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Research Article

Effect of Arbuscular Mycorrhizal Fungi (AMF) and *Rhizobium* Inoculation on Growth and Yield of *Glycine max* L. Varieties

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Biofertilizers are preparations containing living cells that help crop plants in the uptake of nutrients. This study aimed to investigate the effect of coinoculation of arbuscular mycorrhizal fungi and *Rhizobium* species on the growth and nutrient uptake of three varieties of *Glycine max*: Belsa 95, Afgat M5, and Nova E3, in the greenhouse and the field. These varieties were obtained from the Gambela research center of Ethiopia. Commercial *Rhizobium* inoculants were obtained from the Menagesha Biotechnology Institute (MBI), and the previously isolated indigenous AMF inoculants were mass-produced using *Sorghum bicolor* as a trap plant. Two kilograms of sterilized soil and sand in a 2:1 ratio were used for greenhouse treatments, and 2 m × 3 m plots were used for field treatments. In the greenhouse trials, for all the three varieties was recorded better yield plant^{-1} in coinoculated treatments with fertilizer application and without fertilizer application, respectively. The highest root number plant^{-1} (10.0 ± 1.2 and 10.0 ± 1.7) was recorded for variety 1 with the application of only fertilizer and fertilizer + *Rhizobium*, respectively, and the highest values (8.7 ± 1.9 and 4.7 ± 0.8) were recorded for coinoculated treatments with fertilizer application for varieties 2 and 3, respectively. For sole mycorrhiza-inoculated treatments in the greenhouse was recorded higher dry biomass (16.67% for V1, 42.20% for V2, and 22.18% for V3) as compared with the control. Moreover, for combined inoculation of AMF + *Rhizobium* and AMF + *Rhizobium* + fertilizer were recorded 27.01% and 66.99% for V1, 42.20% and 70.33% for V2, and 36.84 and 80.20% for V3, respectively. That means tripartite interactions favor the growth response in association with higher P and N uptake. Finally, it is recommended to apply biofertilizers as the plant-fungi-*Rhizobium* interactions may have a bigger potential role in maintaining sustainable agriculture with effective environmental resilience.

1. Introduction

The indiscriminate use of chemical fertilizer and pesticides has disastrous environmental consequences, promoting research into natural sources of fertilizer, biostimulants, and soil amendments. The soybean crop is one of the world's most important crops. Soybean grains are important as a protein meal and as a source of vegetable oil [1]. It also contains a variety of vitamins and minerals. It provides approximately 60% of the world's supply of vegetable protein and 30% of the world's oil [2]. But, productivity of soybean decreases as a result of a number of factors. This low yield was attributed to a lack of cultivars that were adaptable to specific ecological conditions, poor adoption of technology, and improper soil and nutrient management. The scarcity of cultivars that are adaptable to specific agroecological conditions has greatly contributed to the existing knowledge gap regarding the relationship of traits with seed yield [3].

Ethiopia currently imports vegetable oils for domestic consumption; thus, the introduction of a new oil crop will reduce imports and aid in the self-sufficiency of such critical commodities. To increase legume production, legume inoculation is widely used. *Rhizobium* inoculation of soybean has been shown to increase growth and seed yield [4, 5]. The total number of nodules has increased in the inoculated control compared to the uninoculated control.

Mycorrhiza is a widespread symbiotic association that is commonly described as the result of coevolutionary events between fungi and plants, with both partners benefiting from reciprocal nutrient exchange [6]. Mycorrhizal inoculation increased soybean nodulation, growth, and yield significantly [7]. In a conventional agriculture, diammonium phosphate (DAP)

is the most widely used phosphorus fertilizer in the world, containing 18% N and 46% P_2O_5, and used to produce soybean. But, it has adverse effects on the environmental resilience. Therefore, it is recommended to introduce agricultural technologies that could reduce the application of inorganic fertilizers in crop production.

Soybean is a high nitrogen-demanding crop because the end product is high in protein. The main sources of meeting the nitrogen requirement of high-yielding soybean are biological N_2 fixation and mineral soil or nitrogen fertilizer [8]. Phosphorus deficiency can limit soybean nodulation, growth, and yield, but phosphorus fertilizer application can compensate [9, 10]. In addition to application of phosphorus fertilizer, inoculation of arbuscular mycorrhizal fungi (AMF) is believed to provide soybean with the required phosphorus. These indicate that dual inoculation of *Rhizobium* and arbuscular mycorrhizal fungi provides plants with the two most important nutrients for the plant growth and productivity. However, in Ethiopia, information on the effects of dual inoculation of *Rhizobium* and arbuscular mycorrhizal fungi on plant growth and productivity is so scarce. Therefore, the goal of this experiment was to investigate the tripartite interaction effects of plant, *Rhizobium*, mycorrhiza and application of DAP fertilization on the growth and yield of soybean varieties.

2. Materials and Methods

2.1. Study Design. This study was conducted in the greenhouse and the field of Hawassa University research village. The seeds of three varieties of *Glycine max* were obtained from the Gambela Agricultural Research Center. In the greenhouse, seeds free from visible defects and with uniform size were surface sterilized in sodium hypochlorite and sown in circular polyethylene pots (40 cm height and 30 cm diameter) and were filled with a mixture of sterile garden soil and sand at the ratio of 2:1 (v/v). Before inoculation, the soil substrate to be used was analyzed for its physicochemical properties using standard methods. Seeds were inoculated individually with *Rhizobium* and AM fungi (applied as layering on the soil surface) and a combination of both. Pots were arranged in a completely randomized design (CRD) with 5 treatments replicated three times.

For the field study, the land was prepared by plowing, leveling, and ridging. The spacing between ridges was 50 cm, and then it was divided into plots of $2 \times 3\ m^2$ with three ridges, each three meters long. The inter-row spacing was 5 cm with one seed per hole on the top of the ridges, and gaps were filled by replanting after germination. Irrigation was applied as needed. Weeding was done by hand whenever it was necessary to avoid weed competition. Seeds were inoculated by *Rhizobium* (*Bradyrhizobium japonicum*) and the mixture of two arbuscular mycorrhizal fungi (AMF) morphospecies, *Gigaspora rosea* and *Rhizophagus clarus*, at sowing. DAP fertilizer (18% N + 46% P_2O_5) was added after germination.

