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Rhizophagus irregularis and Nitrogen Fixing Azotobacter with a Reduced Rate of Chemical Fertilizer Application Enhances Pepper Growth along with Fruits Biochemical and Mineral Composition

Meenakshi Sharma [1], Vandana Sharma [2], Anil Kumar Delta [1] and Prashant Kaushik [3,*]

1 Department of Chemistry, Ranchi University, Ranchi 834001, India; meenakshiskkr@gmail.com (M.S.); akumardelta2013@gmail.com (A.K.D.)
2 Department of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut 250110, India; vandana_20sep@yahoo.com
3 Kikugawa Research Station, Yokohama Ueki, 2265, Kikugawa 439-0031, Japan
* Correspondence: prakau@doctor.upv.es

Abstract: Bell pepper is an important vegetable crop containing lots of bioactive compounds. The present study was designed to improve the productivity and quality of bell pepper with the application of arbuscular mycorrhizal fungi (*Rhizophagus irregularis*) and plant growth-promoting bacteria (*Azotobacter chroococcum*) in a combination of chemical fertilizer. Five treatments consisted of 75% chemical fertilizer (T1), 100% chemical fertilizer (T2), 75% chemical fertilizer + *R. irregularis* (T3), 75% chemical fertilizer + *A. chroococcum* (T4) and 75% chemical fertilizer + *R. irregularis* + *A. chroococcum* (T5). Out of 18 morphological parameters, 11 morphometric fruit parameters were recorded in detail by a tomato analyzer. The morphological and biochemical (TSS, ascorbic acid and capsaicin content) attributes of bell pepper were recorded higher in the case of a mixed consortium of chemical fertilizers having *R. irregularis* and *A. chroococcum*. Similarly, the amount of mineral content recorded was highest after 75% chemical fertilizer + *R. irregularis* + *A. chroococcum*, followed by the treatment with only 100% chemical fertilizer. The root mycorrhization (%) and the number of spores were observed highest in 75% chemical fertilizer + *R. irregularis* + *A. chroococcum*, and there was no mycorrhization and spore formation in 75% CF, 100% CF and 75% CF+AC. The treatment involving 75% chemical fertilizer + *R. irregularis* + *A. chroococcum* proved better for pepper's growth, yield and yield-related traits.

Keywords: bell pepper; *Rhizophagus irregularis*; Azotobacter; tomato analyzer; mineral content

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1. Introduction

The bell pepper (*Capsicum annuum* L.) is a member of the Solanaceous family and a globally important vegetable with widespread commercial acceptability for its organoleptic features [1] as well as its high level of bioactive components [2]. The bell pepper is primarily cultivated in tropical and sub-tropical climates [3]. It is the world's second most significant vegetable crop after tomato and is largely non-pungent and mildly pungent with thick flesh and produced in India for its ripe fruits, which are commonly used in stuffing, baking, noodles, salad and soup dishes [4]. The bell pepper is a vital antioxidant-rich food to consume since it can lessen the risk of certain illnesses in humans [5]. Capsicum species have a wide range of therapeutic benefits, which has resulted in their increased cultivation.

Supplying high-quality inputs may boost the yield of any crop. Consequently, various fertilizers are employed in varying concentrations to improve the productivity and quality of Capsicum species. Nitrogen, potassium and phosphorus fertilizers are the most often utilized [6]. The importance of nutrition for the survival or development of capsicum cannot be overstated, as nitrogenous, phosphoric and potassium fertilizers positively affect it [4].

Nutrient storage in the soil significantly impacts crop quality and production. Chemical fertilizers boost growth and crop output [7]. Numerous issues confront modern intensive agricultural techniques, posing severe implications to world food security. Chemical fertilizers and pesticides are used on a broad scale to improve agricultural output in order to meet the nutritional needs of the ever-increasing global population. However, the long-term use of chemical fertilizers contaminates groundwater supplies, alters pH and jeopardizes the health of plant growth-promoting microbes [8]. Organic fertilizers boost crop yield by providing vital nutrients directly or indirectly. Additionally, they stimulate microbial abundance and variety and promote the activities of the primary soil enzymes involved in organic matter breakdown and nutrient recycling [9]. Biofertilizers are a blend of naturally occurring compounds and microorganisms applied to boost soil fertility. Biofertilizers are highly beneficial to soil health, growth and the development of crops.

