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Productivity and Quality of Horticultural Crop Capsicum (*Capsicum Annum* L) Through Co-Inoculation of Novel Microbial Consortium Plant Growth Promoting Rhizobacteria, Glycoprotein Producing AM Fungi and Chemical Fertilizer under Low-Cost Protected Cultivation

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Abstract

Capsicum (*Capsicum annum* L.) is a common vegetable crop with a wide range of culinary utilization worldwide. In horticultural practices, inoculating the planting medium with a beneficial microbial consortium is a novel approach to cultivating high-quality, healthy plants with abundant nutrition. In the following study, inoculation of a selected microbial consortium consisting of PGPR (*Azotobacter*, *Pseudomonas*, *Fraturia*, *Azospirillum*, and *Bacillus* spp.) and glycoprotein producing AM fungi (*Aculospora logula*-15%, *Glomus fasciculatum*-20%, *Glomus intraradices*-40%, *Gigaspora margarita*-15%, and *Scutellospora heterogama*-10%) was inoculated to the planting medium in beds to raise capsicum plants in a low cost protected cultivation. Plant height, stem girth, fruit weight, fruit diameter, number of fruits per plant, and weight of fruit per plant parameters to be considered in the study. Mycorrhizal root colonization, macro, and micronutrient uptake, and quality yield, were increased the most with multiple microbial inoculations. Treatments revealed that plants inoculated with the multiple microbial consortia increased substantially faster than plants treated with chemical fertilizer (100 % RDF) and control. Maximum yield (742.5 q ha^{-1}) was recorded in treatment Absolute's consortium PGPR+ AM Fungi along with maximum values of shelf life (7.10 DAS), TSS (Brix0 5.63), the number of fruit/plant (8.20), fruit length (8.5cm), fruit diameter (7. 81cm) and fruit weight (224g) as compared with control and other biological treatments. The best treatment with respect to projected yield was Absolute consortium PGPR + AM fungi followed by control. Based on the various growth and microbiological parameters studied, it was concluded that inoculation with the multiple microbial consortia (Absolute consortium PGPR+AM fungi) was beneficial for raising healthy, vigorously growing capsicum plants under low-cost protected conditions.

Keywords: Consortium; PGPR; AM Fungi; Chemical Fertilizer; Capsicum

Abbreviations: HH: Healthy Human; CF: Chemical Fertilizers; PGPR: Plant Growth-Promoting Rhizobacteria; IAA: Indoleacetic Acid; PG: Plant Growth; SH: Soil Health; NU: Nutrient Uptake; Psms: Phosphorus Solubilizing Microorganisms; EC: Electrical Conductivity; SOC: Soil Organic Carbon; DAP: Di-Ammonium Phosphate;

IP: Infected Propagules; RRF: Recommended Rate Of Fertilizers; RCDB: Randomized Complete Block Design; RCO: Recommended Cultural Operations; AAS: Atomic Absorption Spectrophotometer; MRC: Mycorrhizal Root Colonization; MA: Microbial Activities.

Introduction

Capsicum (*Capsicum annuum* L.), also known as a bell or sweet pepper, is one of the most widely grown vegetable crops in the world. Because of its richness in vitamins (A, C, E, and K1) and antioxidants, it is commonly regarded as a nutritious food for a healthy human (HH). Its color, scent, flavor, and crisp texture are important factors in its widespread use in a variety of cuisines around the world [1]. In India, it is one of the most significant cash crops. India is the world's greatest producer of chilli, with an annual output of 1.1 million tons. Chilli accounts for roughly 17% of the global spice trade and about 33% in India due to its high economic value and high consumption rate [2]. Tamil Nadu, Karnataka, Uttar Pradesh, and the Deccan Plateau are additional commercially important capsicum-producing regions in India. However, in comparison to the United States, Holland, France, and other capsicum-producing countries throughout the world, there is insufficient supply resulting in lower production rates and limited areas under cultivation. Furthermore, *Phytophthora* and *Colletotrichum* spp. produce damping-off and anthracnose, respectively, which are severe devastative and destructive diseases that drastically lower prospective crop yields [3]. Damping-off is a *Phytophthora*-caused soil-borne fungal disease of seedlings that can be the misery of amateur seed growers that develops on the seeding table while young plants are just starting to grow. Seeds may rot before germinating or seedlings may perish before emergence during pre-emergence damping off. The immature plant seedlings develop rot at the crown if a post-emergence damping-off is present. Later, the tissue becomes soft, causing the plant to wilt and collapse over at the base [4]. Anthracnose, often known as ripe-fruit rot, is another frequent disease caused by the fungus *Colletotrichum*. Within just a few days, the disease reduces the output of ripened sweet pepper fruits and transforms them into rotten garbage [3].

Excessive use of chemical fertilizers (CF) and fungicides can induce various environmental risks as well as carcinogenic and mutagenesis effects in living beings when dealing with these soil-borne diseases and fungal pathogens. Plant protection is thus essential for maintaining long-term crop yields [5-7]. Alternatively, to address production losses caused by serious diseases, biological control measures must be considered (BCAs). Biofertilizers and biocontrol agents are a secure and reliable alternative to chemical fertilizers in the battle against plant pathogens [7-9]. Plant growth-promoting rhizobacteria (PGPR) such as *Bacillus, and Pseudomonas* are well-known for their biological control of soilborne plant pathogens. They colonize the roots of plants and have both direct and indirect impacts. Increased nitrogen uptake, indoleacetic acid (IAA) synthesis, phosphate solubilization, inhibition of soilborne diseases by creating siderophores, and other mechanisms of PGPR promote plant growth (PG) and yield [6,7,10-12]. PGPRs are rhizosphere inhabiting microorganisms that can improve phytoremediation effects. PGPR applied in contaminated soils, improves plant resistance to heavy metals, speeds up nutrient turnover, mitigates the negative effects of some metals, increases plant tolerance to pests and pathogens, and improves soil structure [7,13,14]. Exudates from plant roots provide a range of metabolites and nutrients which are necessary for rhizobacteria to thrive [15]. PG and yield can be increased using a variety of PGPR functions. They have the ability to fix nitrogen from the air, which is linked to the production of various growth-promoting plant hormones [7,16]. Over 90% of the world's plant species have symbiotic relationships with AM fungi [13].

The link is especially critical for agricultural plants, which require a lot of nutrients and water to develop and produce at their best. The AM fungi build a network of thin filaments that attach to plant roots and excrete strong nutrients that break down mineral nutrients, absorb water, inhibit soil pathogens, and bind soil particles together into a porous structure. Different chemical processes reduce the availability of phosphorus for plants, especially in arid and semi-arid soils [12]. AM fungal biofertilizers are a low-cost, long-term solution for improving plant nutrition, yield output, and reducing disease and pests [13,17]. Although AM fungi are normally found in most soils, the number required for improved plant production and repair of the soil structure has declined drastically due to the overuse of CF's insecticides, fungicides, and herbicides [18]. Farmers' primary goals are to increase yield and improve the quality of the crop and soil health (SH). Bell pepper growers face two main issues: poor establishment and poorer production. AM fungal symbiosis can improve PG and nutrient uptake (NU), and there is a growing interest in its use. Understanding the role of mycorrhizae and how they interact with other bio inoculants would help to enhance inoculation techniques and planning so that the benefits of mycorrhizal association can be maximized. Furthermore, inoculating plants with PGPR and AM fungi promotes dry matter deposition and water moisture uptake, enhancing plant tolerance to stressors such as drought and salinity as shown in Figure 1. Utilizing them for plant growth in a variety of biological habitats can make a significant contribution to organic culturing for growth enhancement and yield improvement.