The treatments of this study were as follows:

(1) C = control (without inoculation or fertilizer)

(2) R = inoculation with *Rhizobium* alone

(3) M = inoculation with mycorrhiza alone

(4) RM = inoculation with *Rhizobium* + mycorrhiza

(5) F = application of DAP without biofertilization

(6) RF = inoculation with *Rhizobium* + DAP

(7) MF = inoculation with mycorrhiza + DAP

(8) RMF = inoculation with *Rhizobium* + mycorrhiza + 100 kg/ha (DAP)

2.2. Root Colonization. According to Phillips and Hayman, AMF colonization was evaluated (1970). Root samples were washed several times with tap water before being cleared in 10% (w/v) KOH in a water bath at 90°C for 1-2 hours and then cooled at room temperature. The root samples were washed 3–5 times with tap water after cooling, acidified in 1% HCl for 1 hour, stained with 0.05 percent trypan blue, and finally destained in acidic glycerol.

A compound light microscope (Olympus BX51) with a magnification of 200 times was used to examine the AM fungal structures. The magnified intersection method of McGonigle et al. was used to estimate the total root length colonization as RLC = 100 [(G − N)/G], the percentage of root length colonized by arbuscules, arbuscular colonization, as AC = 100 (A/G), and the percentage of root length colonized by mycorrhizal vesicles, vesicular colonization, as VC = 100 (V/G) (1990), where RLC = total root length colonization, N = no fungal structure, A = arbuscules, V = vesicles, and G = total intersection. All of these were quantified by looking at 100–150 intersections per sample.

2.3. Spore Density. The spore count was processed and determined according to the method in [11]. Accordingly, 100 g of each soil sample was suspended into a 2-liter container and mixed vigorously to free spores from the soil and roots. The supernatant was subsequently decanted through standard sieves (480, 106, 50, and 38 μm) after having been intermittently centrifuged at 2000 rpm for 5 minutes. The last pellet (38 μm) was suspended in 60% sucrose solution and thoroughly mixed and centrifuged at 2000 rpm for 1 minute to collect the spores. The spores and sporocarps were then rinsed with tap water and transferred into plastic Petri dishes. They were counted under a 4x stereomicroscope according to the method in [12], and spore densities were expressed as the number of spores and sporocarps per $100\ g^{-1}$ of dry soil.

2.4. Statistical Analysis. Data on spore abundance and root colonization were log (x) and arcsine (the inverse sine of the square root of the proportion) transformed using PAST3 (version 1.0.0.0) and SPSS software package (version 20.0), respectively, before analysis to meet assumptions of ANOVA such as normality and homogeneity of variance. The significance of differences in AM fungal spore abundance and percentage of root colonization between the samples was tested using Fisher's least significant difference (LSD) at $p < 0.05$ after one-way analysis of variance (ANOVA) with the SPSS software package (version 20.0).

The growth of plants was computed as the percentage dry weight of treated plants over untreated and noninoculated plants [13]. Plant height growth, the numbers and length

(cm) of primary roots, and the number of nodules per plant were measured. Total nitrogen, total phosphorus, and potassium were analyzed from oven-dried ground plant shoot using standard methods. Analysis of variance (ANOVA) was carried out with the SPSS software package (version 20.0). The mycorrhizal dependency (MD) of *Glycine max* varieties was calculated according to the method in [14] as follows: $\text{MD}\ (\%) = [(\text{M} - \text{NM})/\text{M}] \times 100$, where M is the total dry biomass of the mycorrhizal plant and NM is the total dry biomass of the nonmycorrhizal plant.

3. Results and Discussion

3.1. Results

3.1.1. Greenhouse Experiment. The findings of the effect of arbuscular mycorrhizal fungi (AMF) and *Rhizobium* inoculation on the growth and yield of three varieties of *Glycine max* from the greenhouse trials are given in Tables 1–3. The *Glycine max* varieties inoculated either with *Rhizobium* or with AM fungi significantly increased the shoot length, the dry biomass, and the total number of nodules when compared to the control. The dual inoculation of AM fungi and *Rhizobium* showed maximum values in all the tested parameters than plants inoculated with individual endophytes (Table 1). More nodules were recorded for dual inoculated plants when compared to plants inoculated individually with *Rhizobium* and AM fungi and uninoculated control plants. When the three *Glycine max* varieties under study were compared in terms of shoot length, variety 2 showed the highest (88.7 ± 3.8 cm/plant) shoot length, and for varieties 1 and 3 were recorded 68.0 ± 1.7 cm and 49.0 ± 6.0 cm plant^{-1}, respectively.

For coinoculated treatments of the three varieties were recorded the highest shoot length, wet and dry biomass, nodule number, number of roots, pod number, stem girth, and leaf size (Tables 1–3). However, some growth parameters in fertilizer-treated sole inoculation treatments were found to be better favored as compared to those of only fertilizer application treatments and vice versa in all the three varieties of soybean (Tables 1–3).

In the greenhouse trials for all the three varieties was recorded better yield plant^{-1} in coinoculated treatments with fertilizer application (V1, V2, and V3+M + R + F) and without fertilizer application (V1, V2, and V3+R + M), respectively. However, for all control treatments were recorded the lower values when compared with sole, coinoculation, and fertilizer application treatments.

The highest root number plant^{-1} (9.0 ± 0.6, 8.7 ± 1.9, and 4.7 ± 0.8) was recorded for coinoculated treatments with fertilizer application (Tables 1–3). Treatments inoculated with mycorrhiza and *Rhizobium* showed better biomass. However, for sole mycorrhiza-inoculated treatments in the greenhouse was recorded lower dry biomass (16.67% for V1, 42.20% for V2, and 22.18% for V3) as compared with the control. Moreover, for combined inoculation of AMF + *Rhizobium* and AMF + *Rhizobium* + fertilizer were recorded 27.01% and 66.99% for V1, 42.20% and 70.33% for V2, and 36.84 and 80.20% for V3, respectively. Besides, when the sole application of the recommended dose of fertilizer (Hb + F and Cp + F) is compared with that of R + F and M + F, the latter two showed better biomass yield increasing the fertilizer use efficiency.

(1) Mycorrhizal Dependency (MD). The highest MD values were recorded for mixed mycorrhizal species (42.20%) in *Afgat E3* followed by the same mixed species (22.08%) in the *Nova5 soybean variety*. The least value (16.67%) was recorded for Belsa 95 with the mixed mycorrhizal inoculum (Table 4). From these findings, we can understand that variety 3 is of short stature genetically. However, during the growth period, Nova E3 displayed fast flowering and pod setting, within less than three months as compared to Belsa and Afgat varieties that took more than 4 months for flowering and pod setting.

3.1.2. Field Experiment. The findings of the effect of arbuscular mycorrhizal fungi (AMF) and *Rhizobium* inoculation on the growth and yield of three varieties of *Glycine max* from the field trials are given in Tables 5–7. Similar to the greenhouse experiments on the three varieties of *Glycine max*, for trials in the field were recorded significant increases in height, wet and dry biomass, the number of nodules, root number, stem girth, leaf size, and the number of pods plant^{-1} (Tables 5–7). The seed number plant^{-1} and yield plant^{-1} showed a similar pattern.

