPSS software.

Results and Discussion

Results presented separately under the following headings: germination (%), fresh root weight, dry root weight, fresh shoot weight, dry shoot weight, root length, shoot length, number of leaves, pod numbers, spore population and root colonization. The present study was found statistically significant at 25, 35, 45, and 55 days after sowing (DAS). The germination percentage

was significant in T_{12}. The highest fresh shoot weight (2.652 g at 25 DAS, 3.49 g at 35 DAS, 4.326 g at 45 DAS and 4.522 g at 55 DAS) was shown in Table 1. The fresh root weight (0.652 g at 25 DAS, 0.759 g at 35 DAS, 0.757 g at 45 DAS and 0.831 g at 55 DAS) was presented in Table 2. Dry shoot weight (0.878 g at 25 DAS, 0.919 g at 35 DAS, 1.062g at 45 DAS and 1.244 g at 55 DAS) was shown in Table 3. Dry root weight (0.129g at 25 DAS, 0.185 g at 35 DAS, 0.183g at 45 DAS and 0.219g at 55 DAS) was presented in Table 4. The root length (12.25 cm at 25 DAS, 12.75 cm at 35 DAS, 13 cm at 45 DAS and 13 cm at 55 DAS) was presented in the Fig. 1. The shoot length (41.75 cm at 25 DAS, 52.75 cm at 35 DAS, 59.5 cm at 45 DAS and 59.75 cm at 55 DAS) was shown in Fig. 2. The maximum number of leaves (28 at 25 DAS, 28.75 at 35 DAS, 40 at 45 DAS and 40.75 at 55 DAS) was shown in Fig. 3. The highest pod number (4.5 at 35 DAS, 5.75 at 45 DAS, and 5.75 at 55 DAS) were recorded with T_{12} treatment and followed by T_{11} (Fig. 4). The highest spore population (231.3 ± 12.0) and the highest root colonization (30.0 ± 2.9)

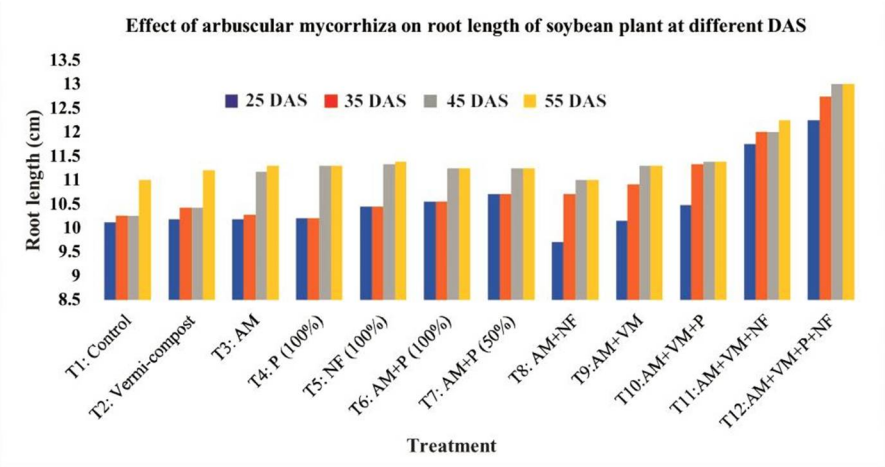

Fig. 1. Effects of arbuscular mycorrhiza on root length of soybean in 25, 35, 45, and 55 DAS.