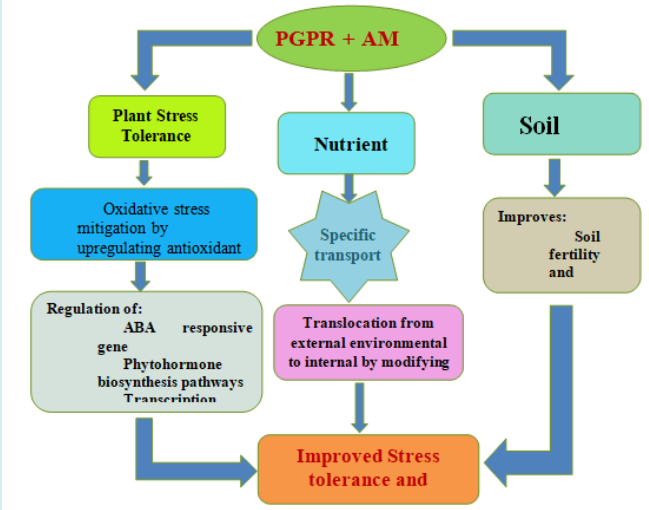

Figure1: A diagrammatic representation of PGPR and AM fungi to regulate various processes in the ecosystem and plant growth promotion under abiotic stress conditions.

Nutrient Acquisition by Way of PGPR and AM Fungi

Fertilizer requirements are often lower in soils with dynamic microbial ecologies and rich organic matter in traditionally treated soils [19]. Bulk microbial activity in soils is frequently considered while controlling the application of organic nutrient sources. Specific plant-microbe interactions that directly help in plant nutrition are beginning to emerge as a result of Phyto microbiome research [20]. Microbes that help plants acquire nutrients (biofertilizers) work in a variety of mechanisms, including increasing the surface area accessible to plant roots, nitrogen fixation, P solubilization, siderophore formation, and HCN production [21,22]. As a result, regulating microbial activity promises a lot of potential for fulfilling crop nutritional requirements. The colonization of AM fungus is also believed to enhance nitrogen absorption in plants (figure 8). It is clear that inoculation with AM fungus can dramatically enhance the concentration of several macro and micronutrients, resulting in higher photosynthate synthesis and thus greater biomass accumulation [23,24]. AM fungi have the potential to increase the absorption of inorganic nutrients, particularly phosphate, in practically the entire plants [7,25]. Free-living N fixing bacteroid fixed atmospheric nitrogen and provider to plant for growth and development. After N, the next most limiting nutrient for crop plants is usually P, according to Liebig's law of the minimum. While most agricultural soils get enough of P, the majority of it exists in insoluble forms. Furthermore, by releasing non-labile phosphorus from its refractory forms, phosphorus solubilizing microorganisms (PSMs) can assist plants in accessing the reservoir of non-labile phosphorus. Organic acids or H+ ions secreted by PSMs can solubilize inorganic P complexed with Ca, Fe, or Al. Many scientists have documented the involvement of AM fungi in the uptake of soil nutrients, particularly N and P, which can help host plants develop more successfully [7,18,26-28]. AM fungi have been proved to sustain P and N absorption, supporting plant development at higher and lower P levels under various irrigation regimes [29,30]. AM fungi are also useful for assisting plants in absorbing nutrients from nutrient-deficient soils [31]. Apart from macronutrients, the interaction of AM fungus has been shown to boost the phyto-availability of micronutrients such as zinc and copper [7,11,12,32]. The surface absorption capacity of host roots is improved by AM fungus [33]. Crop yields might be limited by other nutritional elements such as Fe and Zn. Fe and P can be plentiful in soils yet unavailable to plants. Many bacterial species produce organic acids or siderophores, which increase the availability of Fe [7,12,34-39]. To evaluate the influence of multiple consortium bio inoculants PGPRs and most abundant AM fungi, on growth, quality parameters, yield, and nutrients uptake response of capsicum individually and along with a consortium of rhizobacteria and mycorrhizal inoculants in non-sterilized soils under low cost protected conditions of North India.

Materials and Methods

Location, Climate, and Soil of Experimental Site

The protected cultivation experiment was performed at Delhi NCR, Gurugram, and Haryana, India from July to November. The experimental site is positioned at 28.4211° north, 77.1109° East longitude, and its elevation from sea level is around 300 meters. The weather is sub-tropical with a mean maximum temperature ranging from 20.8 - 40.3°C in summer and a mean minimum between 5-17.7°C in winter. The mean annual rainfall is around 714 mm. The experimental site soil is well-drained sandy loam having good percolation and excessive fertility. The major portion of this location is under the cultivation of different agricultural, horticultural, medicinal, and aromatic crops.

Soil Sampling and Analysis

Soil samples (0-30cm) were collected randomly from each site using the standard conning and quartering method before (Initial time) and at harvest. Air-dried soil samples were used for different Physico-chemical analyses. pH was determined in 1.25 (w/v) solutions of dried soil samples in water and the same was used for the determination of electrical conductivity (EC). Air-dried soil samples were processed (addition of 40% NaOH and distillation) in a Kel Plus Nitrogen estimation system (Class DX, Pelican Equipment's) followed by determination of available nitrogen by titration with 0.02N H_2SO_4 [40,41]. The contents

of soil organic carbon (SOC) were determined using Sims' method [42]. 1.0 g of soil was combined with 10 ml of 1 N $K_2Cr_2O_7$ and 20 ml of concentrated H_2SO_4 in this manner. This suspension was carefully mixed and diluted to 200 ml of distilled water, and then 10 ml of H_3PO_4 and sodium chloride were added. Estimates of total organic carbon (OC expressed as C) are used to assess the amount of organic matter in soils. Available phosphorus was determined by the Olsen method using samples with high pH sodium bi-carbonate as extracting agent [43,44]. Available potassium was determined in a 1N ammonium extract using a flame photometer [44,45].

Experimental Setup

An experiment was designed to investigate the effect of Absolute multiple consortium PGPR (*Azotobacter, Pseudomonas, Fraturia, Azospirillum,* and *Bacillus*)+consortium AM fungi (*Aculospora logula*-15%, *Glomus fasciculatum*-20%, *Glomus intraradices*-40%, *Gigaspora margarita*-15%, and *Scutellospora heterogama*-10%), as well as an Absolute consortium of PGPR and consortium of AM fungi, Chemical fertilizer (CF), Di-ammonium phosphate (DAP), MoP, Urea, also used as control (100% RRF) and control without CF and bioinoculant on the growth parameter such as plant height, stem girth, quality yield, and nutrients uptake of capsicum (bell pepper). In the control (100% RRF) set, no inoculum was added. In each pit single seedling of capsicum was planted and placed in protected conditions. Plants were watered regularly as per needs.