The response of the inoculants on yield and yield components of the three varieties of soybean showed considerable differences among different inoculated treatments for soybean yield-contributing traits (Tables 5–7). Plants inoculated with both, AMF and *Rhizobium*, had recorded a substantially higher number of nodules, pods, and roots as compared with the control. Besides, plant height, dry biomass, stem girth, and leaf size were better for coinoculated treatments. The sole application of DAP had resulted in a less number of nodules as compared with the control. The noninoculated control plants were shorter and had the lowest records when compared with inoculated and fertilizer-treated plants.

(1) Biomass Yield. Biomass yield for the three varieties of *Glycine max* trials in the greenhouse and the field is shown in Table 8. The recorded results of the experiment show that combined inoculation of AMF + *Rhizobium* enhanced biomass yield plant^{-1} by 31.63% in V1, 42% in V2, and 58.47% in V3 over control while 12.83% in V1, 23.77% in V2, and 46% in V3 over sole inoculation of AMF. For sole inoculation of *Rhizobium* were recorded 48.12% in V1, 44.15% in V2, and 62.2% in V3, respectively. Besides, the record indicates that the varieties benefitted more from the sole inoculation of *Rhizobium* as compared to sole inoculation of mycorrhiza in the field and the opposite in the greenhouse (Table 8).

3.1.3. Root Colonization and Spore Density of Glycine max Varieties after Inoculation with Mycorrhiza and Rhizobium

(1) Arbuscular and Vesicular Colonization. For the three varieties of *Glycine max* grown on a sterilized soil + sand (2:1) medium inoculated with *Rhizobium* and arbuscular mycorrhizal fungi, arbuscular and vesicular colonization was recorded from fair to the highest in all treatments except the

Table 1: Effect of AMF and *Rhizobium* inoculation on the growth and yield of variety 1 (Belsa 95) in the greenhouse.

Treatments	H	WW	DW	NN	RN	SG	LS	NP	Spp	Ypp (g)
V1 + R	46.0 ± 1.2b	100.0 ± 0.0b	11.0 ± 0.6ab	5.0 ± 1.0c	5.0 ± 0.6b	0.2 ± 0.0b	60.1 ± 3.8 b	25.2 ± 15.0	2b	18b
V1 + M	48.3 ± 0.9c	100.0 ± 0.0b	12.0 ± 1.0b	12.0 ± 1.5d	5.0 ± 0.6b	0.3 ± 0.0c	69.2 ± 6.8c	18.0 ± 3.2d	2.5bc	20c
V1 + R + M	54.3 ± 2.3d	120.0 ± 0.0c	13.7 ± 1.8c	16.0 ± 2.6e	5.0 ± 0.6b	0.3 ± 0.0c	79.9 ± 0.8d	22.8 ± 3.9f	3c	41e
V1 + F	61.7 ± 2.7e	183.3 ± 16.7g	28.3 ± 3.8d	0.7 ± 0.3a	10.0 ± 1.2e	0.4 ± 0.0d	103.0 ± 5.5f	15.2 ± 4.2b	2b	32de
V1 + R + F	70.7 ± 1.5h	150.0 ± 0.0d	28.3 ± 0.9d	1.8 ± 1.2ab	10.0 ± 1.7e	0.4 ± 0.0d	93.2 ± 8.7e	19.2 ± 5.3e	2b	28d
V1 + M + F	65.0 ± 1.5f	166.7 ± 16.7e	29.3 ± 2.7e	4.0 ± 4.0b	8.0 ± 1.2c	0.3 ± 0.0c	114.3 ± 3.6h	17.0 ± 2.2c	3c	31de
V1 + M + R + F	68.0 ± 1.7fg	176.7 ± 16.7f	30.3 ± 3.9f	5.2 ± 1.2c	9.0 ± 0.6d	0.4 ± 0.0d	126.4 ± 4.4i	18.8 ± 1.2d	3.2cd	42ef
C	38.3 ± 1.5a	68.0 ± 0.0a	10.0 ± 2.6a	4.4 ± 1.9bc	4.0 ± 0.6a	0.1 ± 0.0a	55.0 ± 5.8a	8.7 ± 1.4a	1.2a	15a
$p < 0.05$	Sd	Sd	Sd	Sd	Sd	nd	Sd	Sd	Sd	Sd

Note: H, height; WW, wet weight; DW, dry weight; NN, number of nodules; RN, root number; SG, stem girth; LS, leaf size; NP, number of pods; Spp, seed number per plant; Ypp, yield per plant; Sd, significantly different. Similar letters in columns show no significant difference between treatments at $p < 0.05$.

Table 2: Effect of AMF and *Rhizobium* inoculation on the growth and yield of variety 2 (Afgat M5) in the greenhouse.

Treatments	H	WW	DW	NN	RN	SG	LS	NP	Spp	Ypp (g)
V1 + R	71.7 ± 5.2c	116.7 ± 16.7c	13.3 ± 7.1b	23.7 ± 2.2d	5.0 ± 0.0c	0.3 ± 0.0c	86.0 ± 4.1bc	29.3 ± 4.2c	1.5ab	20b
V1 + M	70.0 ± 2.3b	133.3 ± 16b	17.3 ± 1.2c	23.0 ± 6.4d	4.3 ± 0.9b	0.2 ± 0.0b	111.7 ± 7.1de	26.5 ± 1.3b	2.0b	22c
V1 + R + M	74.3 ± 2.2f	183.3 ± 16.7f	19.7 ± 2.9d	24.5 ± 5.9c	6.3 ± 1.2d	0.2 ± 0.0b	128.4 ± 13.1f	30.5 ± 4.8cd	3c	43d
V1 + F	73.3 ± 1.2d	183.3 ± 16.7f	37.3 ± 0.3g	9.0 ± 2.6a	6.0 ± 2.1d	0.4 ± 0.0d	99.7 ± 3.6c	35.7 ± 2.7d	2.5bc	34d
V1 + R + F	82.3 ± 1.9h	175.0 ± 25.0de	39.3 ± 2.4h	33.0 ± 1.5f	7.0 ± 1.5e	0.3 ± 0.0c	85.4 ± 12.5b	35.5 ± 6.5d	2.5bc	30d
V1 + M + F	81.3 ± 0.7g	166.7 ± 33.3d	31.7 ± 2.3e	21.0 ± 2.1b	4.0 ± 0.5b	0.3 ± 0.0c	107.5 ± 5.4d	46.2 ± 2.6e	3c	33dc
V1 + M + R + F	88.7 ± 3.8i	176.7 ± 16.7de	33.7 ± 3.2ef	25.3 ± 0.9e	8.7 ± 1.9f	0.3 ± 0.0c	122.6 ± 4.6e	50.3 ± 5.4f	3.5cd	44.4ef
C	66.3 ± 4.1a	50.0 ± 0.0a	10.0 ± 1.5a	8.8 ± 2.8a	3.0 ± 0.6a	0.1 ± 0.0a	67.2 ± 7.9a	24.7 ± 4.1a	1.4a	11.7a
$p < 0.05$	Sd	Sd	Sd	Sd	Sd	nd	Sd	Sd	Sd	Sd