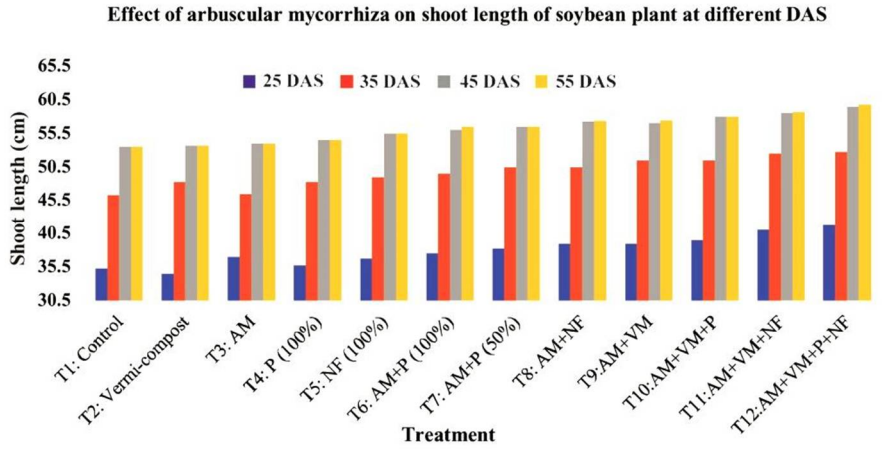

Fig. 2. Effects of arbuscular mycorrhiza on shoot length of soybean in 25, 35, 45 and 55 DAS.

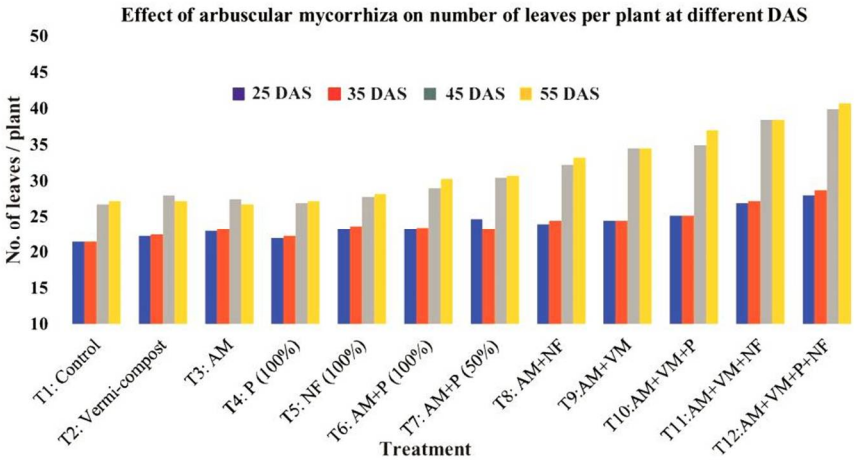

Fig. 3. Effects of arbuscular mycorrhiza on leaves number per plant of soybean.

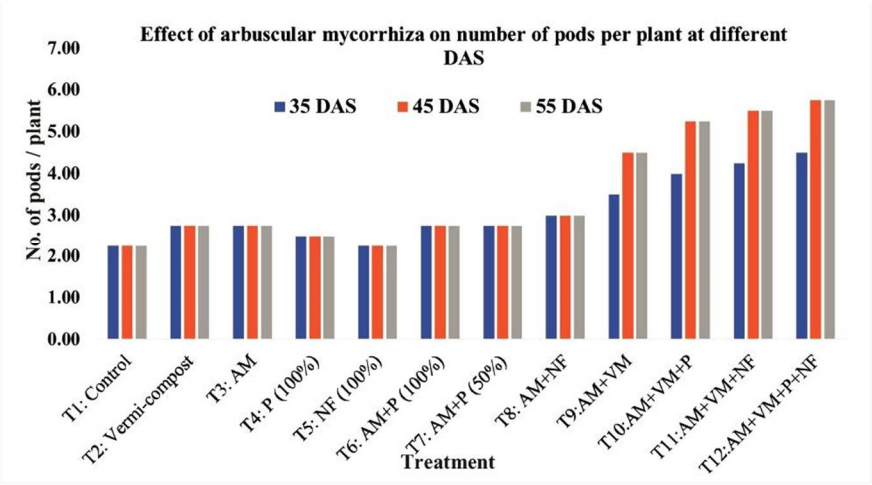

Fig. 4. Effects of arbuscular mycorrhiza on the number of pods per soybean plant.