Microorganisms Applications

Microbial Inoculum Preparation

Preparation of Absolute Consortium PGPR Inoculant: PGPR microbial inoculants (*Azotobacter, Azospirillum, Pseudomonas, Fraturia,* and *Bacillus*) have been proliferated in a nutrient broth medium. Then each PGPR develops eliminated on the top of a logarithmic growth phase and becomes aseptically transferred to plastic containers, which include triple sterile talc powder and then were mixed well. PGPR concentration was adjusted to 1×10^8 CFU/g in all inoculants. Consortium PGPR was prepared and contained *Azotobacter,* Azospirillum, *Pseudomonas, Fraturia,* and *Bacillus* in the same ratio. As per the treatment combination consortium, PGPR @ 1.0 g/plant becomes inoculated across the seedling during the time of transplanting. PGPR has been additionally applied to capsicum growth stages in four equal splits dose.

Preparation of Absolute Consortium of AM fungi Inoculant: The density of Absolute consortium AM fungi mixed with triple sterile talc powder, adjusted with 3000 infected propagules (IP) per gram of inoculant containing growing subtract, infected roots bits, and hyphal and mycelial biomass. AM fungi inoculum contained *Aculospora logula*-15%, *Glomus fasciculatum*-20%, *Glomus intraradices*-40%, *Gigaspora margarita*-15% and *Scutellospora heterogama*-10% infective propagules (IP). AM fungi @500 IP/ plant were applied as a root dipping method at the time of planting. After planting of capsicum, necessary irrigation was given to all treated and untreated plots.

Chemical Fertilizers (CF)

Various level of CF was applied in control treatment in the form of DAP 1.08 q ha^{-1}, MoP 1.0 q $ha^{-1,}$ and Urea 2.275 q ha^{-1} as 100% recommended rate of fertilizers (RRF) as farmers practiced. Urea was also applied as a top dressing in equal splits as per recommendations.

Field Preparation, Nursery Raising, and Experimental Design

The experimental soil was opened with a power tiller and kept exposed to the sun prior to the next plowing. It was prepared afterward by plowing and cross plowing followed by laddering. Well decomposed organic manure at the rate of 20 kg m^{-1} was mixed with all soil. Raised beds were formed after bringing soil to a fine tilth. The bed size was 100 cm wide and 20 cm in height. Between the beds, 50 cm walking space was prepared. Capsicum (Capsicum annuum, family Solanaceae, Capsicum Hybrid Intruder) seedlings were prepared on an experimental field site. Capsicum seeds were treated with Trichoderma species before sowing in the seeding pro tray filled with fine sterilized cocopeat. After 30 days, the uniform size of capsicum seedlings was selected and treated with and without consortium PGPR/AM fungi and transplanted in experimental plots with a spacing of 60cm X 70cm. Planting was done in the raised bed in a pit size of 15cm x 20cm. AM fungi and PGPR treatment were considered as Absolute consortium AM fungi alone, Absolute consortium PGPR alone, and Absolute consortium PGPR + AM fungi and compared to control treatment without microbial inoculant (100% RRF) and control with microbial inoculant and RRF. The plantation was finished in the first week of July and the size of the experimental plot was 2.5X2.5 meters with four replicate in each treatment in a randomized complete block design (RCDB). 100% RRF was added to control treatments only. Recommended cultural operations (RCO) were carried out during the entire cropping period to ensure a healthy crop. The Physico-chemical properties of the soil were estimated at the Initial time and at harvest (Figures 2-7). Nutrient uptake in the shoot was determined after harvest (Figures 8-13).

Morphological and Yield Attributes of Trials

Data Collected and Measurements

The effects of different microbial inoculations observations, randomly 10 plants were selected after one meter of each plot in each replicates for all the characteristics such as the plant height (cm), Stem girth (cm), number of fruit per plant, fruit length (cm), fruit diameter (cm), and fruit weight (g) as well as grass yield (q ha^{-1}).

Plant Height and Stem Girth (cm): Measured from the ground level to the top of a matured plant at 30, 60, 90,120, and 150 days after transplanting (DAT).

No. of Fruit/plant: The number of fruit per plant was counted in different intervals and the average was calculated.

Fruit Length (cm): The average length of the fruit of the plants measured at maturity and expressed in centimeters.

Fruit Diameter (cm): The average fruit diameter was measured using a veneer caliper and expressed in centimeters after harvest.

Fruit Weight (g): Fruit weight was measured using digital balance and recorded and expressed in gm per fruit.

Total Fruit Yield (q ha^{-1}): The marketable and healthy capsicum fruit yields were expressed in q ha^{-1}.

Analysis of TSS

Total Soluble Solids (TSS)

The TSS was determined from fifteen randomly selected bulbs using the procedures [46]. Aliquot juice was extracted using a juice extractor and 50 ml of the slurry was centrifuged for 15 minutes. The TSS was determined by hand refractometer (ATAGO TC-1E) with a range of 0 to 32° Brix and resolutions of 0.2° Brix by placing 1 to 2 drops of clear juice on the prism, washed with distilled water, and dried with tissue paper before use. The refractometer was standardized against distilled water (0% TSS). Amount of total soluble solids present in the bulb expressed in percentage.

Assessment of Shoot Macro and Micronutrient Uptake

Randomly four places were selected for plants samplings in all capsicum plots. For nutrient analysis of the shoot systems, the oven-dried samples were finely ground. Nitrogen (N) in the shoots was determined using an elemental analyzer (EA 3000, Eurovector, Italy). To estimate the phosphate (P) and potassium (K) level in the shoots, 1g of the finely ground sample was subjected to a wet oxidation treatment using tri-acid (HNO_3:H_2 SO_4:$HClO_4$; 10:1:4) digestion in a digestion block (KELPUS, KES121; Pelican Equipment, Chennai, India) at 200°C. Following acid digestion, the samples were diluted and filtered for further nutrient analysis. Shoots P was determined by the Vanado molybdophoshoric acid colorimetric method [47] using a spectrophotometer (Specord 200; Analytik Jena, Germany). K was measured by the ammonium acetate method of Hanway and Heidel [48] by using a flame photometer (Model FP114; Thermo Scientific, USA). To determine iron (Fe), copper (Cu), and zinc (Zn) content in shoots samples were digested in a microwave (Mars 5, CEM). Following the US EPA 3051A method (US EPA 2007), the metal concentration in the acid digestive was determined using atomic absorption spectrophotometer (AAS) (SOLAAR, TJA Solution, UK.

Assessment of Mycorrhizal Root Colonization (MRC) Percentage in Capsicum Root System

Assessment of mycorrhizal root colonization (MRC) percentage in the capsicum root system, approximately 1-2 g of freshly collected fine roots were used for staining and the assessment of MRC percentage. Roots were washed in freshwater, cleared with 10% KOH, acidified with 1N HCl, and stained with 0.05% Trypan blue [49]. Quantification of root colonization for AM fungi was conducted using the gridline intersection method [50] and 100 segments of each sample were observed under a compound microscope (Leica DM750). The presence or absence of AM fungal structure in the root system such as vesicles, arbuscules, and hyphae at particular fixed points was recorded, and the results were expressed as a percentage MRC of observations.

Statistical Analysis

All results were analyzed using Analysis of Variance (ANOVA), followed by a post hoc test through computer software SPSS 11.5 version. Means were then ranked at a $P<0.0$ level of significance using Duncan's Multiple Range Test for comparison.