Note: H, height; WW, wet weight; DW, dry weight; NN, number of nodules; RN, root number; SG, stem girth; LS, leaf size; NP, number of pods; Spp, seed number per plant; Ypp, yield per plant; Sd, significantly different. Similar letters in columns show no significant difference between treatments at $p < 0.05$.

Table 3: Effect of AMF and *Rhizobium* inoculation on the growth and yield of variety 3 (Nova E3) in the greenhouse.

Treatments	H	WW	DW	NN	RN	SG	LS	NP	Spp	Ypp (g)
V1+R	23.7 ± 0.9bc	122.7 ± 15.1b	6.7 ± 1.7ab	11.0 ± 1.0g	2.3 ± 0.3b	0.2 ± 0.0a	64.7 ± 4.3b	10.7 ± 1.8b	1.8b	19b
V1 + M	28.0 ± 3.5b	126.0 ± 17.0bc	7.7 ± 1.2c	10.0 ± 2.5f	2.3 ± 0.9b	0.4 ± 0.2c	65.1 ± 8.0bc	12.3 ± 0.9c	2c	21c
V1 + R + M	30.0 ± 0.6c	206.7 ± 26.2e	9.5.0 ± 0.6d	15.8 ± 5.3h	2.7 ± 0.3bc	0.4 ± 0.1c	65.8 ± 3.2bc	18.7 ± 0.3d	2.5cd	40e
V1 + F	46.7 ± 5.7de	150.0 ± 17.3c	26.7 ± 3.2f	0.8 ± 0.8a	3.3 ± 1.3c	0.4 ± 0.0c	73.2 ± 3.9de	40.0 ± 7.5f	1.5b	30de
V1 + R + F	50.7 ± 13.5ef	155.0 ± 35.0cd	18.3 ± 1.5e	3.3 ± 0.9c	2.3 ± 0.3b	0.3 ± 0.0b	76.2 ± 2.3e	26.3 ± 0.7e	1.5b	29d
V1 + M + F	40.3 ± 1.2d	190.0 ± 18.0d	29.3 ± 1.3g	4.0 ± 1.5d	3.3 ± 1.2c	0.3 ± 0.0b	66.5 ± 3.9c	43.0 ± 0.6fg	3.1e	29d
V1 + M + R + F	49.0 ± 6.0e	218.3 ± 15.9f	30.3 ± 4.3h	5.3 ± 0.9e	4.7 ± 0.8d	0.4 ± 0.1c	72.4 ± 1.9d	49.7 ± 5.8g	3.5ef	41ef
C	22.2 ± 2.7a	65.0 ± 0.0a	6.0 ± 0.6a	1.7 ± 0.9b	1.7 ± 0.3a	0.2 ± 0.0a	30.4 ± 3.9a	9.0 ± 0.6a	1.2a	14a
$p < 0.05$	Sd	Sd	Sd	Sd	Sd	nd	Sd	Sd	Sd	Sd

Note: H, height; WW, wet weight; DW, dry weight; NN, number of nodules; RN, root number; SG, stem girth; LS, leaf size; NP, number of pods; Spp, seed number per plant; Ypp, yield per plant; Sd, significantly different. Similar letters in columns show no significant difference between treatments at $p < 0.05$.

Table 4: Mean mycorrhizal dependency of the three soybean varieties (greenhouse).

Soybean variety	M	NM	MD
Belsa 95	17b	10b	16.67a
Afgat E3	17.3b	10b	42.20c
Nova5	7.7a	6a	22.08b

Note: M, mycorrhizal; NM, nonmycorrhizal; MD, mycorrhizal dependency.

control, sole fertilizer, sole *Rhizobium*, and *Rhizobium* + fertilizer treatments particularly in the greenhouse (Table 9).

(2) Root Length Colonization and Spore Density. All soybean varieties inoculated with sole AMF, AMF and *Rhizobium*, and AMF + *Rhizobium* + fertilizer treatments were found colonized with AMF both in the greenhouse and in the field. But those treatments inoculated with *Rhizobium* alone, R + F, only fertilizer, and the control were found not colonized in the greenhouse (Table 10).

However, the same treatments in the field were found colonized because the soil medium in the field harbors the indigenous arbuscular mycorrhizal fungi propagules. Besides, all control treatments in the greenhouse were found not colonized because the soil was sterilized under heavy

Table 5: Effect of AMF and *Rhizobium* inoculation on the growth and yield of variety 1 (Belsa 95) in the field.

Treatments	H	WW	DW	NN	RN	SG	LS	NP	Spp	Ypp (g)
V1 + R	96.7 ± 6.5b	1001.3 ± 194.2e	348.3 ± 24.3f	11.0 ± 1.0g	4.3 ± 0.9c	11.0 ± 0.5c	108.1 ± 3.8	71.3 ± 10.3e	1a	21b
V1 + M	99.3 ± 3.5cd	807.7 ± 113.8d	207.3 ± 23.8b	10.0 ± 2.5f	3.0 ± 0.6b	10.5 ± 0.8bc	69.2 ± 6.8b	52.3 ± 9.9c	3d	22b
V1 + R + M	111.8 ± 3.9f	706.7 ± 100.9b	264.3 ± 34.5d	15.8 ± 5.3h	4.3 ± 0.3c	9.8 ± 0.8bc	79.9 ± 0.7c	41.3 ± 4.6b	3d	44e
V1 + F	106.0 ± 4.5de	1237.3 ± 85.9g	315.0 ± 58.2e	0.8 ± 0.8b	6.3 ± 1.5ef	11.1 ± 0.2c	103.0 ± 5.5f	77.0 ± 5.0f	2b	33de
V1 + R + F	98.7 ± 4.6c	783.0 ± 124.0c	245.7 ± 35.7c	3.3 ± 0.9e	5.7 ± 2.7d	9.3 ± 0.8b	93.2 ± 8.7d	43.3 ± 6.6bc	2.1b	28c
V1 + M + F	109.3 ± 1.2e	1500.0 ± 0.0h	377.3 ± 14.5g	2.0 ± 0.6cd	6.7 ± 0.9ef	14.2 ± 1.5d	114.2 ± 3.6g	89.3 ± 6.7g	2.5bc	31d
V1 + M + R + F	115.3 ± 6.7g	1066.7 ± 290.9f	348.7 ± 60.4f	1.7 ± 0.3c	6.0 ± 0.6eef	11.0 ± 0.8c	96.4 ± 4.4de	65.0 ± 17.0d	3.5de	45ef
C	66.7 ± 1.8a	550.7 ± 106.7a	180.7 ± 32.2a	0.3 ± 0.3a	2.5 ± 0.4a	7.3 ± 0.5a	55.0 ± 5.8a	31.0 ± 14.5a	1.0a	11a
$p < 0.05$	*	*	*	*	*	*	*	*	*	*

