

Article

***Rhizophagus irregularis* and *Azotobacter chroococcum* Uphold Eggplant Production and Quality under Low Fertilization**

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Abstract: Microorganisms are essential parts of soil and play an important role in mediating many processes and influencing plant health. Arbuscular mycorrhizal fungi (AMF) and nitrogen-fixing bacteria (NFB), the most common of such microorganisms, can benefit plants by enhancing the nutrient-absorbing ability of roots through bio-inoculation, also called biofertilization. Different methods have been tested and proven to be effective in the enhancement of soil nutrient availability. However, the effects of increased application of biological methods with minimal chemical fertilizers are still inconsistent. In this 2-year of fixed-point greenhouse test, we aimed to evaluate the impact of AMF (*Rhizophagus irregularis*) and/or NFB (*Azotobacter*) on growth, quality, and yield of eggplants under different N levels. Data showed that biofertilizer application with reduced chemical fertilizer had the highest impact on eggplant performance and yield. Indeed, low chemical fertilizers combined with adequate amounts of biofertilizers produced a higher plant height, length and width of leaves, dry matter, number of fruits per plant with better morphology, total yield per plant, and total soluble solids (TSS), suggesting that the use of *Azotobacter* and *R. irregularis* as biofertilizers could substantially reduce the use of chemical fertilizers without impairing the quality and yield of eggplant.

Keywords: *Solanum melongena* L.; *Azotobacter*; biological fertilizers; chemical fertilization; rhizosphere microorganisms; sustainable agriculture



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1. Introduction

Eggplant, which belongs to the Solanaceae family, is an economically important crop native to the tropical and subtropical regions of (South) India and Sri Lanka [1] and is widely grown in South/Southeast Asia. Eggplant—as a nutrient-dense food—is an excellent source of proteins and carbohydrates as well as a variety of vitamins and minerals [2–4]. It has high antioxidant levels, especially an anthocyanin called nasunin, high fiber content, and its consumption is believed to prevent cellular damages and risk factors including heart disease and cancers [5–7].

However, the reduced arable land, growing human population, weather patterns, and unpredictability of monsoons that millions of farmers rely on threaten eggplant production and food security. Chemical fertilization, a common agricultural practice to increase the yield, is being used extensively. Fertilization has been found to affect soil microbial biomass, activity, and community composition in agricultural production systems by modifying soil physicochemical properties [8]. Since organic farming is always associated with a lower yield of crops, and is endowed with improved nutritional properties, there is a higher

cost [9,10]. Therefore, the use of chemical fertilizers cannot be eliminated once increased food production is expected.

Use of chemical fertilizers above the threshold level has led to many unexpected effects such as the generation of greenhouse gases, soil toxicity, and waste of mineral resources, resulting in reduced soil permeability [8,11–13], thereby leading to food safety and vegetal quality decline problems [14]. Integrated nutrient management practices by incorporating chemical and organic/biofertilizer nutrient sources are time-demanding tasks for restoring soil health and crop nutritional values. Several studies reported that organic/biofertilizer nutrient sources positively affected crop production and the physicochemical property and microbial biomass of soil [15–17]. Moreover, biofertilizers do not hamper the plant physiological activities [18,19].

The microorganisms, as biofertilizers, stimulate various biochemical and enzymatic processes. Arbuscular mycorrhiza fungi (AMF) are highly efficient for enhancing assimilation, absorption, and translocation of the essential macro- and micro-nutrients required for modulating plant secondary metabolism to overcome biotic stresses [20,21]. For instance, recent studies reported that eggplant, which is susceptible to biotic stresses, colonized by AMF overcame nematodes infestations [22]. Additionally, AMF might help in modulating secondary metabolic rate [23] and enhancing antioxidant activity coupled with the increase in antioxidant allergens [24], generally recognized as phytochemicals [22].

Inoculation with AMF is an effective strategy for yield stability and crop quality improvement due to their benefits to plants, such as improved nutritional supply, disease resistance, and abiotic tolerance [25–27]. Additionally, applying plant growth-promoting bacteria (PGPB) increased nutrient use efficiency [28,29]. Previous findings show that agronomic yields are considerably improved by the PGPB application to arable soils [30,31]. *Azotobacter*, a well-known nonsymbiotic nitrogen fixer, can solubilize inorganic nitrogen from insoluble compounds that can be utilized for plants to increase their yield [32,33]. Owing to the production of PGP substances, biofertilizers increase, among others, the availability of accumulated P (by solubilization) and the efficiency of fixing biological N₂ [34,35].