Microorganisms linked with plants play a critical function in agricultural productivity. Numerous studies showed that the existence of a microbial consortium comprising two or more interacting microorganisms benefits plants. However, it is becoming more evident that the presence of a microbial consortium consisting of two or more interacting microorganisms results in additive or synergistic effects [10]. There are various microbial inoculants (bacteria + fungi) comprising *Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas* and *R. irregularis* that were found to be successful for crop production compared to conventional mineral and chemical fertilizers. The commonly used biostimulants are AMF (arbuscular mycorrhizal fungi) and PGPB (plant growth-promoting bacteria) [11,12]. Peppers are a rich source of important vitamins and minerals content, making them highly beneficial to human health [13], and the inoculation of PGPB and AMF increases the mineral nutrient content and enhances the growth and yield of the crop [14].

Azotobacter chroococcum was found to be important in agricultural production for enhancing germination percentage, plant nutrition and soil fertility [15]. The significance of AMF as a bio-fertilizer has the potential to improve plants' ability to adapt to changing environments. AMF may help host plants up-regulate tolerance mechanisms or prevent down-regulating critical metabolic reactions [16]. Because AMF are natural root symbionts, they deliver vital plant inorganic nutrients to host plants, enhancing growth and production in stressed and unstressed conditions [13,17]. Combined organic and inorganic fertilizers have gained significant importance for vegetable cultivation [18]. So many nutrients are essential for the high throughput production of edible crops, but fertilizer alone cannot sustain the productivity of soils under highly intensive cropping systems. In addition, using biofertilizers as an integrated nutrient management strategy can help mitigate several nutrient deficits [19]. Therefore, in the present investigation, we examine the effects of *R. irregularis* (AMF) and *A. chroococum* (PGPB) with a combination of chemical fertilizer on the morphological and biochemical parameters of bell pepper.

2. Materials and Methods

2.1. Experimental Setup

The present investigation was conducted in greenhouses at the Agriculture Research Farm, Haryana, India (29.94° N 76.89° E) during October 2018–19. The standard temperature was 22.5 ± 6.0 °C. The relative humidity was 50–68%. Bell pepper (*Capsicum annuum*) cv. California Wonder was chosen as the experimental material, and the seeds were sown in plastic germinating trays containing a mixture of cocopeat and perlite (2:1 v/v). After 30 days (2–4 true leaf stages), germinated seeds in uniform size were selected and transplanted into each plastic pot filled with sieved and autoclaved growing soil. The soil characteristics were 70.5% sand, 24.8% silt, 4.7% clay, 0.048% N, 0.020% available P, 0.05% organic carbon and pH 7.2 as estimated using an auto discrete analyzer SmartChem® 200 (KPM Analytics, Frepillon, France). There were five plants in each replication, and each treatment was replicated 5 times.

2.2. Treatments

The treatments were designed as 75%CF (T1): 75% chemical fertilizer with conventional package and practices, 100%CF (T2): 100% chemical fertilizer with conventional package and practices, 75%CF+RI (T3): 75% CF + AMF (*R. irregularis*), 75%CF+AC (T4): 75% CF + PGPB (*A. chroococum*) and 75%CF+RI+AC (T5): 75% CF + *R. irregularis* + *A. chroococum*. A fertilization rate of 50 kg of nitrogen (110 kg of urea), 25 kg of phosphorus (172 kg of Superphosphate) and 12 kg of potassium (200 kg of Muriate of Potash) was recommended per acre basis as defined elsewhere [20]. The chemical fertilizer applied in the pots was consistent with the local farmers' fertilization practice; half of N and full P and K dosages were provided at the sowing time, and the remaining half of N was administered 20 days after transplantation, before blooming and following the first harvest. In order to ensure an equal distribution of fertilizers across the whole amount of soil, we started with a small amount of fertilizer and progressively increased the amount of soil in each pot. Potassium dosage was supplied in accordance with the package and practices recommended by the university for 1 acre cultivation of the selected variety for approximately 16,000 pepper plants should be supplied with 200 kg of muriate of potash, which translates to 12.5 g of muriate of potash being supplied to each plant.

The 75% chemical fertilizer in this study was primarily based on the threshold level below which the interaction between AMF/PGPB and fertilizer could not yield consistent nutrient uptake when compared to the non-inoculated fertilizer rates [21]. DORA (Zoutleeuw, Flemish Brabant, Belgium) provided A. chroococum and M/S ShriRam Solvent Extractions Pvt. Ltd. (Jaspur, India) provided R. irregularis at a CFU count of 100 spores/g. Prior to transplantation, the AMF-inoculated plants were given (100 g) of material comprising infective propagules (mycelium, spores and roots). To match the 'organic matter' in the pots, non-mycorrhizal treatments received an equivalent amount of non-inoculated (and non-mycorrhizal) *Zea mays* L. roots and filtered inoculum to restore other soil free-living microbes that accompanied the AMF.