Results

Physico-chemical Properties of Soil (Initial time and at Harvest)

Comparing the Physico-chemical properties of the soil initial time and at harvest of experimental crops were presented in Figures 2-7. A slight alteration of pH was

recorded in treatments (Figure 2). Microbial-mediated capsicum showed a significant (P<0.05) decrease in electrical conductivity (Figure 3). The factors are responsible for the change in pH and EC in the continuous variations of equilibrium between cations and anions present in the soil. Plant uptake of soluble salts by crops and or leaching of cations such as calcium, and magnesium can decrease the pH and at the same time chloride accumulation on the surface due to capillary action can be responsible for the decrease in EC [7,11,12,51,52]. Available N in the soil is directly associated with soil organic matter (SOM). The gradual increase in N is due to an increase in SOM and the microbial activities (MA) which make N available from organic matter (OM) to microbial inoculants treated plots. A maximum significant increase (P<05) in available N (0.0079%) was recorded from multiple Absolute consortium PGPR + AM fungi treated plots (Figures 4 & 5) and minimum in control (0.0058%). Available P content was noticed significantly lower in treatment T5 (31.45ppm) as compared to treatment T1 (43.24ppm) soil and it may be due to P mobilizing activity of mycorrhiza, added during plantation activities (Figure 6). A sharp decrease in K content in soil was noticed in all microbial treated capsicum compared to control (Figure 7). Maximum decrease of K has been recorded in Absolute consortium PGPR + AM fungi treatment T5 (189.67ppm) and minimum in control (121.25 ppm) as compared to initial time (88.25ppm). Various factors including weathering, upward translocation of soluble ions through capillary action, and involvement from the degradation of plants litters can be responsible for such variation of K content in different treatments of capsicum crop [11,12,14,53].

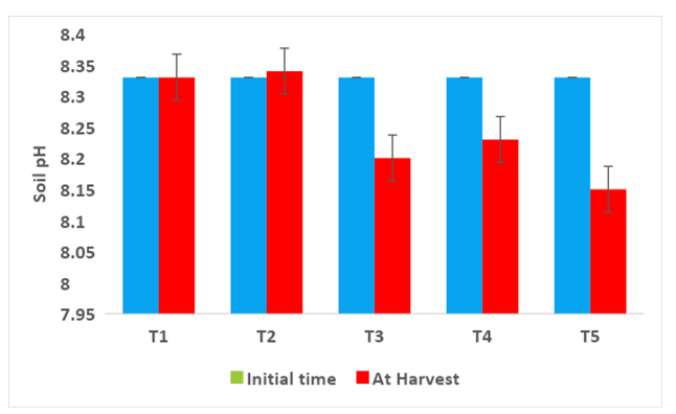

Figure 2: Soil pH at Initial time and after crop harvest (R-4).
T1- Control; T2- Control (100% RDF); T3- Consortium AM Fungi; T4- Consortium PGPR; T5- Absolute Consortium PGPR+ AM Fungi

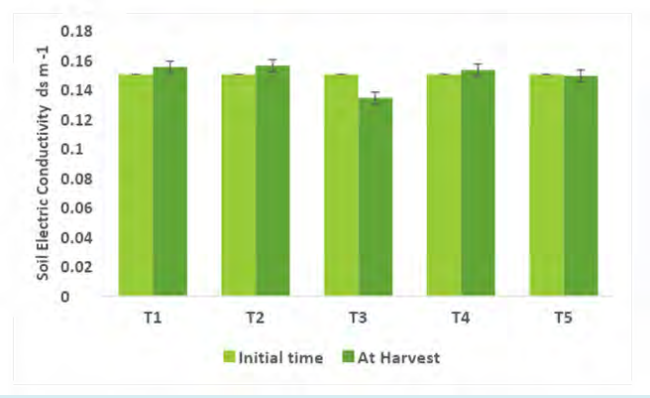

Figure 3: Soil Electric Conductivity at Initial time and after crop harvest (R-4).
T1- Control; T2- Control (100% RDF); T3- Consortium AM Fungi; T4- Absolute Consortium PGPR; T5- Absolute Consortium PGPR+ AM Fungi

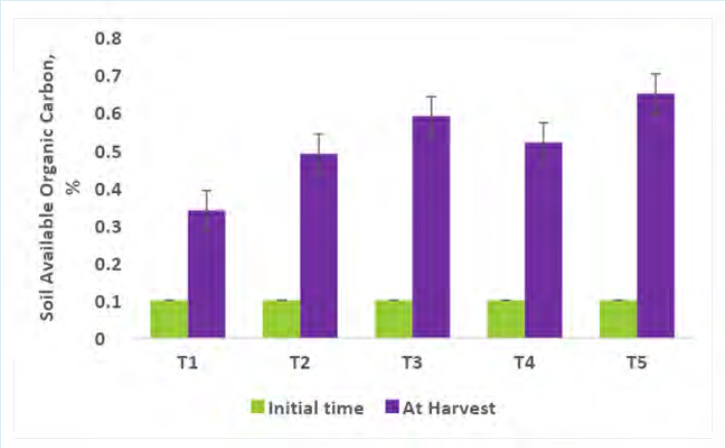

Figure 4: Soil organic carbon at the initial time and after crop harvest (R-4).
T1- Control; T2- Control (100% RDF); T3- Consortium AM Fungi; T4- Consortium PGPR; T5- Absolute Consortium PGPR+ AM Fungi

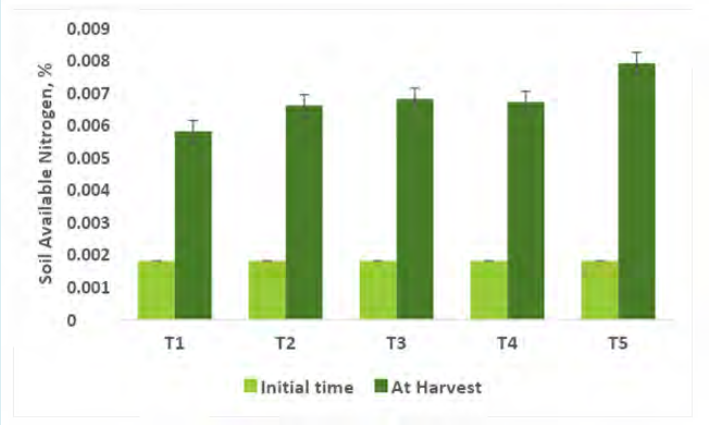

Figure 5: Soil available nitrogen concentration at an initial time and after crop harvest (R-4).
T1- Control; T2- Control (100% RDF); T3- Consortium AM Fungi; T4- Consortium PGPR; T5- Absolute Consortium PGPR+ AM Fungi

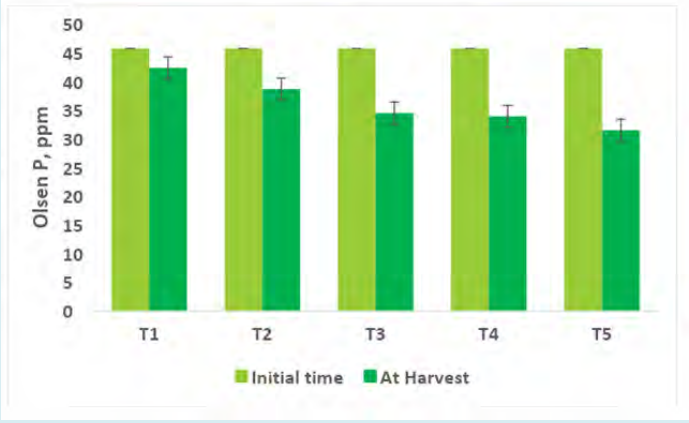

Figure 6: Soil Olsen's P concentration at the initial time and after crop harvest (R-4).
T1- Control; T2- Control (100% RDF); T3- Consortium AM Fungi; T4- Consortium PGPR; T5- Absolute Consortium PGPR+ AM Fungi