However, co-inoculation reports of *Azotobacter* and AMF are scarce and very little study has been undertaken on these guidelines, especially in vegetables. To discover the impact of NFB and AMF on vegetable crop yields, comprehensive studies are urgently needed. Rather than sole chemical fertilizers, we showed that the eggplant production strategy should be combined using the application of chemical and biofertilizer for sustainability due to environmental issues created by over-use and higher manufacturing expenses of fertilizers. Therefore, this study aimed to evaluate the significance of single and dual inoculation of AMF and PGPB combined with reduced rates of chemical fertilizer to produce eggplant yield and fruit quality equivalent.

2. Material and Methods

2.1. Experimental Layout

The experiment was performed under Greenhouse located at Ranchi University, Jharkhand, India, for consecutive 2 years, viz. 2018 and 2019 (23°22'18" N, 85°19'27" E). In both years, the experiment was conducted in a randomized complete block design (RCBD), and there were three replicates of each treatment, each of which consisted of 15 plants. The average temperature recorded was 22.5 ± 6.0 °C and the relative humidity was 50–68%. We selected eggplant (*Solanum melongena*) cv. Pusa Kranti (IARI, New Delhi) for the material used in the experiment and the plastic-germinating trays comprising a cocopeat and perlite (2:1 v/v) mixture were used for sowing the seeds. The seeds that germinated uniform in size after 30 days (2–4 true leaf stage) were carefully selected and transplanted into pots each filled with sieved and growing soil.

The characteristics of the soil were sand (70.5%), silt (24.8%), clay (4.7%), nitrogen (0.048%), available phosphorus (0.020%), organic carbon (0.05%), and a pH of 7.2. The pH content of the soil was determined using the Orion pH meter (Model 420A, Orion Research

Inc., Franklin, MA, USA) by suspending the samples in water at a ratio of 1:2.5 soil to water. The treatments designed for the experiment were as follows: (1) CF, 100% chemical fertilizer with conventional package and practices; (2) 75%CF, 75% chemical fertilizer; (3) 75%CF + RI: 75%CF + *Rhizophagus irregularis* (AMF); (4) 75%CF + AC: 75%CF + *Azotobacter chroococcum* (PGPB); and (5) 75%CF + RI + AC: 75%CF + *Rhizophagus irregularis* + *Azotobacter chroococcum*.

According to the conventional package and practices, the recommended dosage of chemical fertilizer per acre consists of 55 kg urea, 155 kg single superphosphate, and 20 kg muriate of potash. The chemical fertilizers were incorporated in the soil at the time of transplanting [36]. The 75% CF used for the experiment was mainly based on the minimum levels, below which the AMF/PGPB and fertilizer interaction could not result into a constant nutrient uptake as compared to the non-inoculated fertilization rates [9].

A. chroococcum was obtained from DORA (Zoutleeuw, Flemish Brabant, Belgium), while *R. irregularis* was provided by M/S Shri Ram Solvent Extractions Pvt. Ltd., (Jaspur, India) at 100 spores/g CFU count. For AMF, prior to transplanting, the plants were provided with 100 g of a material having infected propagules (mycelium, roots, and spores). For non-mycorrhizal, an equal amount of non-inoculated and non-mycorrhizal *Z. mays* roots were provided to match the organic matter in pots, and inoculum filtered to refurbish other free-living micro-organisms in the soil known to accompany the AMF. We used *Z. mays* as we previously described it as a good carrier medium that is both cost-effective and economical, and that can be used by farmers as part of environmentally responsible cultural practices to produce AMF inoculum on their farm [37].

The mycorrhizal inoculum was passed through a layer of 15–20 µm filter paper (Whatman, GE Healthcare, Buckinghamshire, UK) in 100 mL of distilled water to obtain the filtrate from each pot. For PGPB, the seedlings were dipped in a suspension of *Azotobacter chroococcum* (40 g/L) for 30 min. The experiment was performed repeatedly for five replicates. The AMF spore number was estimated with 'Gridline Intersect method' defined by Kennedy et al. [38]. The extent of root colonization (%) was estimated with the Phillips and Hayman [39] staining method, subsequently by the 'Giovannetti and Mosse' [40] method, with the help of a Lab Digital Trinocular Compound LED Microscope (Omax 40×–2500×). The percentage AMF infested root segments was obtained using the following formula: 100/(number of root segments colonized/total number of root segments).