Each pot's filtrate was made by passing the mycorrhizal inoculum through a layer of 15–20 m filter sheets in 100 cm^3 of distilled water (Whatman, GE Healthcare, Buckinghamshire, United Kingdom). For PGPB treatment, the seedlings were dipped in a suspension of *A. chroococum* (40 g dm^{-3}) for 30 min. As Kennedy et al. [22] defined, the number of AMF spores was determined using the Gridline Intersect method, whereas the extent of root colonization (%) was determined using a Lab Digital Trinocular Compound LED Microscope (Omax 40X–2500X) [23,24]. The AMF% in infected roots was recorded with the formula: 100/(number of root segments colonized/total number of root segments).

2.3. Soil and Plant Sampling

At flowering time, days to 50% flowering, fruit length (cm), fruit width (cm), number of marketable fruits per plant, average fruit weight (g) and marketable yield per plant were among the seven characteristics measured manually. The plant height was measured from the ground level to the tip of the plant and measured in centimeters (cm), whereas at commercial maturity, fruit length and fruit circumference were recorded. In the same direction, the perimeter, area (cm^2), width mid-height (cm), maximum width (cm), height mid-width (cm), maximum height (cm), curved height, shoulder height, pepper pericarp boundary, pepper pericarp area and pepper pericarp thickness were measured with the Tomato Analyzer. Ten fruit samples were collected from each replication, and five fruits were split longitudinally and transversally and scanned at a resolution of 300 dpi with an Epson Perfection V19 J371A picture scanner (Epson, Amsterdam, The Netherlands). Tomato Analyzer version 3 software was used to analyze image data for morphometric and colorimetric purposes [25,26].

Inductively coupled plasma (ICP) optical emission spectrometry (Integra XL; GBC Co., Melbourne, Australia) was used to quantify calcium, magnesium, phosphorus, sulphate, iron and sodium following the MFDS Food Code [27]. A solution comprising nitric acid and sulfuric acid was used for the hydrolyzation of the sample before proceeding with the instrumental analysis of ICP. Ascorbic acid estimation was conducted according to the Klein and Perry [27] method. Methanolic extraction (20 mg) was carried out using 10 cm^3 1% of meta-phosphoric acid (Union) at room temp for about 45 min, followed by filtration using 'Whatman No. 4' filter paper. The filtrate (1 cm^3) obtained was then mixed with 9 cm^3 2,6-dichloroindophenol (Sigma, St. Louis, MO, USA). The absorbance of the sample was determined at 515 nm within 15 s against a blank solution. Finally, the ascorbic acid content was evaluated based on the authentic L-ascorbic acid calibration curve [28]. TSS (total soluble solids) was measured through a hand refractometer and expressed in brix. Capsaicin content (mg/100 g as dry weight) of bell pepper fruit was measured as described by Popelka et al. [29]. The capsaicin content was analyzed through HPLC, which involved sample extraction and investigation through liquid chromatography. For the extraction of the sample, fresh as well as dried material were cut down into small pieces, after which the extraction was conducted with ethanol in a ratio of 1:10, then sonication for 30 min and finally, maceration of 4 h showing 90% efficiency of extraction. For HPLC analysis, Ascentis Express RP Amide column 2.7 µm, 100 × 2.1 mm was used, gradient Acetonitrile and 0.2% HCOOH in the ratio 30:70 at 0 min and subsequently 71:29 after 10 min, a flow rate of 0.5 mL/min, volume injected 1 µL, temperature maintained 40 °C and UV detection was conducted at 254 nm and 280 nm.

2.4. Data Analysis

The results obtained were expressed as the mean values ± standard deviation and were subsequently calculated and statistically scrutinized with the help of variance analysis and a Newman–Keuls multiple-range test. Statistical significance was marked at $p < 0.05$ unless stated otherwise. Most tests were conducted using Statgraphics Centurion XVIII software (StatPoint Technologies, Warrenton, VA, USA). Similarly, the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method of hierarchical clustering, Principal Component Analysis (PCA) and Pearson correlation coefficient of pairwise correlation analysis and the Statgraphics Centurion XVIII software package (version 18, StatPoint Technologies, Warrenton, VA, USA) were used in the present study.

3. Result

The combination of chemical fertilizer and bio-fertilizers significantly influenced the growth and yield and was statistically significant over 75% CF (T1).

3.1. Influence of Chemical Fertilizers and Bio-Fertilizer on Growth Attributes of Bell Pepper

The perimeter ranged from 25.85 cm (T1) to 45.72 cm (T5). Among all the treatments, T5 recorded a significantly larger perimeter (45.72 cm), followed by T4 with 41.44 cm (Table 1). Similarly, the T2 and T3 treatments gave 30.48 and 47.58% more perimeter than the T1 treatment. The area of fruit was increased as the use of CF increased, or 75% CF use with different combinations of *R. irregularis* + *A. chroococum*. The minimum area was 19.94 cm^2 in T1, and the maximum was in T5 (72.57 cm^2) (Table 1).