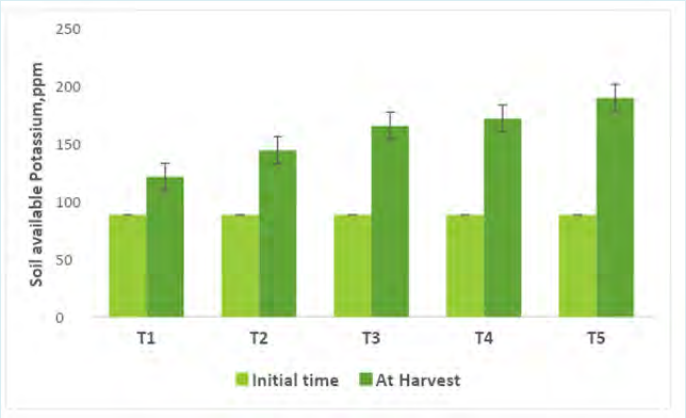

Figure 7: Soil available Potassium concentration at an initial time and after crop harvest (R-4).
T1- Control; T2- Control (100% RDF); T3- Consortium AM Fungi; T4- Consortium PGPR; T5- Absolute Consortium PGPR+ AM Fungi

Measurement of Plant Growth Parameter (MPGP)

The effect of the Absolute consortium PGPR, Absolute consortium AM fungi single and in combination, and CF on the growth and yield of capsicum differed significantly (Tables 1-4). Change in plant height was significant in all the treatments and maximum plant height was observed in the Absolute consortium PGPR + AM Fungi followed by Absolute consortium of AM Fungi and Absolute consortium of PGPR alone *i.e.,* 35.76-116.70 cm, 31.03-111.97 cm, and 27.6o-108.60 cm respectively, while CF (100% RRF) had 22.36-103.37 cm height, which was much higher than the uninoculated plants (control) having 99.30 cm, after 30- 150 DAT from the DAT of capsicum (Table 1). A significant difference was observed between the treatments. Additionally, multiple Absolute consortium PGPR+ AM Fungi resulted from the maximum increase in capsicum plant stem girth followed by an Absolute single consortium of AM fungi and a single consortium of PGPR such as54.32 mm, 54.21 mm, and 54.15 mm respectively, while CF (100% RRF) having 53.29 mm, whereas uninoculated plant (control) having 52.29 mm stem girth, 150 DAT, which was very poor in comparison of the multiple consortia of PGPR + AM fungi and alone consortium of AM fungi or PGPR (Table 2). Statistically significant ($P<0.05$) differences were observed between the treatment. Similarly, the multiple Absolute consortium PGPR + AM Fungi in capsicum has the maximum weight per fruit followed by a single consortium of AM fungi and a single consortium of PGPR i.e., 224 gm, 216 gm, and 207.0 gm respectively, while CF (100% RRF) having the 196.0 gm, apart from that uninoculated plant (control) of capsicum having the 173.0 gm weight of fruit. While the maximum number of fruits/plant, fruit diameter, and fruit length were also found in the multiple Absolute consortium PGPR + AM Fungi, 8.20, 7.81 cm, and 8.5 cm respectively, followed by single consortium AM fungi (7.89 cm, 6.1 cm, and 8.03 cm) and single consortium PGPR was 6.80, 7.82 cm and 5.98 cm whereas CF (100% RRF) was 6.10cm, 5.98 cm, and 7.53 cm respectively, apart from that uninoculated plant (control) was a weight of fruits 173.0 gm, a number of fruits per plant 4.90, fruit diameter 4.63 cm and fruit length 6.47 cm which was very low in comparison to the multiple Absolute consortium PGPR + AM Fungi and single consortium AM fungi or consortium PGPR (Table 3).

Treatment	Plant Height (cm)				
	30 DAT	60 DAT	90 DAT	120 DAT	150 DAT
Control	18.46	32.87	49.47	71.77	**99.3**
Control (100% RRF)	22.36	36.77	53.37	75.77	103.37
Consortium AM Fungi	31.03	45.37	61.97	84.37	111.97
Consortium PGPR	27.6	42	58.6	81	108.6
Absolute Consortium PGPR+ AM Fungi	35.76	50.1	66.73	89.1	116.7
F Test	S	S	S	S	S
C.D.at 5%	2.12	2.21	2.22	2.36	2.29

Table1: Plant height of Capsicum under Low-Cost Protected Cultivation (n=10).
DAT-Days after transplanting

Treatment	Stem Girth (mm)				
	30 DAT	60 DAT	90 DAT	120 DAT	150 DAT
Control	11.66	21.78	33.96	45.34	52.94
Control (100% RRF)	11.99	22.11	34.29	45.69	53.29
Consortium AM Fungi	13.01	23.13	35.31	46.71	54.21
Consortium PGPR	12.64	22.76	34.94	46.34	54.15
Absolute Consortium PGPR+ AM Fungi	13.37	23.49	35.7	47.07	54.32
F Test	**S**	**S**	**S**	**S**	**S**
C.D.at 5%	**1.23**	**1.77**	**1.79**	**1.69**	**1.7**

Table2: Morphological observation of capsicum stem girth (mm) under Low-Cost Protected Cultivation (n=10).
DAT-Days after transplanting

Treatment	Quantity Parameters			
	No. of fruit/plant	Fruit length (cm)	Fruit diameter (cm)	Weight Fruits (g)
Control	4.9	6.47	4.64	173
Control (100% RDF)	6.1	7.53	5.88	196
Consortium AM Fungi	7.89	8.03	6.1	216.99
Consortium PGPR	6.8	7.82	5.98	207.99
Absolute Consortium PGPR+ AM Fungi	8.2	8.5	7.81	224
F Test	**S**	**S**	**S**	**S**
CD at 5%	**0.02**	**0.46**	**0.35**	**0.38**

Table 3: Quantity parameters of capsicum influenced by different doses of biofertilizers under Low-Cost Protected Cultivation.
DAT-Days after transplanting