2.2. Plant Characterization

At the time of flowering, plant height, primary branches per plant, days to 50% flowering, and leaf length and width were assessed. After commercial maturity, the fruit length and the fruit circumference were measured. Total yield (kg) was measured as the harvest per plant and number of fruits/plant were determined manually. Dry matter, which was found after drying the samples at 80 °C until a constant weight, and total soluble sugar (TSS) with a handheld refractometer (RA-130-KEM, Kyoto Electronics Manufacturing Co., Ltd., Kyoto, Japan) were measured.

2.3. Data Analysis

The means of the treatments for each year and the grand mean of both years were analyzed for differences using the ANOVA. The Student-Newman-Keuls test was employed as a post hoc test in the Statgraphics Centurion XVIII (Stat Point Technologies, Warrenton, VA, USA) software to determine whether there was a statistically significant difference between treatment averages. Further, the Pearson correlation coefficient for the pair-wise correlation analysis was performed using Statgraphics Centurion XVIII software to assess the eggplant performance.

3. Results

3.1. Effects of Different Fertilizer Treatments on Eggplants Performance

Significant differences among the five treatments (<0.01) were observed for the 14 traits studied in the present investigation. In contrast, the effects of years were not significant

(Table 1). The treatment effects over the 2 years were significant only for two traits, plant height and leaf length. The effects of the mycorrhizal fungus *R. irregularis* and/or the nitrogen-fixing bacterium *A. chroococcum* and/or chemical fertilizer on the growth and development of eggplant were investigated (Table 2). Data indicated that microbial fertilizers with reduced rates of chemical fertilization (75% of the conventional application) (75%CF + RI + AC) produced eggplant biomass and growth better than those obtained using the 100% or 75% of the chemical fertilizer alone. In addition, the dual combination 75%CF + RI and 75%CF + AC positively affected the measured traits (Table 2).

Table 1. Variance analysis of the effects of different treatments on morphological and biochemical traits of eggplant.

Trait	Treatments	Year	Treatments X Year	Residuals
df	4	1	4	20
Plant height (cm)	155.24	18.28	87.06	20.29
F	76.84	0.90	4.29	
p	<0.001	0.35	0.01	
Primary Branches/Plant	7.07	0.17	1.07	0.73
F	9.68	0.24	1.46	
p	<0.001	0.63	0.25	
Leaf length (cm)	32.75	5.83	8.68	3.03
F	10.82	1.92	2.87	
p	<0.001	0.18	0.05	
Leaf width (cm)	15.36	0.03	2.69	1.85
F	8.29	0.02	1.45	
p	<0.001	0.9	0.25	
Flowers/Cluster	4.14	0.09	0.29	0.38
F	10.78	0.22	0.76	
p	<0.001	0.64	0.56	
Days to 50% Flowering	139.03	31.79	3.70	17.40
F	7.99	1.83	0.21	
p	<0.001	0.19	0.93	
Fruit length (cm)	11.86	0.43	1.37	1.38
F	8.58	0.31	0.99	
p	<0.001	0.58	0.44	
Fruit circumference (cm)	336.07	10.50	2.30	5.03
F	66.82	2.09	0.46	
p	<0.001	0.16	0.76	
Number of Fruits/Plant	111.52	27.28	1.15	3.67
F	13.25	2.76	10.22	
p	<0.001	0.18	0.54	
Yield (Kg)	32.75	4.04	3.87	2.13
F	8.10	5.18	2.01	
p	<0.001	0.21	0.49	
Dry matter%	10.39	4.36	1.76	1.34
F	7.75	3.25	1.31	
p	<0.001	0.09	0.30	
TSS (°Brix)	4.79	0.78	0.55	0.34
F	13.98	2.29	1.61	
p	<0.001	0.15	0.21	
AM spore number	19,072.03	9.63	4.63	10.63
F	1793.61	0.56	0.44	
p	<0.001	0.49	0.78	
Root colonization (%)	7558.31	5.35	2.09	2.82
F	2681.44	2.14	0.74	
p	<0.001	0.22	0.57	

Table 2. Effects of different treatments on morphological and biochemical traits of the eggplant.