As shown in Table 1, the width mid-height and maximum width varied from 3.36 to 8.88 cm and 5.21 to 9.19 cm, respectively, from T1 to T5. The maximum increment in width mid-height and the maximum width was observed after the application of T5 (Table 1). In comparison to T1, the width mid-height was increased up to 86.31, 134.23, 142.56 and 164.29% after the application of T2, T3, T4 and T5 treatments. Similarly, the maximum width was increased to 38.77%, 57.77%, 59.88% and 76.39%, respectively, after T2, T3, T4 and T5 (Table 1).

Table 1. Growth and biochemical attributes studied among the different treatments for the bell pepper in the present study.

Variables	T1 (75%CF ± SD)	T2 (100%CF ± SD)	T3 (75%CF + RI ± SD)	T4 (75%CF + AC ± SD)	T5 (75%CF + RI + AC ± SD)
Perimeter	25.85 ± 2.83 e *	33.73 ± 0.63 d	38.15 ± 1.05 c	41.44 ± 0.73 b	45.72 ± 1.10 a
Area (cm^2)	19.94 ± 3.80 e	51.83 ± 1.07 d	59.13 ± 0.95 c	63.15 ± 0.44 b	72.57 ± 0.18 a
Width Mid-height	3.36 ± 0.79 d	6.26 ± 0.50 c	7.87 ± 0.11 b	8.15 ± 0.05 b	8.88 ± 0.10 a
Maximum Width	5.21 ± 0.43 d	7.23 ± 0.18 c	8.22 ± 0.02 b	8.33 ± 0.02 b	9.19 ± 0.24 a
Height Mid width	6.42 ± 0.63 e	8.37 ± 0.01 d	8.99 ± 0.14 c	9.96 ± 0.21 b	11.07 ± 0.73 a
Maximum Height	6.61 ± 1.15 d	9.37 ± 0.05 c	10.14 ± 0.09 c	11.09 ± 0.08 b	12.48 ± 0.08 a
Curved Height	7.84 ± 0.91 e	10.97 ± 0.18 d	12.60 ± 0.09 c	13.49 ± 0.10 b	14.35 ± 0.48 a
Shoulder Height	0.01 ± 0.00 c	0.24 ± 0.05 b	0.48 ± 0.02 a	0.50 ± 0.00 a	0.51 ± 0.00 a
Pepper Pericarp Boundary	18.12 ± 1.73 e	26.08 ± 0.29 d	29.22 ± 0.57 c	32.40 ± 0.60 b	35.87 ± 0.91 a
Pepper Pericarp Area	25.82 ± 2.14 e	41.12 ± 0.85 d	52.37 ± 0.78 c	58.67 ± 0.67 b	63.33 ± 0.78 a
Pepper Pericarp Thickness	0.69 ± 0.33 c	1.38 ± 0.02 b	1.54 ± 0.01 ab	1.65 ± 0.04 a	1.74 ± 0.00 a
Days to 50 flowering	40.06 ± 2.64 a	36.39 ± 0.53 a	36.22 ± 5.29 a	32.01 ± 1.76 b	28.39 ± 0.95 b
Number of marketable fruits per plant	11.41 ± 0.89 d	22.34 ± 0.27 c	24.60 ± 3.04 b	26.14 ± 0.97 b	29.40 ± 0.91 a
Fruit length (cm)	4.82 ± 0.58 d	6.57 ± 0.04 c	6.85 ± 0.03 bc	7.10 ± 0.10 ab	7.34 ± 0.09 a
Fruit width (cm)	4.76 ± 0.24 c	6.94 ± 0.05 b	6.77 ± 0.11 b	7.01 ± 0.34 ab	7.33 ± 0.05 a
Average fruit weight (g)	62.57 ± 6.84 d	79.24 ± 3.41 c	83.59 ± 1.41 c	109.18 ± 1.54 b	133.73 ± 1.25 a
Marketable yield per plant (kg)	0.70 ± 0.10 e	1.70 ± 0.10 d	1.94 ± 0.05 b	1.83 ± 0.06 c	2.69 ± 0.01 a
Plant height cm.	51.75 ± 0.50 c	71.75 ± 0.50 b	74.68 ± 0.90 b	73.10 ± 0.29 b	80.41 ± 5.35 a
TSS (%)	2.82 ± 0.56 b	4.12 ± 0.16 a	4.33 ± 0.06 a	4.29 ± 0.03 a	4.56 ± 0.14 a
Ascorbic Acid	128.50 ± 0.95 c	131.33 ± 1.06 c	149.00 ± 5.59 b	153.50 ± 4.84 ab	160.50 ± 5.64 a
Capsaicin content	3.55 ± 0.09 d	4.48 ± 0.20 c	5.44 ± 0.16 b	6.11 ± 0.10 a	6.31 ± 0.19 a

* According to the Student-Newman-Keuls test, the means within rows separated by different letters are statistically significantly different.