Note: H, height; WW, wet weight; DW, dry weight; NN, number of nodules; RN, root number; SG, stem girth; LS, leaf size; NP, number of pods; Spp, seed number per plant; Ypp, yield per plant; *significantly different. Similar letters in columns show no significant difference between treatments at $p < 0.05$.

Table 6: Effect of AMF and *Rhizobium* inoculation on the growth and yield of variety 2 (Afgat M5) in the field.

Treatments	H	WW	DW	NN	RN	SG	LS	NP	Spp	Ypp (g)
V1 + R	100.0 ± 2.9bc	1166.7 ± 440.9e	361.7 ± 109.4f	12.0 ± 0.6f	3.7 ± 0.9c	11.2 ± 0.7d	86.0 ± 4.1b	81.3 ± 17.7e	2.1b	23b
V1 + M	108.7 ± 5.2d	1166.7 ± 166.7e	265.0 ± 14.8bc	10.3 ± 2.3e	4.0 ± 0.6d	9.4 ± 0.2c	111.7 ± 7.1d	56.0 ± 6.1cd	2.6cd	22b
V1 + R + M	98.3 ± 5.8b	1833.3 ± 166.7g	348.3 ± 50.6e	22.2 ± 2.5 g	4.7 ± 0.3de	9.5 ± 0.4c	108.4 ± 13.1cd	62.0 ± 3.1de	3.2ef	44d
V1 + F	106.3 ± 1.7cd	1083.3 ± 83.3c	262.0 ± 25.5b	0.8 ± 0.8c	2.3 ± 0.3c	9.0 ± 0.9bc	99.7 ± 3.6cd	54.7 ± 3.2c	2.2c	33cd
V1 + R + F	101.7 ± 3.3bc	866.7 ± 185.6b	271.7 ± 35.5c	3.3 ± 0.9d	1.7 ± 0.3b	8.7 ± 0.4b	85.4 ± 12.5b	43.0 ± 9.2b	2c	30c
V1 + M + F	109.3 ± 6.4d	1016.7 ± 60.1d	266.0 ± 28.7bc	0.3 ± 0.3b	5.0 ± 0.6e	8.9 ± 0.3b	107.5 ± 5.4 cd	46.7 ± 13.4b	3e	33 cd
V1 + M + R + F	105.7 ± 7.3c	1400.0 ± 100.0f	306.0 ± 35.4d	3.0 ± 1.0d	2.3 ± 0.3c	8.9 ± 0.3b	90.6 ± 4.6c	60.7 ± 8.9d	3.5f	46de
C	78.7 ± 2.3a	483.3 ± 109.3a	202.0 ± 29.6a	0.2 ± 0.3a	0.7 ± 0.3a	6.3 ± 0.9a	73.2 ± 7.9a	37.3 ± 4.7a	1.2a	16a
$p < 0.05$	*	*	*	*	*	*	*	*	*	*

Note: H, height; WW, wet weight; DW, dry weight; NN, number of nodules; RN, root number; SG, stem girth; LS, leaf size; NP, number of pods; Spp, seed number per plant; Ypp, yield per plant; *significantly different. Similar letters in columns show no significant difference between treatments at $p < 0.05$.

Table 7: Effect of AMF and *Rhizobium* inoculation on the growth and yield of variety 3 (Nova E3) in the field.

Treatments	H	WW	DW	NN	RN	SG	LS	NP	Spp	Ypp (g)
V1 ± R	52.7 ± 1.8e	1156.7 ± 340.9e	365.0 ± 112.5f	11.7 ± 1.8f	3.0 ± 0.6b	0.3 ± 0.0c	64.7 ± 4.3c	46.0 ± 4.7g	2b	17b
V1 + M	50.0 ± 2.1d	1156.7 ± 106.7e	255.0 ± 17.0bc	9.7 ± 2.3e	4.0 ± 0.6c	0.2 ± 0.0b	65.1 ± 8.0d	38.3 ± 7.2f	2b	19bc
V1 + R + M	53.0 ± 0.6de	1813.3 ± 176.7g	332.3 ± 36.9e	19.8 ± 1.6g	3.0 ± 0.6b	0.5 ± 0.1e	65.8 ± 3.2d	33.7 ± 0.7d	3c	39d
V1 + F	53.3 ± 1.3de	1073.3 ± 83.3d	254.0 ± 15.7b	4.2 ± 1.0 cd	4.3 ± 1.2 cd	0.4 ± 0.0d	73.2 ± 3.9e	34.3 ± 1.7e	2b	32cd
V1 + R + F	52.3 ± 2.3de	766.7 ± 75.6b	268.3 ± 28.2c	5.0 ± 0.6d	3.7 ± 0.3bc	0.4 ± 0.0d	76.2 ± 2.3f	33.3 ± 3.9d	2b	28c
V1 + M + F	42.7 ± 4.1c	1015.7 ± 60.1c	262.7 ± 28.0c	0.7 ± 0.3a	4.0 ± 0.0c	0.3 ± 0.0c	66.5 ± 3.9de	27.3 ± 1.8c	3c	30 cd
V1 + M + R + F	26.7 ± 1.7b	1390.0 ± 100.0f	297.0 ± 31.5d	4.0 ± 1.2c	6.3 ± 0.9d	0.5 ± 0.1e	32.4 ± 1.9b	18.7 ± 0.3b	3c	40de
C	15.7 ± 0.9a	403.3 ± 99.3a	137.7 ± 18.8a	0.2 ± 0.3b	1.7 ± 0.3a	0.1 ± 0.0a	28.4 ± 2.9a	15.0 ± 1.0a	1.5a	12a
$p < 0.05$	*	*	*	*	*	*	*	*	*	*

Note: H, height; WW, wet weight; DW, dry weight; NN, number of nodules; RN, root number; SG, stem girth; LS, leaf size; NP, number of pods; Spp, seed number per plant; Ypp, yield per plant; *significantly different. Similar letters in columns show no significant difference between treatments at $p < 0.05$.