	Years	100%CF	75%CF	75%CF + RI	75%CF + AC	75%CF + RI + AC
Plant height (cm)	2018	60.31 ± 4.53 d	42.86 ± 1.66 e	68.42 ± 0.87 c	74.98 ± 2.77 b	84.15 ± 3.76 a
	2019	67.46 ± 2.33 b	39.86 ± 0.87 c	79.27 ± 3.78 a	66.88 ± 3.25 b	85.07 ± 11.23 a
	Overall	63.88 ± 5.07 C	41.36 ± 2.03 D	73.84 ± 6.43 B	70.93 ± 5.20 B	84.61 ± 7.51 A
Primary branches/Plant	2018	3.56 ± 0.61 c	3.66 ± 0.66 c	5.48 ± 1.57 b	6.02 ± 0.34 a	6.02 ± 0.51 a
	2019	4.75 ± 1.67 b	3.15 ± 0.72 c	4.79 ± 0.42 b	5.13 ± 0.27 b	6.15 ± 0.31 a
	Overall	4.15 ± 1.30 BC	3.41 ± 0.68 C	5.14 ± 1.10 AB	5.58 ± 0.56 A	6.09 ± 0.38 A
Leaf length (cm)	2018	16.41 ± 2.51 a	15.16 ± 1.76 b	16.33 ± 1.17 a	17.29 ± 1.26 a	19.72 ± 0.92 a
	2019	17.91 ± 2.49 a	12.15 ± 1.48 b	19.52 ± 0.63 a	19.60 ± 0.68 a	20.13 ± 2.80 a
	Overall	17.16 ± 2.38 B	13.65 ± 2.20 C	17.93 ± 1.94 AB	18.45 ± 1.56 AB	19.93 ± 1.88 A
Leaf width (cm)	2018	12.81 ± 1.27 b	11.78 ± 0.39 b	12.89 ± 2.50 a	12.57 ± 1.93 a	15.53 ± 1.19 a
	2019	11.57 ± 0.27 b	10.66 ± 0.98 b	13.78 ± 1.28 a	14.46 ± 0.68 a	15.43 ± 1.48 a
	Overall	12.19 ± 1.07 BC	11.22 ± 0.91 C	13.33 ± 1.85 B	13.52 ± 1.66 B	15.48 ± 1.20 A
Flowers/cluster	2018	4.11 ± 0.34 a	3.02 ± 0.60 b	3.95 ± 0.89 a	3.97 ± 0.46 a	4.93 ± 0.71 a
	2019	3.99 ± 0.55 a	2.47 ± 0.41 b	4.43 ± 0.40 a	4.44 ± 0.67 a	5.18 ± 0.89 a
	Overall	4.05 ± 0.42 B	2.74 ± 0.55 C	4.19 ± 0.67 B	4.21 ± 0.57 B	5.05 ± 0.73 A
Days to 50% Flowering	2018	75.44 ± 2.90 ab	65.96 ± 2.00 d	70.47 ± 5.51 bc	68.43 ± 6.49 cd	78.60 ± 2.59 a
	2019	73.71 ± 3.66 ab	63.37 ± 3.49 d	70.27 ± 4.90 bc	67.06 ± 5.58 cd	74.19 ± 1.31 a
	Overall	74.57 ± 3.10 AB	64.66 ± 2.92 D	70.37 ± 4.66 BC	67.75 ± 5.46 CD	76.39 ± 3.03 A
Fruit length (cm)	2018	9.18 ± 0.45 b	7.96 ± 0.80 c	9.02 ± 0.29 b	9.68 ± 2.37 b	12.71 ± 1.31 a
	2019	9.11 ± 1.13 b	8.19 ± 0.76 b	9.94 ± 1.27 b	9.02 ± 0.97 b	11.10 ± 1.06 a