In addition, the maximum height mid-width was recorded after T5, which was 72.43% higher than T1, followed by 30.37%, 40.03% and 55.14% with T2, T3 and T4, respectively. Likewise, the maximum height of fruit was recorded in treatment T5, which was 88.80% higher than in the T1 treatment (Table 1). The minimum curved height (7.84 cm) was recorded at T1 and the maximum (14.35 cm) at T5; however, T4 (13.49 cm) also showed significant results. In the case of T2 and T3, curved height was found to be increased up to 39.92% and 60.71%, respectively. The shoulder height was highest for T5 (0.51 cm), followed by T4 (0.50 mm), T3 (0.48 mm), T2 (0.24 mm) and T1 (0.01 mm) (Table 1).

The pepper pericarp boundary was at a minimum (18.12 cm) when the plants were treated with T1 and found to be increased up to 43.93%, 61.26%, 78.81% and 97.97% after T2, T3, T4 and T5, respectively. Similarly, the maximum pepper pericarp area recorded was 63.33 cm^2 in T5, followed by 58.67 cm^2, 52.37 cm^2, 41.12 cm^2 and 25.82 cm^2 with T4, T3, T2 and T1, respectively (Table 1). The pericarp area recorded in T5 was 145.27% higher than in T1. In addition, pepper pericarp thickness also increased maximum up to 152.17% after T5, followed by 139.13%, 123.19% and 100.0% after T4, T3 and T2, respectively. The minimum number of days to 50% flowering was recorded in T5 (28.39), and the maximum was in T1 (40.06) (Table 1). It was observed that the number of days to 50% flowering was lower in T2 (9.16%), T3 (9.59%), T4 (20.09%) and T5 (29.13%). Further, the number of marketable fruits per plant was also recorded as higher after T5 application (Table 1). In comparison to T1, the number of marketable fruits per plant increased up to 157.67% in T5, followed by 129.10% in T4, 115.60% in T3 and 95.79% in T2 (Table 1).

The highest fruit length (cm) was observed after the application of T5, which exhibited 52.28% differences with T1 alone. Similarly, the mixed combination of T4 (47.30%), T3 (42.12%) and T2 (36.31%) showed more fruit length than T1. In addition, fruit width was maximum in the case of T5 (7.33 cm), followed by T4 (7.01 cm) (Table 1). Still, the application of T2 presents better results than T3 and T1, exhibiting 3.57% and 45.80%, respectively. The increment in average fruit weight was also observed to be the maximum in T5 (133.73 g), followed by T4 (109.18 g), T3 (83.59 g), T2 (79.24 g) and T1 (62.57 g), as shown in Table 1. As observed, T5, T4, T3 and T2 showed 113.73%, 74.49%, 33.59% and 26.54% more fruit weight than T1 (Table 1).

Furthermore, in the combination of T5, the marketable yield per plant was also recorded as the highest (2.69 kg). The combination of T3 also represented a superior marketable yield per plant than T4, T2 and T1 (Table 1). Similarly, the incensement in plant height (cm) was further justified in the T5 treatment, 55.38% more than T1. Meanwhile, T3 also revealed a fine plant height higher than T4 and T2 (Table 1).

3.2. Influence of Chemical Fertilizers and Bio-Fertilizer on Chemical Attributes of Bell Pepper

Like other parameters, the maximum value of TSS (4.56) was recorded with the T5 treatment. Additionally, the data recorded in Table 1 indicated that the combination in T3 showed a higher value of TSS than T1, T2 and T4. In comparison to T1, the increased percentage of TSS was 61.70, 53.55, 52.13 and 46.10% in T5, T3, T4 and T2, respectively (Table 1). While all the treatments offer a remarkable increment in ascorbic acid in contrast to T1, the highest percentage of ascorbic acid was found in T5 (24.90%), followed by 19.46% in T4, 15.95% in T3 and 2.20% in T2 (Table 1). Moreover, the capsaicin content varied from 3.55 to 6.31 after treatment with T1 to T5. Among all the treatments, T5 recorded a maximum value of capsaicin content as compared to T1, which was 77.75%, followed by T4 (72.11%), T3 (53.24%) and T2 (26.20%) (Table 1).