Table 8: Biomass yield of the three varieties.

Treatments	Greenhouse			Field		
	V1	V2	V3	V1	V2	V3
R	9.1a	24.81a	10.45a	48.12de	44.15cd	62.27e
M	16.67b	42.20b	22.18b	12.83a	23.77ab	46ab
R + M	27.01c	49.24bc	36.84c	31.63c	42c	58.47d
F	64.67d	73.19def	77.53e	42.63d	22.90a	45.79a
R + F	64.67d	74.55ef	67.21d	24.26b	25.63bc	48.68bc
M + F	65.87d	68.45d	79.52ef	52.11f	24.06b	47.58bc
M + R + F	66.99de	70.33de	80.20g	48.18de	51.49d	53.64c

V1, Belsa 95; V2, Afgat M5; V3, Nova E3; R = inoculation with *Rhizobium* alone; M = inoculation with mycorrhiza alone; F = application of DAP without biofertilization.

Table 9: Arbuscular and vesicular colonization of AMF- and *Rhizobium*-inoculated soybean varieties in comparison with the control.

Treatments	Arbuscular colonization						Vesicular colonization					
	Greenhouse			Field			Greenhouse			Field		
	V1	V2	V3	V1	V2	V3	V1	V2	V3	V1	V2	V3
V1 + R	−	−	−	++	+++	++	−	−	−	++	+++	+++
V1 + M	++++	+++++	+++	++++	+++++	+++	+++	+++	++++	+++	+++	+++
V1 + R + M	++++	+++++	+++	++++	++++++	+++	++++	+++++	++++	++++	+++++	+++
V1 + F	−	−	−	+	++	++	−	−	−	++	++	++
V1 + R + F	−	−	−	++	+++	+++	−	−	−	+++	++++	+++
V1 + M + F	+++	+++	++	+++	+++	+++	+++	+++	+++	++++	++++	+++
V1 + M + R + F	++++	+++++	+++	++++	+++++	+++	++++	+++++	++++	++++	++++++	+++
C	−	−	−	++	++	++	−	−	−	++	++	++

Note: V1, Belsa 95; V2, Afgat M5; V3, Nova E3; −, absent; +, fair; ++, moderate; +++ and above, high; R = inoculation with *Rhizobium* alone; M = inoculation with mycorrhiza alone; F = application of DAP without biofertilization.

pressure and at 121°C for 15 minutes in an autoclave. In both the greenhouse and the field was also recorded better AMF root colonization in sole AMF inoculation and M + R and M + R + F treatments.

The highest RLC (72 ± 4.1, V1; 74 ± 3.8, V2; 76 ± 30.21, V3) was recorded for R + M + F treatment, while the lowest (54.0 ± 5.5, V1; 45.0 ± 5.0, V2; 40.7 ± 4.3, V3) was recorded for M + F treatment (Table 10). As to what concerns spore density, it follows a similar pattern with greenhouse treatments. In the field, the lowest values were recorded for fertilizer and control treatments. Besides, recorded analysis of variance (ANOVA) shows that results for all treatments are significantly different at $p < 0.05$.

(3) Plant Nutrient Contents. All inoculated and fertilizer-treated plants showed an increase in plant tissue nutrients (Table 11). Plant tissue phosphorus, nitrogen, and potassium concentration was higher in the inoculated plants than in noninoculated ones. For all coinoculation treatments were recorded the higher nutrient uptake values as compared with sole mycorrhizal or rhizobial inoculation treatments. The highest N% concentration was recorded for M + R and M + R + F coinoculation treatments (Table 11).

3.2. Discussion. The application of biofertilizers in agricultural systems is believed to increase the fertilizer use efficiency and helps to discover the most suitable and sustainable alternative to the fertilizer and minimize crop dependency on fertilizer. The use of inorganic fertilizer not only brings a surge in the production cost but may also prove in creating a large number of environmental problems alongside. Climate change and ecosystem degradation likely inflict new restrictions; accordingly, sustainable agriculture and organic sources of nutrients have an essential function to perform in conserving natural resources. To solve problems like this, the use of legumes for increasing soil fertility is considered to be the only viable solution for not only increasing crop production and yield but also playing an important role in ameliorating soil fertility and productivity.

Furthermore, to enhance the nutrient (nitrogen and phosphorus) fixing ability of various bacteria and fungi, it is essential to treat seeds with proper inoculums before sowing. The dual inoculation of seed with arbuscular mycorrhizal fungi (AMF) and *Rhizobium* enhanced the growth parameters such as the shoot length, stem girth, root number, and leaf size of haricot bean and chickpea [15].

The findings of the current study on the three varieties of *Glycine max* inoculated with arbuscular mycorrhizal fungi and *Rhizobium* showed a significant increase in shoot length, dry biomass, the number of nodules, the number of pods, stem girth, leaf area, and seed yield per plant as compared with uninoculated treatments.

Similarly, research studies in the past few decades on various aspects of root symbiosis have shown that the dual interaction of AM fungi and *Rhizobium* has improved the growth, nodulation, and yield [16]. Similarly, seed inoculation and soil treatment also showed significantly higher protein, carbohydrates, fats, crude fibers, and dry matter of soybean than uninoculated plants. Studies [17, 18] also reported that arbuscular mycorrhizal fungi had shown promising effects on crude protein, fat, moisture, and ash contents in mycorrhizal plants except for carbohydrates.

The findings of the current study also correlate with those of Samanhudi et al. [19], who studied the effect of AMF inoculation on the Temulawak plant and observed that mycorrhizal inoculation improves the yield of the studied plant. Our results are in accordance with the findings of Lokshman and Kadam [20] that plants inoculated with both rhizobial and mycorrhizal symbiosis improved growth, nodulation, and nitrogen fixation. Our results are in line with the findings of Jarande et al. [21] who stated that treatments had higher values of growth parameters including plant height, the number of seeds per plant, and pod length. Our results are also in line with the findings of the studies [22–26]. Our findings were supported by Al-Zalzaleh et al. [27] who reported significant effects of biofertilizer on plant height.

The same observations were also reported by Mortimer et al. [23] stating that synergistic effects of the combined application of *Rhizobium* and AM fungi enhance plant growth to a greater extent than singular inoculation. Coinoculation of the three varieties of *Glycine max* ((R + M) and (R + M + F)) with and without fertilizer showed a better increase in all growth parameters of the plants.

Table 10: (Mean ± SEM) Root length colonization and spore density of mycorrhiza- and *Rhizobium*-inoculated soybean varieties.