	Overall	9.14 ± 0.77 B	8.08 ± 0.71 B	9.48 ± 0.97 B	9.35 ± 1.66 B	11.90 ± 1.38 A
Fruit circumference (cm)	2018	10.87 ± 1.63 c	7.68 ± 2.22 c	14.47 ± 3.49 b	14.48 ± 1.70 b	27.08 ± 0.46 a
	2019	11.89 ± 1.10 cd	7.71 ± 2.52 d	17.75 ± 4.27 b	15.11 ± 0.80 bc	28.02 ± 0.98 a
	Overall	11.38 ± 1.36 C	7.69 ± 2.13 D	16.11 ± 3.93 B	14.79 ± 1.24 B	27.55 ± 0.86 A
Number of Fruits/Plant	2018	11.70 ± 0.13 bc	9.56 ± 0.37 c	12.44 ± 0.15 b	13.29 ± 0.19 a	14.33 ± 0.25 a
	2019	12.41 ± 0.29 b	8.36 ± 0.14 d	12.28 ± 0.31 c	13.84 ± 0.36 b	15.12 ± 0.47 a
	Overall	12.05 ± 0.49 C	8.90 ± 0.84 D	12.30 ± 0.14 C	13.40 ± 0.56 B	14.70 ± 0.46 A
Yield (Kg)	2018	2.46 ± 0.45 b	1.15 ± 0.13 d	2.03 ± 0.06 c	2.90 ± 0.07 a	3.05 ± 0.66 a
	2019	2.31 ± 0.12 b	1.63 ± 0.10 d	2.18 ± 0.11 c	2.68 ± 0.21 b	3.26 ± 0.09 a
	Overall	2.38 ± 0.10 C	1.40 ± 0.34 D	2.10 ± 0.13 C	2.80 ± 0.15 B	3.16 ± 0.17 A
Dry matter (%)	2018	8.64 ± 1.48 b	12.59 ± 0.48 a	10.11 ± 0.78 b	10.05 ± 1.44 b	9.00 ± 0.40 b
	2019	10.91 ± 2.12 b	12.84 ± 1.03 a	9.44 ± 0.92 b	10.99 ± 1.23 b	10.02 ± 0.50 b
	Overall	9.78 ± 2.05 B	12.72 ± 0.73 A	9.77 ± 0.85 B	10.52 ± 1.30 B	9.51 ± 0.69 B
TSS (°Brix)	2018	5.68 ± 0.16 b	5.35 ± 0.56 b	6.23 ± 1.00 b	7.21 ± 0.38 a	7.51 ± 0.58 a
	2019	6.05 ± 1.05 b	5.04 ± 0.41 b	5.66 ± 0.50 b	6.01 ± 0.21 a	7.60 ± 0.27 a
	Overall	5.87 ± 0.70 CD	5.19 ± 0.47 D	5.94 ± 0.77 BC	6.61 ± 0.71 B	7.56 ± 0.40 A
AM spore number	2018	0.00 ± 0.00 c	0.00 ± 0.00 c	94.00 ± 4.35 b	0.00 ± 0.00 c	108.33 ± 6.35 a
	2019	0.00 ± 0.00 c	0.00 ± 0.00 c	98.00 ± 6.24 b	0.00 ± 0.00 c	110.00 ± 4.58 a
	Overall	0.00 ± 0.00 C	0.00 ± 0.00 C	96.00 ± 5.29 B	0.00 ± 0.00 C	109.16 ± 5.03 A
Root colonization (%)	2018	0.00 ± 0.00 c	0.00 ± 0.00 c	59.55 ± 1.26 b	0.00 ± 0.00 c	67.55 ± 4.54 a
	2019	0.00 ± 0.00 c	0.00 ± 0.00 c	62.00 ± 1.73 b	0.00 ± 0.00 c	69.33 ± 1.52 a
	Overall	0.00 ± 0.00 C	0.00 ± 0.00 C	60.77 ± 1.90 B	0.00 ± 0.00 C	68.35 ± 1.27 A