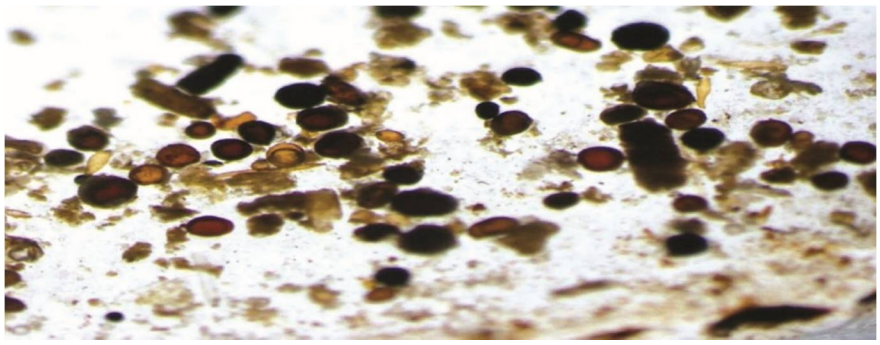

Fig. 5. Mycorrhizal spores (treatment T_{12} at 55 DAS) under stereomicroscope.

Table 1. Effects of arbuscular mycorrhiza on fresh shoot weight of soybean.

Treatment	Fresh shoot weight (g)			
	25 DAS	35 DAS	45 DAS	55 DAS
T_1: Control	2.5685c	3.2260de	3.6352f	3.7598e
T_2: Vermicompost	2.5730c	3.0648f	3.7425ef	3.9210d
T_3: AM	2.5593c	3.1768e	3.7085ef	3.9378d
T_4: P (100%)	2.6428a	3.3613bc	3.7278ef	4.0305c
T_5: NF (100%)	2.5665c	3.2065de	3.8028de	4.2188b
T_6: AM+P (100%)	2.5810bc	3.2948cd	3.9213bc	4.2733b
T_7: AM+P (50%)	2.6473a	3.3465bc	3.9013cd	4.2943b
T_8: AM+NF	2.6180ab	3.2873cde	3.8985cd	4.2580b
T_9: AM+VM	2.5902bc	3.3165bcd	3.9275bc	4.2710b
T_{10}: AM+VM+P	2.6445a	3.3885abc	4.0318b	4.4943a
T_{11}: AM+VM+NF	2.6360a	3.4123ab	4.2312a	4.5197a
T_{12}: AM+VM+P+NF	2.6520a	3.4908a	4.3267a	4.5222a
CV (%) SE (±)	1.08	2.36	2.03	1.39
	0.0199	0.0551	0.0560	0.0414

In a column, having common letters does not differ significantly at 5% level of DMRT.

Table 2. Effects of arbuscular mycorrhiza on fresh root weight of soybean in 25, 35, 45, and 55 DAS.

Treatment	Fresh root weight (g)			
	25 DAS	35 DAS	45 DAS	55 DAS
T_1: Control	0.5553f	0.6390e	0.7170de	0.7418e
T_2: Vermicompost	0.5853de	0.6730d	0.7253cd	0.7508de
T_3: AM	0.6365ab	0.6968bc	0.7032e	0.7613d
T_4: P (100%)	0.5893de	0.7028bc	0.7150de	0.7933bc
T_5: NF (100%)	0.6058cd	0.7143b	0.7298bcd	0.7865c
T_6: AM+P (100%)	0.5668ef	0.6888cd	0.7583a	0.8030b
T_7: AM+P (50%)	0.5913de	0.6873cd	0.7470ab	0.8208a
T_8: AM+NF	0.6385ab	0.7153b	0.7313bcd	0.8265a
T_9:AM+VM	0.6310abc	0.7393a	0.7440abc	0.8262a
T_{10}:AM+VM+P	0.6215bc	0.7420a	0.7310bcd	0.8282a
T_{11}:AM+VM+NF	0.6430ab	0.7470a	0.7438abc	0.8315a
T_{12}:AM+VM+P+NF	0.6520a	0.7590a	0.7578a	0.8313a
CV (%) SE (±)	3.36	2.14	1.84	1.33
	0.0145	0.0107	9.534e-03	7.503e-03