3.3. Influence of Chemical Fertilizers and Bio-Fertilizer on Mineral Content (mg/100 g Dry Weight), Root Mycorrhization (%) and Number of Spores

As presented in Table 2 regarding mineral content, calcium was recorded minimum (42.21 mg/100 g dry weight) when the plants were under treatment T1 and found to be increased up to 109.24%, 34.33%, 44.75% and 133.64%, with T2, T3, T4 and T5, respectively (Table 2). Similarly, the minimum magnesium content was 38.22 mg/100 g dry weight in T1, and the maximum recorded 131.57 mg/100 g dry weight in T5, followed by 113.0 in T2, 94.44 in T3 and 90.35 mg/100 g dry weight in T4 (Table 2). The magnesium content in T5 was 244.24% higher than in T1 (Table 2). In addition, phosphorus content also increased maximum up to 184.37% after T5, followed by T3 (173.92%), T2 (145.18%) and T4 (115.51%). The maximum value of sulfate (264.0 mg/100 g dry weight) was recorded with T5 treatment, and the minimum in T1 (66.15 mg/100 g dry weight) (Table 2).

The data recorded in Table 2 further indicated that the treatment T2 showed a higher value of sulfate than the T1, T3 and T4 applications. Compared to T1, the increased percentage of sulfate was 299.09%, 293.5%, 217.46% and 202.34% in T5, T2, T3, and T4, respectively (Table 2). The minimum content of iron (2.88 mg/100 g dry weight) was observed in T1, whereas the maximum content (7.12 mg/100 g dry weight) was observed in T2 (Table 2). In comparison to T1 (5.50 mg/100 g dry weight), the increment in sodium content was observed to be maximum with T4 (21.21 mg/100 g dry weight) followed by T5 (21.08 mg/100 g dry weight), T3 (19.22 mg/100 g dry weight) and T2 (18.34 mg/100 g dry weight) (Table 2). As is apparent from Table 2, the root mycorrhization and number of spores were observed at maximum in T5 and minimum in T3. However, no root mycorrhization and number of spores were found in the T1, T2 and T4 combination (Table 2).

Table 2. Mineral content (mg/100 g dry weight), root mycorrhization (%) and number of spores among the different treatments studied for the bell pepper in the present study.

Variables	T1 (75%CF ± SD)	T2 (100%CF ± SD)	T3 (75%CF + RI ± SD)	T4 (75%CF + AC ± SD)	T5 (75%CF + RI + AC ± SD)
Calcium (mg/100 g dry weight)	42.21 ± 3.81 e *	88.32 ± 12.10 b	56.70 ± 9.12 c	61.10 ± 3.57 d	98.62 ± 10.45 a
Magnesium (mg/100 g dry weight)	38.22 ± 12.15 d	113 ± 20.23 b	94.44 ± 13.65 c	90.35 ± 9.12 c	131.57 ± 4.56 a
Phosphorus (mg/100 g dry weight)	102.22 ± 18.06 d	250.62 ± 7.81 b	280 ± 32.12 a	220.29 ± 16.22 c	290.68 ± 12.80 a
Sulfate (mg/100 g dry weight)	66.15 ± 5.18 c	260 ± 10.32 a	210 ± 20.75 b	200 ± 4.56 b	264 ± 5.80 a
Iron (mg/100 g dry weight)	2.88 ± 0.98 d	7.12 ± 1.95 a	5.58 ± 4.12 c	6.88 ± 2.11 b	7.00 ± 3.12 a
Sodium (mg/100 g dry weight)	5.50 ± 1.50 c	18.34 ± 1.75 b	19.22 ± 4.12 b	21.21 ± 2.15 a	21.08 ± 1.88 a
Root Mycorrhization (%)	0.00 ± 0.00 c	0.00 ± 0.00 c	45.72 ± 8.62 b	0.00 ± 0.00 c	55.31 ± 12.17 a
Number of Spores	0.00 ± 0.00 c	0.00 ± 0.00 c	81.36 ± 5.88 b	0.00 ± 0.00 c	112.25 ± 9.22 a

* According to the Student-Newman-Keuls test, the means within rows separated by different letters are statistically significantly different.

3.4. UPGMA Clustering and PCA Analysis

UPGMA analysis grouped treatments T2 and T4 together as a single group. Treatments T3 and T5 were also grouped together in the same way (Figure 1). As seen in the Figure, the treatments with bioinoculants (T1) and (T2) were significantly different from one another (T3, T4 and T5) (Figure 1). In addition, the PCA was utilized to assess differences between treatments and investigate relationship-based clustering. The cluster analysis revealed that the treatment T1 was distinct from the other treatments in the study (Figure 2). The figure shows that samples corresponding to the first two principal components (PCs) exhibited a solid propensity for segregation (70.22% of the total variance; Figure 2). Whereas the correlations among the different traits are provided in the Figure S1 and Figure S2, respectively.