Means followed by the same letters either in upper or lower case are not significantly different at $p < 0.05$ (Newman-Keuls test).

Indeed, we observed a significant enhancement in plant height by ca. 32% in 75%CF + RI + AC, 16% in 75%CF + RI, and 11% in 75%CF + AC compared to C plants (Table 2). Similarly, primary branches per plant increased by ca. 47% in 75%CF + RI + AC, 34% in 75%CF + AC, and 25% in 75%CF + RI than in C plants. Furthermore, 75%CF + RI + AC presented the most effective treatment to increase leaf length and width by 16% and 27%, respectively, compared to non-inoculated C plants (Table 2). The application of the tripartite

combination yielded the highest values of flowers' number per cluster, ca. 25% of the C values, followed by the dual combinations (75%CF + RI and 75%CF + AC) (Table 2). The biomass in the microbial-added treatments was much higher than with the full-rate chemical fertilizer treatment (C), with the highest value ca. 30% recorded in the tripartite treatment.

Application with the inoculant (RI and/or AC) combined with 75%CF led to an increase in fruits traits compared to the control (Table 2). Fruit length was approximately 30% higher in 75%CF + RI + AC than that of the C fruits. Similarly, the fruit circumference increased by ca. 42% with 75%CF + RI, 30% with 75%CF + AC, and 142% in the microbial material added to the reduced chemical fertilizer treatment. Number of fruits per plant increased by 22% with 75%CF + RI + AC and 11% with 75%CF + AC, while other treatments were not as effective for this parameter (Table 2).

Likewise, yield per plant increased by 33% with 75%CF + RI + AC and 17% with 75%CF + AC. Interestingly, the dual combination 75%CF + RI and 75%CF + AC decreased the days to 50% flowering by 6% and 9%, respectively, compared to the C treatments, while no significant changes were recorded in the 75%CF + RI + AC treatment (Table 2). The TSS contents increased significantly in the tripartite and dual (75%CF + AC) treatments than in control plants. The application of 75%CF did not inhibit the AM spore number nor root colonization after the mycorrhizal amendment. AM colonization and spore number were higher in eggplant inoculated with AMF combined with PGPB than AMF alone (Table 2).

3.2. Pearson Correlations of the Effects of Different Fertilizer Treatments on Eggplant Performance and Fruit Parameters

In total, 66 correlations among the plant performance, flowering time, and fruit characteristics were detected (Figure 1). Of these correlations, 62 had a significance level of <0.05 (Figure 1). In particular, the primary branches/plant trait was found to be positively correlated with plant height (0.722), leaf length (0.641), leaf width (0.505), number of flowers per cluster (0.662), fruit length (0.583), TSS (0.827), and fruit circumference (0.597). Leaf length has a positive correlation with plant height (0.727), leaf width (0.563), number of flowers per clusters (0.703), days to 50% flowering (0.394), fruit length (0.459), TSS (0.539), and fruit circumference (0.604) (Figure 1).

Similarly, leaf width was positively correlated with plant height (0.703), the number of flowers per cluster (0.601), days to 50% flowering (0.366), fruit length (0.566), TSS (0.49), and fruit circumference (0.689). Further, flowers/clusters were significantly correlated with plant height (0.799), days to 50% flowering (0.592), fruit length (0.595), TSS (0.66), fruit circumference (0.689) in a positive direction. The days to 50% flowering showed a positive internal link with plant height (0.505), fruit length (0.583), TSS (0.478), and fruit circumference (0.504). Fruit length showed strong positive links with plant height (0.717), TSS (0.671), and fruit circumference (0.694).

The TSS has a strong positive connection with fruit circumference (0.664) (Figure 1). Further, yield was positively correlated with plant height (0.803), primary branches/plant (0.645), leaf length (0.601), leaf width (0.614), flowers/cluster (0.701), days to 50% flowering (0.47), fruit length (0.641), fruit circumference (0.756), and TSS (0.723). Likewise, number of fruits per plant exhibited a positive correlation with plant height (0.89), primary branches per plant (0.739), leaf length (0.811), leaf width (0.717), flowers/cluster (0.796), days to 50% flowering (0.53), fruit length (0.608), fruit circumference (0.797), TSS (0.746), and yield (0.872). In contrast, dry matter was negatively correlated with all parameters detected in this work, being significantly correlated with plant height (-0.606), leaf length (-0.482), flowers/clusters (-0.473), days to 50% flowering (-0.469), fruit circumference (-0.46), leaf width (-0.422), yield (-0.508), and number of fruits per plant (-0.536) (Figure 1).