In a column, having common letters does not differ significantly at 5% level of DMRT.

were recorded at 35 days in T_{11} which was significantly higher over all other treatments (Table 5). The present results indicated that when AMF, VC, P, and NF were applied combinedly, it increased all parameters. The maximum growth and yield were observed in the T_{12} treatment, spore population and root colonization were observed in T_{11} and the lowest was observed in the

control. However, inoculation with AMFs has been identified as an eco-friendly approach to improve soil fertility (Dalcortivo *et al.* 2018). AMF is the most widespread soil microorganisms that form a symbiotic relationship with more than 80% of plants (Prasad *et al.* 2017). The increase in the host plant nutrient uptake is due to the characteristics of AMF mycelium. These mycelia or hyphae absorb nutrients osmotically and explore more surface area compared to non-mycorrhizal roots (Duponnois *et al.* 2011). In return, AMF benefits carbohydrates from the host plants (Diagne *et al.* 2020). AMF obtains up to 20% of photosynthetic carbohydrates from the host plant (Kaiser

Table 3. Effects of arbuscular mycorrhiza on dry shoot weight of soybeans in 25, 35, 45 and 55 DAS.

Treatment	Dry shoot weight (g)			
	25 DAS	35 DAS	45 DAS	55 DAS
T_1: Control	0.7543b	0.7630d	0.8350d	0.8315f
T_2: Vermicompost	0.7763b	0.7615d	0.8463d	0.8508ef
T_3: AM	0.7653b	0.7940cd	0.8518d	0.8593ef
T_4: P (100%)	0.6928c	0.8010cd	0.8570d	0.8615def
T_5: NF (100%)	0.7555b	0.8242bcd	0.8578d	0.9080cdef
T_6: AM+P (100%)	0.7697b	0.8585abc	0.8735cd	0.9138cde
T_7: AM+P (50%)	0.7560b	0.8520abc	0.9342bc	0.9423bcd
T_8: AM+NF	0.7685b	0.8618abc	0.9587b	0.9560bc
T_9:AM+VM	0.7718b	0.8658abc	0.9565b	0.9710bc
T_{10}:AM+VM+P	0.8540a	0.9070a	0.9715b	1.0185b
T_{11}:AM+VM+NF	0.8590a	0.8988ab	1.1318a	0.9753bc
T_{12}:AM+VM+P+NF	0.8788a	0.9192a	1.0625a	1.2445a
CV (%) SE (±)	3.11	6.22	5.33	6.05
	0.0172	0.0370	0.0350	0.0404

In a column, having common letters does not differ significantly at 5% level of DMRT.

Table 4. Effects of arbuscular mycorrhiza on dry root weight of soybean in 25, 35, 45 and 55 DAS.

Treatment	Dry root weight (g)			
	25 DAS	35 DAS	45 DAS	55 DAS
T_1: Control	0.0955c	0.1203b	0.1348d	0.1335d
T_2: Vermicompost	0.0928c	0.1270b	0.1248d	0.1215d
T_3: AM	0.1050bc	0.1333b	0.1313d	0.1390d
T_4: P (100%)	0.1168ab	0.1295b	0.1700bc	0.1718c
T_5: NF (100%)	0.0935c	0.1142b	0.1688bc	0.1710c
T_6: AM+P (100%)	0.0953c	0.1273b	0.1670c	0.1723c
T_7: AM+P (50%)	0.1233a	0.1183b	0.1808ab	0.1855bc
T_8: AM+NF	0.1200ab	0.1360b	0.1890a	0.1860bc
T_9:AM+VM	0.1193ab	0.1660a	0.1870a	0.1865bc
T_{10}:AM+VM+P	0.1220a	0.1778a	0.1862a	0.1932b
T_{11}:AM+VM+NF	0.1140ab	0.1813a	0.1875a	0.1955b
T_{12}:AM+VM+P+NF	0.1292a	0.1850a	0.1833a	0.2198a
CV (%) SE (±)	10.39	11.06	5.45	7.75
	8.119e-03	0.0112	6.455e-03	9.478e-03

In a column, having common letters does not differ significantly at 5% level of DMRT.