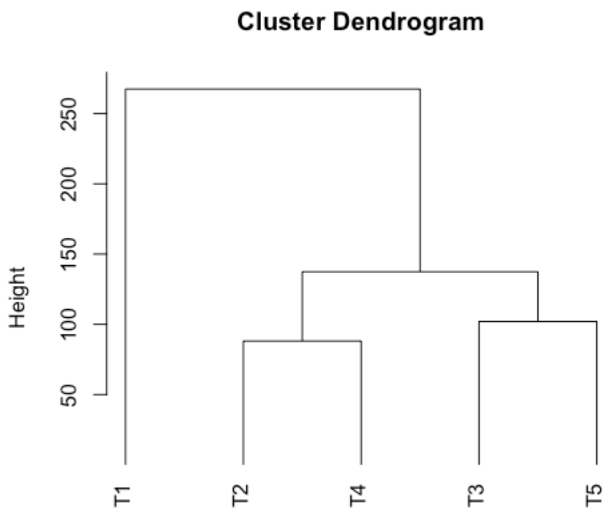

Figure 1. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering of parental different treatments applied to pepper. The cophenetic correlation coefficient of clustering is 0.9.

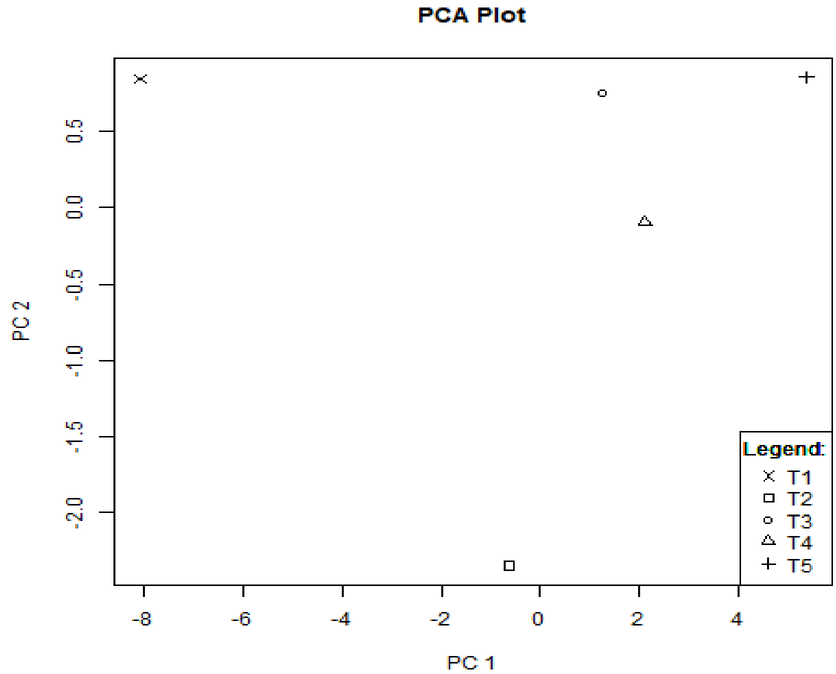

Figure 2. Diagram of PCA of different treatment groups.

4. Discussion

The statistical analysis revealed significant ($p < 0.05$) differences in the agronomic variables of fertilized bell pepper plant growth and productivity with the application of two concentrations of chemical fertilizer (75% and 100%) individually and in combination with the *R. irregularis* + *A. chroococum*. The combination of organic and inorganic fertilizers is essential for enhancing soil health, nutrient absorption and ensuring the long-term production of bell peppers [3,30]. Millions of microbial colonies, such as AMF and PGPB, live in natural soil and play an essential function in growth and development [31].

The combination of 75% CF + *R. irregularis* + *A. chroococum* in treatment T5 manifests the best results among all the given treatments. In this investigation, perimeter and maximum perimeter were noted to be increased. *R. irregularis* and *A. chroococum* enhance soil fertility by fixing nitrogen and phosphorus and ultimately promoting the parameters' quality [8,32]. T5 was further proved better in the traits related to the fruit size and shape such as width mid-height, maximum width, height mid-width maximum height, curved height and shoulder height. Similarly, the pepper pericarp boundary, area and thickness were found to increase in all the treatments compared to T1. These results support the previous findings on the morphological characteristics of pepper [4]. Generally, the consolidated use of AMF and PGPB improves the quality of the fruit [33,34] by enhancing phytoremediation, fertilizer nutrient useability and lowering the chemical fertilization application requirements [35].