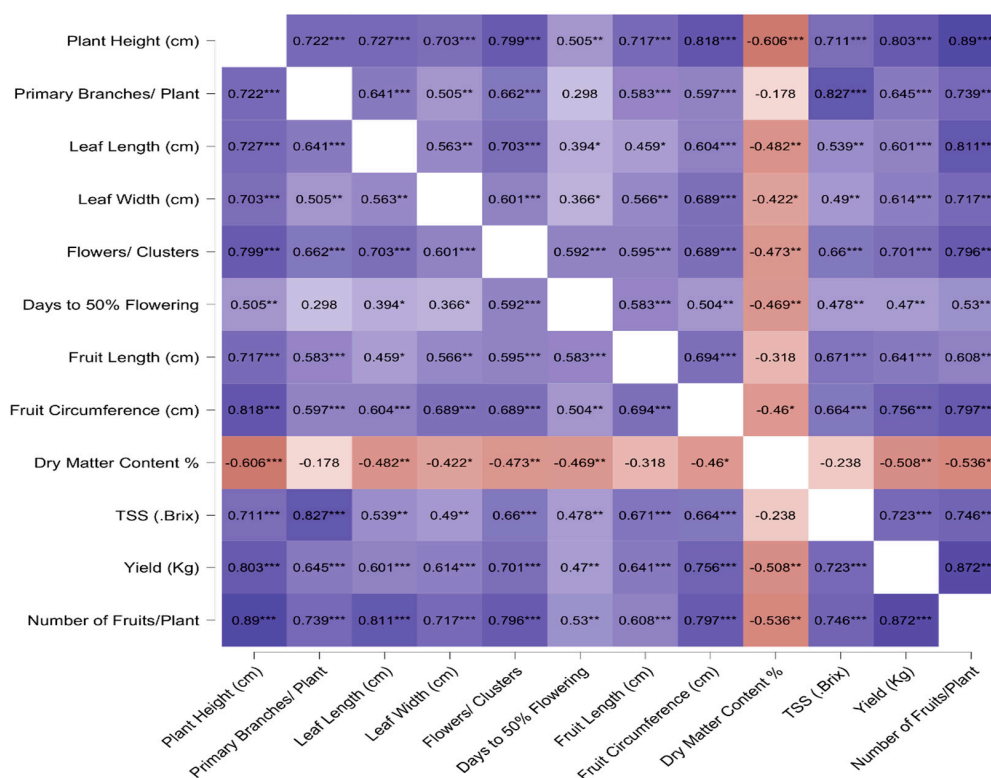


Figure 1. Pearson linear correlations of the studied parameters. Where, ***, **, * indicate significant at $p < 0.001$, $p < 0.01$, or $p < 0.05$, respectively.

4. Discussion

As it is associated with growth and production, the plant microbiome is developing as a good complement of chemical fertilizers for a sustainable resource for agricultural productivity. This “assemblage” of plant-associated microbes have likely shaped this association and impact plant fitness. Besides, the high microbe densities spotted on the plant tissues indicate that the interactions among microbes are also significant selective forces carving complex microbial assemblies and their significance for plant health. The reduced application of fertilizers on agricultural soils without compromising productivity is an achievable but difficult task. However, the consequences of bio-inoculation with reduced levels of chemical fertilizer remains scarce.

The utilization of AMF inoculum developed on-farm is associated with increased crop yields with minimal modifications to typical farm management strategies [41,42]. In accordance, our data showed that plant height increased following the *R. irregularis* and/or *A. chroococum* application, along with reduced fertilizers. These observations confirmed our supposition that the AMF and PGB-enriched bioorganic fertilizer combination with reduced chemical fertilizers (75%) could potentially yield eggplant performance either comparable to or even more than that obtained with 100% chemical fertilizer (Tables 1 and 2).

Rose et al. [43] reported that biofertilizers could replace 23–52% of N fertilizer without yield loss [43]. Studies showed that AMF could potentially enhance water uptake along with the absorption of essential minerals, thereby producing higher growth in terms of plant height and weight in inoculated eggplant [20,21,44]. Similar results regarding PGPB and AMF application showed an improvement of root architecture and localized systemic resistance and enhanced microbial rhizospheric soil-flora. Moreover, AMF and *Azotobacter* can easily undergo colonization with plant roots in addition to the symbiotic association with other beneficial microbes [45,46]. The observed increases in leaf length and width were also in accordance with several reports indicating that, in addition to the absorption of essential minerals, AMF and *Azotobacter* could promote the synthesis of chlorophyll and chloroplast proteins [47–49]. This later might be involved in an increase of photosynthesis

and other functions in leaves, which are associated with enhanced growth and uptake of C, N, and P due to microbial application that promote the development of eggplants.