Treatments	Percentage root length colonization						Spore density 100 g^{-1} soil plus sand substrate					
	Greenhouse			Field			Greenhouse			Field		
	V1	V2	V3	V1	V2	V3	V1	V2	V3	V1	V2	V3
V1 + R	–	–	–	65.2 ± 4.1^{e}	73.3 ± 3.4^{c}	$62.0 \pm 2.8.0^{d}$	–	–	–	498.3 ± 201.4^{d}	489.0 ± 34.1^{e}	621.7 ± 172.2^{e}
V1 + M	60.2 ± 0.1^{b}	65.3 ± 5.7^{b}	70.7 ± 3.2^{b}	70.2 ± 0.2^{c}	74 ± 3.8^{d}	76 ± 3021^{e}	535.0 ± 44.4^{b}	670.0 ± 75.7^{a}	455.5 ± 97.9^{a}	535.0 ± 44.4^{e}	546.7 ± 139.8^{f}	665.0 ± 63.5^{f}
V1 + R + M	68.0 ± 1.2^{c}	72.4 ± 4.6^{c}	74.0 ± 3.12^{c}	72 ± 4.1^{cd}	75.3 ± 4.7^{e}	80.7 ± 2.2^{f}	572.3 ± 46.4^{bc}	776.7 ± 139.8^{c}	621.7 ± 172.2^{c}	572.3 ± 46.4^{df}	729.0 ± 30.3^{g}	741.7 ± 36.3^{g}
V1 + F	–	–	–	45.0 ± 4.0^{b}	33.3 ± 4.9^{a}	29.9 ± 0.5^{a}	–	–	–	258.3 ± 79.5^{a}	155.0 ± 141.5^{a}	245.3 ± 39.9^{a}
V1 + R + F	–	–	–	50.0 ± 5.0^{c}	34.3 ± 4.9^{ab}	32.9 ± 0.5^{b}	–	–	–	374.0 ± 40.4^{e}	270.0 ± 75.7^{b}	255.5 ± 97.9^{b}
V1 + M + F	54.0 ± 5.5^{a}	45.0 ± 5.0^{a}	40.7 ± 4.3^{a}	54.0 ± 5.5^{d}	45.0 ± 5.0^{b}	40.7 ± 4.3^{c}	458.3 ± 79.5^{a}	755.0 ± 141.5^{bc}	545.3 ± 39.9^{b}	458.3 ± 79.5^{cd}	355.0 ± 141.5^{c}	345.3 ± 39.9^{c}
V1 + M + R + F	72 ± 4.1^{d}	74 ± 3.8^{d}	76 ± 30.21^{d}	78.0 ± 1.6^{f}	82.4 ± 3.6^{f}	84.0 ± 2.12^{g}	578.0 ± 40.4^{bc}	829.0 ± 30.3^{d}	741.7 ± 36.3^{d}	574.0 ± 40.4^{f}	770.0 ± 75.7^{h}	755.5 ± 97.9^{h}
C	–	–	–	33 ± 1.2^{a}	45 ± 4.5^{b}	40 ± 3.5^{c}	–	–	–	325 ± 55.4^{b}	445 ± 35.4^{ef}	400 ± 76.5^{d}

Note: V1, Belsa 95; V2, Afgat M5; V3, Nova E3. Similar letters in columns show no significant difference between treatments at $p < 0.05$.

Table 11: (Mean ± SEM) Effect of AMF and *Rhizobium* inoculation on nutrient uptake of *Glycine max* varieties.

Treatments	Nutrient uptake						Nutrient uptake					
	Greenhouse			Field			Greenhouse			Field		
	N%	P	K	N	P	K	N	P	K	N	P	K
V1, V2, V3 + R	0.58c	0.15ab	0.10a	0.52de	0.12ab	0.15b	0.28c	0.11ab	0.12ab	0.15ab	0.12ab	0.05a
V1, V2, V3 + M	0.62d	0.21c	0.11ab	0.56e	0.13ab	0.22c	0.45de	0.14ab	0.26bc	0.24bc	0.17b	0.17c
V1, V2, V3 + R + M	0.83e	0.29e	0.27a	0.65e	0.32c	0.48e	0.90f	0.16b	0.56c	0.32c	0.27cd	0.31d
V1, V2, V3 + F	0.46b	0.25cd	0.15b	0.28b	0.13ab	0.32d	0.21b	0.21c	0.20b	0.20b	0.11a	0.12bc
V1, V2, V3 + R + F	0.46b	0.14b	0.15b	0.32bc	0.14abc	0.16bc	0.32d	0.24d	0.22bc	0.21bc	0.19bc	0.11b
V1, V2, V3 + M + F	0.58c	0.26d	0.27c	0.36c	0.21b	0.22c	0.40de	0.18bc	0.24bc	0.24bc	0.23 cd	0.12bc
V1, V2, V3 + M + R + F	1.2f	0.46f	0.62d	1.28f	0.50d	0.50f	1.76g	0.35e	0.58c	1.22d	0.47d	0.32d
V1, V2, V3 control	0.35a	0.13a	0.11ab	0.25a	0.11a	0.13a	0.18a	0.10a	0.11a	0.14a	0.11a	0.12bc

Note: V1, Belsa 95; V2, Afgat M5; V3, Nova E3. Similar letters in columns show no significant difference between treatments at $p < 0.05$; R = inoculation with *Rhizobium* alone; M = inoculation with mycorrhiza alone; F = application of DAP without biofertilization.

Our result agrees with the findings of Jia et al. [28] who reported that inoculation with AM fungi promoted biomass production and photosynthetic rates in *Vicia faba* because of the enhanced P supply due to AM fungi inoculation. Also, our findings were in line with those of Hussain et al. [29] who studied the influence of phosphorus fertilization and *Rhizobium* inoculation on the growth and yield parameters of mung bean.

The present results indicated that AMF produced significantly better growth attributes when combined with NPK fertilizer or *Rhizobium* as compared to the yield and yield attributes over the rest of the biofertilizers and NPK fertilizer alone. The significant increase in the leaf area per plant, plant height, pods plant^{-1}, and seeds per plant due to either single or dual inoculation of seed with AMF and *Rhizobium* was also supported by the findings of Balachandran et al. [30] who reported that the effect of inoculation with *Rhizobium* and phosphate-solubilizing bacteria (PSB) significantly increased plant height, several leaves, and leaf area.