The complex carbon and nutritional need patterns in plant organs lead to blooming [36]. The existing system for flowering regulation states that the process begins when all the needed elements, such as nutrients and phytohormones, are present in the shoot apex to become an inflorescence apex. Hormone synthesis and photosynthate availability are affected by AMF and PGPB, which has an indirect effect on flowering duration [37]. In this study, early flowering was observed in the T5 treatment. The feasible cause for quick blooming may be the continued degradation of organic manures after application, resulting in increased rhizosphere temperature. The rise in temperature and elevated potassium levels may have hastened the initiation of blooming [38].

PGPB improved nutrient absorption and mobilization within the plant and promoted vegetative development, resulting in more branches, yielding more fruits and boosting production [39]. The consortia treatment (T5) showed the highest number of marketable fruits in our study. In parallel, fruit length and width enhancement were observed under the influence of T5. Likewise, the maximum incensement in plant height was recorded in T5. Inoculation with PGPB and AMF provides a viable biofertilizer resource option and helps to increase the plant height [40]. Increments in fruit size, yield and yield-related traits of red capsicum were also observed using an effective combination of chemical and organic fertilizer [41,42]. The incensement in plant height of *Capsicum annuum* was also reported earlier [43]. The release of fixed nitrogen might also be a factor responsible for the rise in plant height, which increases the amount and accessibility of nitrogen in the root zone because protein synthesis, plant growth and development are all enhanced by nitrogen [44].

Total soluble solids (TSS) and ascorbic acid content are critical quality indicators in the processing industry and nutritional elements [45]. The highest value of TSS, ascorbic acid and capsaicin content was recorded in treatment T5, receiving 75% CF + *R. irregularis* + *A. chroococum* (Table 1). The fixed nitrogen increases the concentration and availability of nutrients in roots, explaining the greater TSS in this treatment [46]. The increment in TSS and ascorbic acid content in a mixed consortium of inorganic and biofertilizers was also reported by other researchers [47]. The application of biostimulants provides a variety of health advantages to plants by modulating secondary plant metabolism, improving biosynthesis and accumulating phytochemicals [48].

Capsaicin is a bioactive compound that provides a distinctive sharp taste to pepper fruit. Biological agents promote the absorption of nutrients such as nitrogen, phosphorus and micronutrients and the faster rate of nitrogen absorption increases the overall concentration of alkaloids such as capsaicin [49]. Bell peppers contain various macro and micronutrients such as potassium, magnesium, iron, calcium, zinc and bio-functional qualities. They boost yield and yield components via different kinds of mechanisms [50]. It increases chlorophyll concentration and stimulates photosynthetic activity and auxin production, resulting in improved crop growth and development [51]. It was previously observed that the combined inoculation of PGPB and AMF in crops resulted in greater macro and micronutrient absorption [52–54].

Similarly, in the present study, higher uptake of macronutrients and micronutrients was recorded in the individual treatment as well as co-inoculated with PGPB and AMF. The percentage of root mycorrhization and spore formation was observed only in T3 and T5 treatment combinations. It was observed that the plants inoculated with rhizobia were found to have a higher number of spores and root mycorrhization [55,56]. Overall, in this study, we found that T5 was the most suitable treatment for the growth of bell pepper, followed by T4 and T3.

5. Conclusions

Bell pepper was treated with a different combination of chemical fertilizer containing *R. irregularis* and *A. chroococum*. The 100% chemical fertilizer showed a better result than the 75% chemical fertilizer, but treatment five was found to be the most effective treatment of all other treatments. The integrated application of *R. irregularis* and *A. chroococum* with 75% chemical fertilizers increased the fruit quality and yield attributing characteristics and was even found to enhance the mineral content of bell peppers. By using AMF and PSB on peppers, we expect that we may reduce the rate at which chemical fertilizers are applied while simultaneously improving pepper growth as well as the biochemical and mineral composition of pepper fruits.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14095653/s1, Figure S1. Pearson's correlation coefficients for growth and biochemical attributes studied among the different treatments for the bell pepper in the present study with significant values $p < 0.05$ flagged (*), $p < 0.01$ (**) and $p < 0.1$ (***), respectively. Figure S2. Pearson's correlation coefficients mineral content (mg/100 g dry weight), root mycorrhization (%) and number of spores among the different treatments studied for the bell pepper in the present study with significant values $p < 0.05$ flagged (*), $p < 0.01$ (**) and $p < 0.1$ (***), respectively.

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