The extra radical mycelium may efficiently enhance nutrient-uptake, improving the growth and development of different plant parts [50]. In addition, better growth of the root structure impacts the soil microbiome by releasing extra exudates, i.e., organic acids and sugars. In turn, the soil microbes respond with higher interactions and more availability of nutrients that may affect the plant growth [51]. In this study, at reduced CF, the dual inoculation consistently enhanced the (primary) branching number, the number of flowers per cluster, and early flowering and fruiting, as well as the quality of fruit.

Aseri et al. [52] showed that an amalgamation of AMF (*Glomus mosseae*) and nitrogen fixing bacteria (*Azotobacter chroococcum*) enhanced the yield of the pomegranate fruits under field conditions [52]. *Azotobacter* turns nitrogen into ammonia, which is then absorbed by plants, thereby supplying crop plants with accessible nitrogen.

Similarly, Bona et al. [53] revealed that PGP pseudomonas and AMF inoculum (*R. intraradices*, *G. aggregatum*, *G. viscosum*, *C. claroideum* and *C. etunicatum*) increase the rate of flowering, fruit production, as well as the vitamin content in strawberries cultivated at lower levels of nitrogen and phosphorus [53]. This may be demonstrated through Pearson correlations, showing a correlation of biomass and flowering/fruiting traits, as in Figure 1. The beneficial effects of bacteria on several crops have been elucidated by their ability to produce substances like auxin and cytokinin, in addition to solubilization of phosphates [54]. Similarly, AMF secretes strigolactones (iron-chelating agent) to increase the communication between plant and the interacting organisms-inducing several responses in AMF (e.g., spore germination, hyphal branching pattern, production of chitin oligosaccharides) [55–57].

Besides, the inoculation of AMF stimulated the biochemical reactions involved in phytohormones biosynthesis, especially gibberellins, cytokines, and/or auxins (or molecules with auxinic activity) responsible for fruit quality enhancement [58,59], which could, at least partly, improve the eggplant fruit characteristics (fruit length and fruit circumference) observed in our study. In fact, fruit production is caused by auxin and gibberellins [60,61], the production of which by PGPB is generally documented in the literature.

Ferrol et al. [62] and Salvioli et al. [63] also revealed that AMF up-regulated the mitochondrial genes regulating ATP synthesis required for plant growth and development [62,63]. This further correlates with chitin formation and phytohormone synthesis and eventually intensifies the symbiotic association [64].

The AM spore number and root colonization (%) were significantly pronounced in *R. irregularis* treated plants as compared to controls; however, the treatments CF, 75%CF, and 75%CF + AC presented no AM spore number and root colonization (%). Yadav et al. [65] reported that the increment of AMF (*Glomus mosseae* and *Acaulospora laevis*) in the sesame plant was correlated positively to AM root colonization [65].

In general, *G. mosseae* and *A. laevis* show different magnitudes of root colonization owing to the difference in the extent of water and mineral absorption, which could result in variations in different plant-growth parameters. In addition, after inoculation with AMF, plants displayed longer and thinner root architecture for nutrient absorption [66,67]. Hyphae of AMF-infected roots can enter deep into the phosphorus depleted zone, making it easily accessible to plants [68]. Inferential statistical analysis of the Pearson correlation coefficient helped to test the statistical hypotheses. More specifically, in the present study, we determined the statistically significant connection between the primary branches per plant trait and plant height, leaf length, leaf width, and TSS. These results are consistent with our previous studies [65,69].

5. Conclusions

Altogether, although chemical fertilizers are primarily needed to overcome nutrient deficits and in facilitating the growth and development of vegetable crops, their disproportionate use may prove to be harmful to the environment and human health. Bioinoculants

such as AMF and *Azotobacter* have been tested for their efficacy in crops to minimize chemical fertilization. This study observed that the proper and judicious application of bioinoculants provides enough nutrients and other substances to the host eggplants that may improve their growth, nutritive value, and yield.

In addition, our results imply that the dual application of AMF and *Azotobacter* biofertilizers, rather than single application, together with the CF could help avoid the overuse of full chemical fertilizers and results in sustainable agricultural production. Therefore, considering the beneficial effects of AMF and *Azotobacter* (in terms of biofertilization and bioremediation) on crop productivity and functioning of the ecosystem, their agricultural application should be promoted.

Although plant bioinoculants appear to be an innovative and a potential class of agricultural inputs capable of substituting chemical fertilizers, additional research will be required to decipher the mechanisms at molecular, cellular, and/or physiological levels, which will undoubtedly facilitate these bio-products into the management strategies in agriculture.

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