In a similar trend, the multiple consortium PGPR + AM Fungi showed the maximum yield and quality parameter (Fruit Yield (1.83 kg plant^{-1}), total Fruit Yield (742.5 q ha^{-1}), Shelf life7.10 days, and TSS (5.63, Brix^{0}), followed by single consortium AM Fungi (Fruit Yield kg plant^{-} (1.64 kg plant^{-1}), total fruit yield (622.85 q ha^{-1}), Shelf life 6.70 days and TSS (5.60 Brix^{0}) and a consortium of PGPR (fruit yield (1.33kg plant^{-1}, total fruit yield (537.3 q ha^{-1}), Shelf life (6.5 days and TSS(5.55 Brix^{0}). CF (100% RRF) (Fruit Yield,1.32 kg plant^{-1}), total fruit yield 535.95 q ha^{-1}, shelf life 6.2days, and TSS (5.52 Brix^{0}), but uninoculated plants (control) showing fruit yield (0.84 kg plant^{-1}), total fruit yield (341.55 q ha^{-1}), shelf life 5.40 days and TSS (5.34 Brix^{0}) which was very poor in compared with multiple consortium PGPR + AM Fungi and single consortium PGPR or AM fungi (Table 4). Significant differences were recorded between the treatments and the control. Prasad K [11] reported that when *S. tuberosum* cultivars (*Kufri pukhraj, Kufri sindhuri, and Kufri laukar*) and tomato were inoculated with consortium AM fungal inoculant (*Aculospora logula, Glomus fasciculatum, Glomus intraradices, Gigaspora margarita, and Scutellospora heterogama*), the percent of edible tubers and tomato increased due to improvement of root volume and macro and micronutrients absorption by plants. The yield of capsicum can be affected by the interaction of mycorrhizae, PGPR, and fertilizers (Table 4). Mean assessments indicated that in mycorrhiza and PGPR inoculated capsicum with increasing of K, P and other minerals rate than capsicum yield increased. The highest yield was noticed in Absolute consortium PGPR + AM fungi treated cucumber followed by consortium AM fungi, consortium PGPR, and the lowest in control (Table 4). It is widely assumed that the capsicum plant benefits positively from PGPR and AM fungi symbiosis [46,54,55], it makes little growth without mycorrhiza unless heavily fertilized [7,11,13,56,57]. The consortium PGPR and AM fungi alone and with combination-treated capsicum performed better than untreated control (100% RRF) and control. Significant differences were recorded between the treatments. Consortium PGPR and AM fungi-treated capsicum showed a significant increase in plant height, and the number of leaves/plant, compared to non-microbial control (100% RRF, Farmer's practiced). Multiple consortium PGPR and AM fungi association has also positively correlated with plant growth and productivity. It is expected that the capsicum

plant benefited positively from *Pseudomonas, Azotobacter, Fraturia, Azospirillum, Bacillus* PGPR microbes, and AM fungi symbiosis in an early application.

Treatment	Yield Parameters			
	Fruit Yield kg plant^{-1}	Fruit Yield q ha^{-1}	Shelf life (Days after harvest)	TSS (Brix0)
Control	0.84	341.55	5.4	5.34
Control (100% RDF)	1.32	535.95	6.2	5.52
Consortium AM Fungi	1.64	662.85	6.7	5.6
Consortium PGPR	1.33	537.3	6.5	5.55
Absolute Consortium PGPR+ AM Fungi	1.83	742.5	7.1	5.63
F Test	**S**	**S**	**S**	**S**
CD at 5%	**0.015**	**1.16**	**0.55**	**0.17**

Table 4: Yield and quality parameters of capsicum influenced by different doses of biofertilizers under Low-Cost Protected Cultivation.

Macronutrients Translocation by Capsicum Shoot System

Nitrogen Translocation by Capsicum Shoot System

PGPR and AM fungi can improve plant growth by improving macro and micronutrient absorption. To determine the effect of multiple consortiums of PGPR + AM fungi as well as a single consortium of PGPR or AM fungi inoculants on the uptake of macronutrients state of pepper plants and its detoxifying role, N, P, K, were examined (Figures 8-10). An assessment of data indicates that N translocation (Figure 8) through capsicum shoot systems shows that all the microbial treatments had a significant influence on N uptake as compared to NMC (100% RRF) and control treatment. The maximum N uptake (2.34% at harvest) was obtained under treatment T5 (Absolute consortium PGPR + AM fungi) where the six microbial inoculants were applied. However, the lowest value of N uptake (2.5% at harvest) by capsicum shoot was recorded under control treatment. The uptake of N by the capsicum plants went on increasing with the successive microbial application because the uptake is a resultant of strength and biological yield.

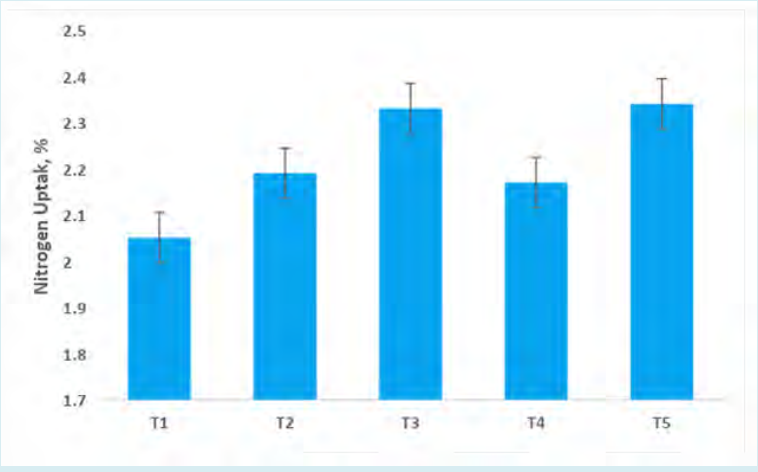

Figure 8: Nitrogen uptake in capsicum at harvest (R-4).
T1- Control; T2- Control (100% RDF); T3- Consortium AM Fungi; T4- Consortium PGPR; T5- Absolute Consortium PGPR+ AM Fungi

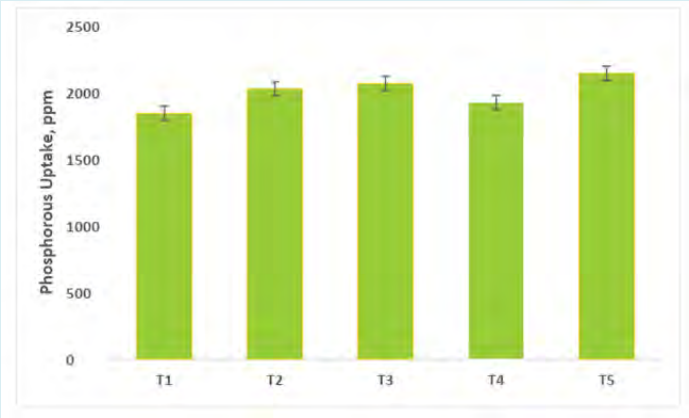

Figure 9: Phosphorous uptake in capsicum at harvest (R-4).
T1- Control; T2- Control (100% RDF); T3- Consortium AM Fungi; T4- Consortium PGPR; T5- Absolute Consortium PGPR+ AM Fungi

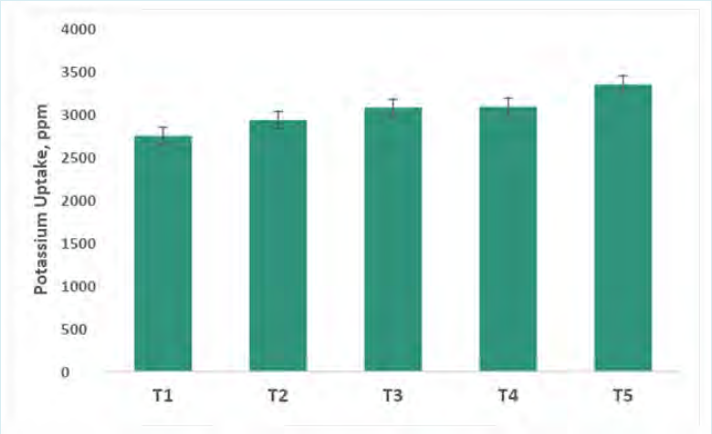

Figure 10: Potassium uptake in capsicum at harvest (R-4).
T1- Control; T2- Control (100% RDF); T3- Consortium AM Fungi; T4- Consortium PGPR; T5- Absolute Consortium PGPR+ AM Fungi

Phosphorus Translocation by Capsicum Shoot System

A glance at data in figure 9 shows the highest uptake of P (2145ppm) by the capsicum under treatment T5 (consortium PGPR + AM fungi). The minimum P uptake was recorded under T1 (1845.12 ppm at harvest). The effect of microbial consortium PGPR + AM fungi inoculation on P uptake was significant (Figure 9). P uptake increased may be due to improved absorption and utilization of available soil P at higher rates.