Table 5. Effects of arbuscular mycorrhiza on root colonization and spore population of soybean in 35 DAS.

Treatment	Spore number per 100 g soil[a]	Root colonization[a] (%)
T_1: Control	93.3 ± 5.3	20.0 ± 2.9
T_2: Vermicompost	105.3 ± 10.1	20.0 ± 2.9
T_3: AM	184.6± 9.3	30.0 ± 2.9
T_4: P (100%)	108.6 ± 8.5	20.0 ± 2.9
T_5: NF (100%)	107.6 ± 15.6	30.0 ± 2.9
T_6: AM+P (100%)	160.6 ± 8.6	20.0 ± 2.9
T_7: AM+P (50%)	175.0 ± 12.7	20.0 ± 2.9
T_8: AM+NF	131.6 ± 13.6	20.0 ± 2.9
T_9:AM+VM	160.6 ± 5.0	20.0 ± 2.9
T_{10}:AM+VM+P	181.0 ± 15.1	20.0 ± 2.9
T_{11}:AM+VM+NF	231.3 ± 12.0	30.0 ± 2.9
T_{12}:AM+VM+P+NF	206.6 ± 11.0	20.0 ± 2.9

[a]Per cent root colonization & spore population are the means. S.E. of four independent counts.

et al. 2015). Moreover, Bhuiyan *et al.* (2012) found a similar result on cabbage inoculated by AMF, (*Glomus intraradices*). In that study, it was observed that the effect of AMF which was important in cabbage concerning morphological parameters (fresh root weight) increased in the root. It is revealed from the results that AMF application with VC, P and NF exerted some effect on increasing the fresh shoot weight of shoot. Similarly, there was an increase in plant height of 42.58 cm in case of soybeans (Subaedah *et al*. 2024). AMF does not only have an impact on plant growth and production but it has been also reported that they improve some soil characteristics, such as soil aggregation, soil nutrients availability, water retention, microbial activities, nitrogen, carbon, and phosphorus cycling, and soil acidity correction (Parihar *et al*. 2020). Moreover, another result was observed by Akkopur and Demir (2005) on tomato inoculated by *G. intraradices*. They observed that the effect of AMF which was important in tomato concerning morphological parameters (fresh root weight) increased in the root. Result of this study concurred with Wang (2006) who worked on spinach inoculated by *G. intraradices*. He observed that the positive effect of AMF which was important in spinach concerning morphological parameters (dry root weight) increased in the root. The maximum number of mycorrhizal spores in treatment was found in the treatment T_{12} at 45 and 55 DAS under stereomicroscope (Fig. 5). Our result concurred with these researchers. Lastly, it can be concluded that all the physical parameters gave positive responses due to the combined application of AM, VC, P and NF in T_{11} and T_{12} treatments where T_{12} is the best. The minimum response was found in T_1 as (control).

The findings of the present study may conclude that AMF influences positively various growth, yield, and biochemical parameters in comparison to the control. However, T_{11} and T_{12} showed optimum results among different treatments and T_{12} was the best between these two treatments. The AMF effectively extends the root area of soybeans and exists by taking sugars from plants in exchange for moisture and nutrients gathered from the soil by the fungal strands. The symbiotic nature of AMF in soybean is significant. Utilizing these bio-enhancers conserves soil health and plays a vital role in increasing the soybean yield by increasing the number of pods.

Acknowledgments

The authors would like to thank the Chairman of the Department of Botany, Jagannath University, Dhaka, the Director of the Soil Science Division, Bangladesh Agricultural Research Institute (BARI) for technical support and materials and University Grants Commission (UGC), Dhaka, Bangladesh, for their financial assistance in conducting this research.

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(Manuscript received on 05 January 2025; revised on 12 March, 2025)