The highest numbers of seeds pod^{-1} were found in either dual or single inoculation than the control. These findings are in accordance with those of the studies [20, 31] that concluded that dual inoculation, *Rhizobium*, and AMF increased grain yield, over no inoculation. Similar results were obtained by Jarande et al. [21] who stated that treatments which included rock phosphate and seed treatment with PSB and *Rhizobium* application of rock phosphate and PSB + *Rhizobium* recorded higher values of growth as well as yield.

The results of this study are also supported by Meghvansi and Mahna [32], who found that dual inoculation of *Rhizobium* + AMF was superior to single inoculation. Similarly, our results correlate with those of [33, 34] that reported better results in coinoculation of *Rhizobium* and AMF. It is evident from the results that the efficacy of AMF fungi was influenced by coinoculation with *Rhizobium* and DAP fertilizer, increasing the fertilizer use efficiency.

In this study, roots of all *Glycine max* varieties inoculated with AM fungi were colonized, and M alone, M + F, R + M, and R + M + F were found colonized as compared with all other treatments including the control. Besides, the highest root colonization was recorded for coinoculation treatments with the fertilizer. The rate of AM colonization is normally attributed to crop species and environmental factors. Smith and Read [35] reported that the extent to which typical AM fungi colonize root systems varies with plant species. This fact is also revealed in the current study in which variety 3 was better colonized than varieties 1 and 2.

The extent of AM infection in root systems is also known to be influenced by environmental conditions, the most important being the age of the plants, the level of phosphate (P) in the soil relative to the requirements of the plant, and the capacity of the population of AMF propagules in the soil to form AMF. In this study, the highest percentage of root colonization was recorded for the 3rd variety of *Glycine max*, and as the soil in the field harbors indigenous AMF propagules, all treatments in the field were found colonized.

Although all AMF-inoculated plant species have produced spores both in the greenhouse and in the field, despite their high colonization and production of spores in the field, for AMF + fertilizer treatment both in the greenhouse and in the field were recorded both low percentage root colonization and spore density. For V1, the spore density ranged from 458.3 in M + F to 578 spores/100 g dry soil (the highest) in M + R + F in the greenhouse trial and from 325 (control) to 574 (M + R + F) in the field.

For V2 in the greenhouse were recorded 670 spores/100 g dry soil (M) to 829 spores for M + R + F in the greenhouse, and for the same variety in the field were recorded 155 spores/100 g (F) dry soil to 770 spores in M + R + F treatment.

A similar pattern was also recorded for the V3 variety. For M treatment, 455.5 spores/100 g dry soil and 741.7 were recorded for M + R + F in the greenhouse, while 245.3 (the lowest) was recorded for F treatment and 755.5 which was the highest was recorded for M + R + F treatment in the field.

In this investigation, the higher nitrogen concentration in M + R + F treatments in both the greenhouse and field treatments could be attributed to a higher nutrient absorption rate by mycorrhizal plants. The higher plant tissue nitrogen content in inoculated plants could be attributed to hypha uptake. It has been reported that the existence of extraradical hyphal bridges between individual plants permits the transfer of nutrients such as nitrogen, and Marschner and Dell [36] have reported that about 24% of the total nitrogen uptake in mycorrhizal plants could be attributed to uptake and delivery by the external hyphae.

Besides, the highest nitrogen and phosphorus uptake in the current study is due to AMF and *Rhizobium* coinoculation from which the plants were benefited from nitrification by *Rhizobium* and absorption of P by AMF.

There is also evidence that AMF hyphae take up nitrogen from inorganic ammonium sources [37], so the higher nitrogen concentration in mycorrhizal plants could be attributed to hypha uptake. The same could be said of inoculated plants' higher potassium concentration. Li et al. [38] demonstrated in a compartment pot experiment that hyphal uptake and transport accounted for approximately 10% of total potassium uptake in mycorrhizal couch grass.

4. Conclusion

Tripartite interactions of legumes with arbuscular mycorrhizal (AM) fungi and rhizobial bacteria have a higher implication to maintaining sustainable agriculture. Most of the previous research literature focused on studying interactions between legumes and one of the symbionts, either AM fungus or rhizobial bacteria. However, an understanding of legumes with only one of the symbionts at a time does not provide enough information about the dynamics of the nutrient exchange process between symbiotic partners, as a legume in natural conditions forms symbiotic relations simultaneously with AM fungi and rhizobial bacteria forming tripartite interactions. The main goal of this study was to study the tripartite interactions of legumes in association with AM fungi and rhizobial bacteria.

Current studies have demonstrated that the tripartite interactions significantly facilitate the plant growth response along with phosphate and nitrogen uptake of the plant. We found that the nutrient demands of the host and the fungal access to nutritions are important factors that control the carbon allocation to individual root symbionts in tripartite interactions. The host plant allocated more photosynthetic carbon to nodulated root half under nitrogen demand conditions. However, the host plant strategically allocated more carbon to AM root half when exogenous nitrogen was supplied to the plant. This discriminatory capability of the host plant to allocate its carbon to the most beneficial partner supports previous findings of biological market dynamics in plant-beneficial microbe interactions.

Tripartite interactions have a synergistic effect on the host plant growth response as AM fungi deliver phosphate from soil beyond root access and rhizobial bacteria provide nitrogen through the biological nitrogen fixation process to the host plant. We tested the effects of indigenous fungal and commercial rhizobial inocula on plant growth parameters including the seed yield of three soybean varieties in greenhouse and field conditions. We found that the application of AM and rhizobial inoculum increased plant biomass and seed yield in greenhouse and field conditions.

In a simple pot experiment, we observed the lowest growth response of AM plants compared with non-AM plants despite higher phosphate nutrition to the host. However, higher growth response was recorded for those coinoculation treatments signifying that both arbuscular mycorrhizal fungi and *Rhizobium* contribute both phosphorus and nitrogen, necessary for the growth of soybean varieties. Further investigation should be addressed to figure out what are the probable factors for growth variability. These factors could explain the variability: volume of soil/pot size, duration of the experiment, and nutritional profile of the soil substrate. Additionally, measurement of carbon and nitrogen in root-free soil before and after the experimental period may provide some information on growth variability. In a larger context, the role of AM fungi in carbon sequestration in the soil also can be quantitatively addressed in future studies.

The application of AM fungi and rhizobia to increase crop productivity in field conditions could be an alternate option to reduce dependency on chemical fertilizer. Furthermore, crop plants including soybean, corn, and alfalfa have many biotic stressors in the field during the growing period.

Data Availability

The datasets generated during and/or analyzed during the current study are not publicly available due to the problem of plagiarism but are available from the author upon reasonable request.

Conflicts of Interest

The author declares that he has no conflicts of interest.

Authors' Contributions

BD designed the study; collected, analyzed, and interpreted data; was involved in the write-up; and reviewed and corrected the manuscript.

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