Potassium Translocation by Capsicum Shoot System

Potassium translocation in capsicum plants has been presented in figure 10. An inquisition of data indicates that maximum K uptake (3345 ppm) by capsicum shoot was recorded in consortium PGPR + AM fungi treatment, where the six consortium microbial stimulants were applied followed by T4 (consortium PGPR), T3 (consortium AM Fungi), T2 (control (100% RRF), and T1 (control). K uptake was increasing may be due to improved absorption and utilization of potassium at higher rates of available soil potassium.

Micronutrient Translocation by Capsicum Shoot System

Copper Translocation by Capsicum Shoot System

The glimpse of data presented in Figure 11 indicates the highest uptake of copper (56.78 ppm) by the capsicum

shoot system was recorded in the treatment consortium PGPR + AM fungi.The effect of six consortium bio inoculants treatments was noticed to exert a significant effect on the copper removal by capsicum plants. The minimum copper (39.1ppm) uptake was recorded under control without a treatment plant at harvest.

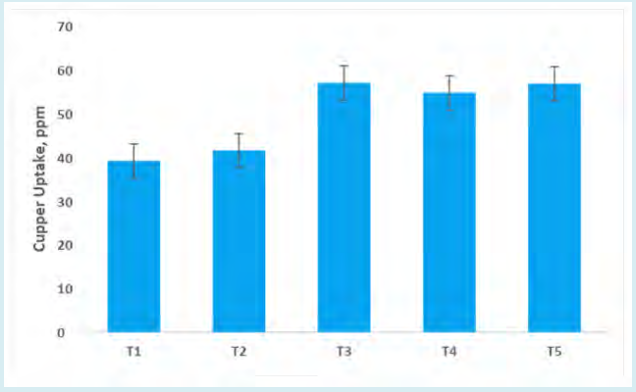

Figure 11: Copper uptake in capsicum at harvest (R-4). T1- Control; T2- Control (100% RDF); T3- Consortium AM Fungi; T4- Consortium PGPR; T5- Absolute Consortium PGPR+ AM Fungi

Iron Translocation by Capsicum Shoot System

The perusal of data presented in Figure 12 reveals the highest uptake of iron (745.34 ppm) by the capsicum plant was recorded in treatment T5 (Absolute consortium PGPR + AM fungi). The effect of multiple cultures of PGPR and AM fungi in treatments was noticed to exert a significant effect on the iron removal by capsicum shoot followed by control there no fertilizers and bioinoculant was applied (657ppm).

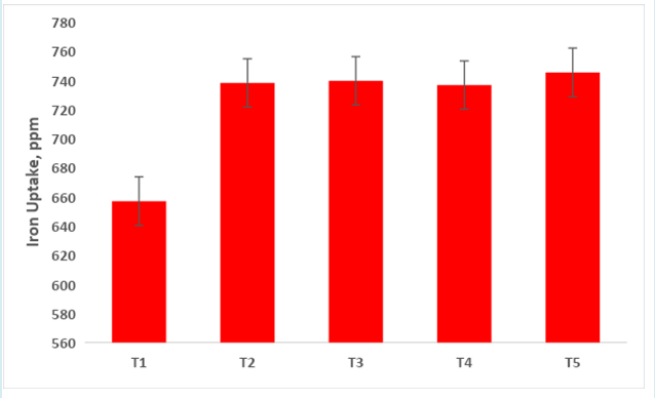

Figure 12: Iron uptake in capsicum at harvest (R-4). T1- Control; T2- Control (100% RDF); T3- Consortium AM Fungi; T4- Consortium PGPR; T5- Absolute Consortium PGPR+ AM Fungi

Zinc Translocation by Capsicum Shoot System

The data presented in Figure 13 reveals that maximum Zn translocation (98.78 ppm) by capsicum shoot system recorded under treatment T5 (Absolute consortium PGPR + AM fungi) followed by T3 (94.76ppm), T4 (91.31ppm), T2 (70.79ppm), T1 (65.78). The microbial inoculants such as PGPR and AM fungi facilitated capsicum plants were found to impose a significant effect on the Zn uptake.

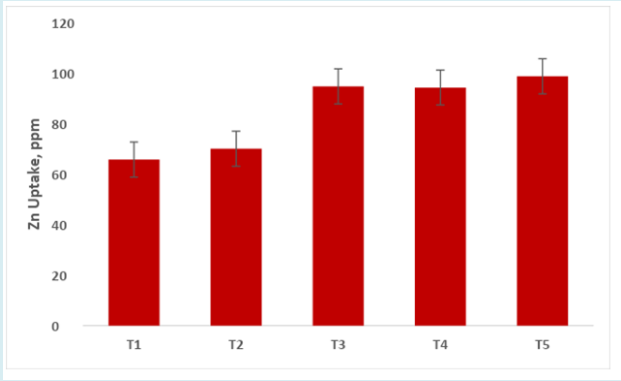

Figure 13: Zink uptake in capsicum at harvest (R-4). T1- Control; T2- Control (100% RDF); T3- Consortium AM Fungi; T4- Consortium PGPR; T5- Absolute Consortium PGPR+ AM Fungi

Mycorrhiza Root Colonization (MRC) Percentage In Capsicum

MRC percentage in the capsicum root system was observed in all treatments including control plants (Figure 14). Maximum 89.56% of MRC was observed in T5 (Absolute consortium PGPR + AM fungi) bioinoculant mediated capsicum crop followed by T3 (Absolute consortium AM fungi, 88.56%), T4 (Absolute consortium PGPR, 54.45%), T2 (control, 100% RRF, 53.56%) and T1 (control, 43.89%) plants at harvest (Figure 15). Statistically significant ($P<0.05$) differences were observed between the treatment. MRC was increased with additionally added PGPR with mycorrhizae. In general, AM fungal strains arbitrated plants have been encouraged by higher water and mineral nutrients uptake from the soils because they increased the total root surface [6,11,13,18,58,59]. The colonization potential of AM fungi decreases in control treatment due to 100% RRF applied in capsicum whereas increases in PGPR application. MRC percentage was affected with an added in PGPR and values were statistically different compared to NMC (100% RRF) and control [58]. The results in this study revealed increased plant growth parameters, nutrients concentration in soil, nutrient translocation, and AM fungal root colonization percentage when consortium PGPR and AM fungi inoculated plants, and this was comparable to uninoculated plants

treated with high dosages of fertilizer (100% RRF) and without fertilizer. The influence of consortium PGPR and AM fungi in decreasing fertilizer demand of major crop species was reported by [6,11,12,56,60]. It has been proven that PGPRs and AM fungi have the potential to reduce the high application rate of fertilizer needed to produce high capsicum yield [57]. Moreover, the capsicum plant benefits positively from AM fungal symbiosis. It creates small growth without mycorrhiza unless severely fertilized [55,61]. Reduction in plant growth characteristics, shoot nutrient content, root colonization, and yield of control plant with improvement in fertilizer function. From the above results, it appears that capsicum should be integrated with a consortium of *Azotobacter, Pseudomonas, Fraturia, Azospirillum, Bacillus* in combination with a consortium of AM fungi for better growth, yield, and quality. For increasing capsicum shelf life, the combination of PGPR + AM fungi is effective. Though the Absolute consortium PGPR + AM fungi bioinoculant produced the best result compared to different combinations of biofertilizers and recommended rate of CFs. The next may be a particular level with certain considerations of sustainability in production and environmental protection.

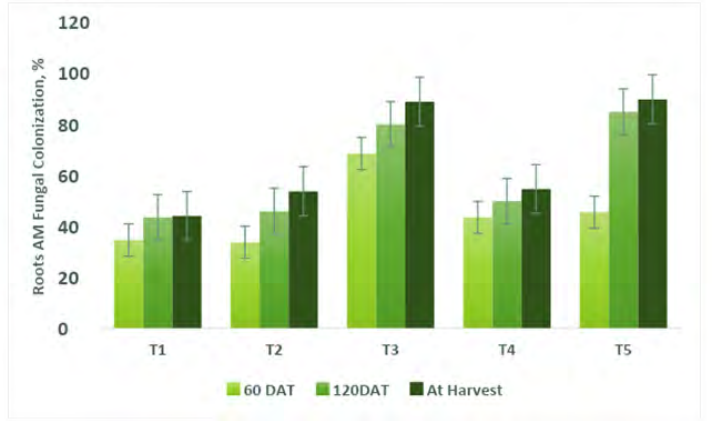

Figure 14: Mycorrhiza root colonization percentage in Capsicum at 60 DAT, 120 DAT, and at harvest (R-4).
T1- Control; T2- Control (100% RDF); T3- Consortium AM Fungi; T4- Consortium PGPR; T5- Absolute Consortium PGPR+ AM Fungi

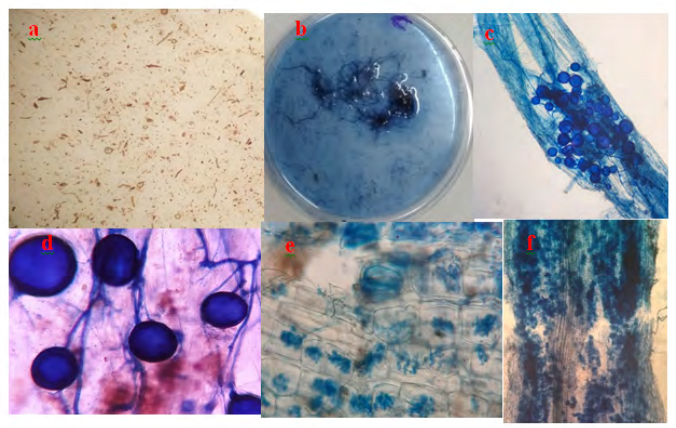

Figure 15: a. Consortium AM fungal spores b. AM fungi root colonization staining; c. Vesicles and mycelium formation; d. Mature vesicles and mycelium; e. Arbuscules and intra and extracellular hyphae; f. Vesicle, arbuscles, and mycelium.

Discussion

In general, capsicum responded better to different treatments for different characters as compared to the control. In this experiment, none of the single inoculation treatments had a significant effect on all of the experimental parameters or showed consistent plant growth-promoting effects as observed in multiple bio inoculation; however, the observed growth parameters were found to be highest in both the consortium PGPR + AM fungi and the alone consortium

PGPR and AM fungi for inoculating capsicum plants. AM fungi are known to have the capacity to increase plant nutrient intake by creating an association with roots [62], as well as promote the growth of other rhizospheric microbes (PGPR), resulting in increased PG [63]. Another reason could be that once the AM fungus invades the host roots, they alter the root exudates produced and create the phosphatase enzyme in the rhizosphere. Extraradical hyphae of AM fungus produced phosphatases that could hydrolyze extracellular phosphate ester linkages, making P accessible to plants [13,18,64]. The root system was shown to be considerably more compatible with the consortium AM fungi + consortium PGPR in terms of absorbing and translocating nutrients through a large consortium. AM fungi aid in the uptake of diffusion-limited nutrients such as P, Zn, Cu, and others, provide drought resistance and pathogen protection, produce plant growth regulators, and interact synergistically with PGPR [7,65].

Improved plant growth may lead to an increase in fresh shoot and root growth, as well as increased biomass build-up, which enhances P uptake. Because phosphorus is required for nitrogen fixation, consortium inoculation may have altered the plant's P and N uptake in this experimentation as well. The P and N levels of the plants followed a similar pattern to that shown in previous studies on other crops [66]. Finally, the growth parameters (plant height, stem girth, yield, and quality) of the inoculated capsicum plant were considerably higher than those of the uninoculated (control) plant. This is owing to the presence of AM fungus, as well as the PGPR's efficiency. Plant pathogens are protected by phytohormones produced by PGPR. In this study, it was found that inoculating the field with mixed consortium AM fungus + PGPR at the time of transplanting not only improved plant growth and production but also reduced CF application and improve SH as well as reduced pollution and save the environment.

Conclusion

Based on the findings; maximum growth and quality yield were noticed in Absolute consortium PGPR + AM fungi treatment followed by the single biological consortium PGPR, AM fungi NMC, and control treatments. On the other hand, these treatments showed had the maximum nutrients uptake by the shoot. A significant difference ($P<0.05$) was noticed in nutrient uptake by capsicum. It has been determined that the maximum utmost was within the consortium PGPR + AM fungi treatment and the minimum was discovered within the PGPR treatment that was higher than NMC (100% RRF) and control treatment. One of the physiological processes which can markedly alter or cut back the nutritionary quality of the various plant products consumed by humans is oxidative stress. The environmental factors that induce oxidative stress in plants include air pollution, herbicide/pesticide utilization, heavy metal contamination, drought, salinity, injuries, UV light, unfavorable temperatures, and photoinhibition from excessive solar radiation [67,68]. The utilization of the consortium of multiple PGPR and AM fungi could also be due to the ability to reduce the negative effects of environmental stress and improved capsicum quality. The production of plant growth regulators (PGR) by PGPR microorganisms is another vital mechanism usually related to growth stimulation [69]. AM fungi are identified to have an effect on PG and health by increasing resistance to the tolerance of biotic [70,71] and abiotic stress [11,72].

Based on the responses of different characters such as PG, nutrient uptake (P, N, K, Zn, Fe, Cu), and yield, it can be concluded that the multiple consortium PGPR + AM fungi are the best consortia of microorganisms for inoculating capsicum plants, followed by the single consortium AM fungi and the consortium PGPR. Inoculation with a microbial community like this could result in healthy, aggressively growing capsicum seedlings and plants. Because it is simple and environmentally safe any nurseryman or farmer can use it to inoculate capsicum seedlings in the nursery and in the field. As a result, this study suggests that farmers with similar soil and other environmental conditions add Absolute consortia PGPR + AM fungi to their farms and fields at the transplanting stage instead of other dangerous chemicals to improve crop establishment, production, and nutrients. This development combines long-term control with pathogen control and provides ISR with a defense mechanism.

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