

Endophytes and AM Fungi collegial outcome on growth and nutrient status of *Capsicum frutescens* L. in tropical region

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Abstract

Capsicum frutescens L. (Pepper) is a valuable source of nutrition and widely consumed as vegetable as well as a spice crop all over the globe. Although it is well recognized to be extensively grown in warm, temperate, tropical, and subtropical regions, this crop's flavor, tanginess, and tantalizing aroma vary depending on the variety grown in the different parts of India. The current research aimed to investigate the effects of AM fungi and endophytic bacteria on the growth and nutrient acquisition of *C. frutescens* in Northwestern Indian region. A dual collaboration of AM Fungi and endophytes showed enhanced response with respect to plant growth in *C. frutescens* compared to plants that were not inoculated. AMF inoculation demonstrated a considerable enhancement in nutrient uptake in comparison to control. Moreover, introducing endophytes resulted in significant growth and yield, demonstrating that improved plant growth and nutrient absorption relied on the specific combination and synergism of endophytic bacteria and AM Fungi being utilized. The purpose of carrying out these experiments in rural cultivable areas was also to raise farmers' awareness about the potential of biological agents and to motivate them towards organic farming. It was demonstrated that for capsicum cultivation, the combination of favorable and competent microbes is crucial in achieving the desired plant growth and improved productivity.

Keywords

Capsicum, Endophytes, Mycorrhiza, Plant growth parameters, Nutrient uptake.

Introduction

The *Capsicum* spp. a member of the family Solanaceae, is one of the significant commercial and horticultural farms produces in the Asiatic countries and its varied varieties are cultivated as spices, vegetables, seasonings, etc., which are added chiefly for enhancing taste, coloring, and tanginess¹. Peppers are laden with Vitamin A, C and E, beta carotene, and capsaicin, improving the therapeutic properties of the staple diet of different territories. Hence, *Capsicum* finds diverse utility, including medicinal and herbal aspects; besides this, it is an imperative marketable cash crop. India is the principal consumer and producer of pepper, contributes about 25% to worldwide cultivation, and exports 39% of its total produce; therefore, it retains the key position as a *Capsicum* exporter, earning about 30.0 million US\$ annum⁻¹ 2,3. The agroclimatic conditions of Western Uttar Pradesh, India are apposite for farming lucrative magnitudes of diverse vegetables and the area could also be professed as an important area for several aboriginal chili species⁴.

The *Capsicum* crop responds well to the application of both organic manures as well as inorganic inputs.

Since many decades, bio-fertilizers are gaining much importance because of being cost effective, augmenting plant growth and nutrients, have no residual toxicity and capacity to enhance soil fertility in addition to higher returns under favorable weather conditions^{5,6}. Therefore, in that respect, there is a huge demand in the international grocery store for organically produced *Capsicum*⁷.

Arbuscular mycorrhizal fungi (AM Fungi) belonging to phylum Glomeromycota are the main useful and efficient constituent in any agroecosystems and form synergetic connections with the economically valuable plants⁸, including horticultural and vegetable crops⁹. AM Fungi are considered to augment plant growth and development by boosting the absorption of macro and micro elements along with water and also provide physico-chemical and biotic stress tolerance^{10,11}. The field crops primarily rely on AM Fungi symbiotic relationships. In different types of soils, inoculation of these fungi has demonstrated a positive impact on crop productivity¹². Nevertheless, the influence of AM Fungi on plant maturation and development in both horticultural and agricultural farm crops is not generally uniform, owing to the intricate interactions between the

host plant genomes and AM Fungi, the type of inoculation techniques, and varying settings of the environment^{9,13}.

Employment of microbial inoculums such as potential rhizobacterial strains, endophytes and AM Fungi termed as bioinoculants, biostimulants, and biofertilizers, has drawn universal consideration owing to the undesirable effects of unwarranted application of chemical pesticides and fertilizers. Injudicious usage of chemical fertilizers and pest repellents, along with deep tillage and close rotations, have caused a diminution in fertile soils and diverse microbiota in rigorous farming systems¹⁴. The microflora and biological activity in traditionally managed soils were discovered to be inferior to those in less exhausted or organically cultivated soils¹⁵. Moreover, to move towards sustainable organic agriculture, opting the biofertilizers is a better option¹⁶. Further, the resourceful collaboration between the rhizospheric microbiome and crop plants is well established^{6,17,18}. Amongst the bioinoculants, phosphate solubilizing microbes (PSM), plant growth-stimulating rhizobacteria (PGPR), and AM Fungi consortium have acquired noteworthy attention owing to the mutualistic favorable impact as natural growth stimulators to enhance the growth of horticulture and agriculture farm crops^{6,19,20}. From this perspective, phosphate mobilizing, and nitrogen-fixing bacteria are prevalent biostimulants employed to boost the development of varied farm crop plants²¹. These beneficial bacteria encourage plant development by making nutritional elements accessible to plants, synthesizing phytohormones, facilitating phosphate solubilization, siderophores synthesis, and excretion of lysins and antimicrobials that act as a biocontrol against pathogenic microbes^{22,23}.

These bacterial inoculants aid AM Fungi in stimulating mycorrhizal establishment and function. Earlier research has exhibited that inoculation of *Azotobacter chroococcum* and *Pseudomonas* sp. together with AM Fungi, boosts the maturation, development and productivity in numerous species of plants^{5,6,20}.

The endophytic microbiome associated with plants is considered as microorganisms that

reside within intracellular host plant tissues, without causing any evident harm to the host plant²⁴. The association between endophytic bacteria and plants is believed to be established during evolutionary progression^{25,26}. Endophytic bacterial strains universally colonize the interior plant tissues, coexisting in almost every plant worldwide. Some potential endophytic bacteria are capable of stimulating plant maturation. The beneficial endophytic strains exhibiting the mechanisms for growth enhancement in plants are comparable to the rhizospheric bacterial mechanisms, e.g., the acquirement of supplies needed for the maturation and development of plants^{27,28,29,30}. Comparable to growth-stimulating rhizospheric bacteria associated with plants, the plant growth-stimulating endophytic bacteria can also act as a biostimulant to expedite plant growth in agro-ecosystem along with environmental cleanup strategies (i.e., phytoremediation)^{31,32,33,34}.

Keeping the above in mind, the present study was planned to employ AM Fungi (to mobilize macro and micronutrients) and competent endophytic bacterial strains (to provide plant growth attributes) individually and together to augment the plant maturation and nutrients in *C. frutescens* L.

Material and methods

Site description

The study was carried out during the winter season (November-February) in the Western Uttar Pradesh district of Meerut, India (28.9845°N, 77.7064°E). The sites were selected on the basis of field already under cultivation of vegetables, which were used to carry out experiments with *C. frutescens* L. The sites of experiments are mentioned as L1, L2, L3, L4 (Figure 1), all the four locations were cultivable lands in rural areas. Four locations have been selected to bring awareness among the farmers about the use of biofertilizers as well as for assessing the effect of bioinoculants on *Capsicum* crop at different locations. The average mean temperature of the site during cultivation of *Capsicum* crop was 13 to 25°C. The soil physiochemical properties of all four locations are mentioned in Table 1. Before

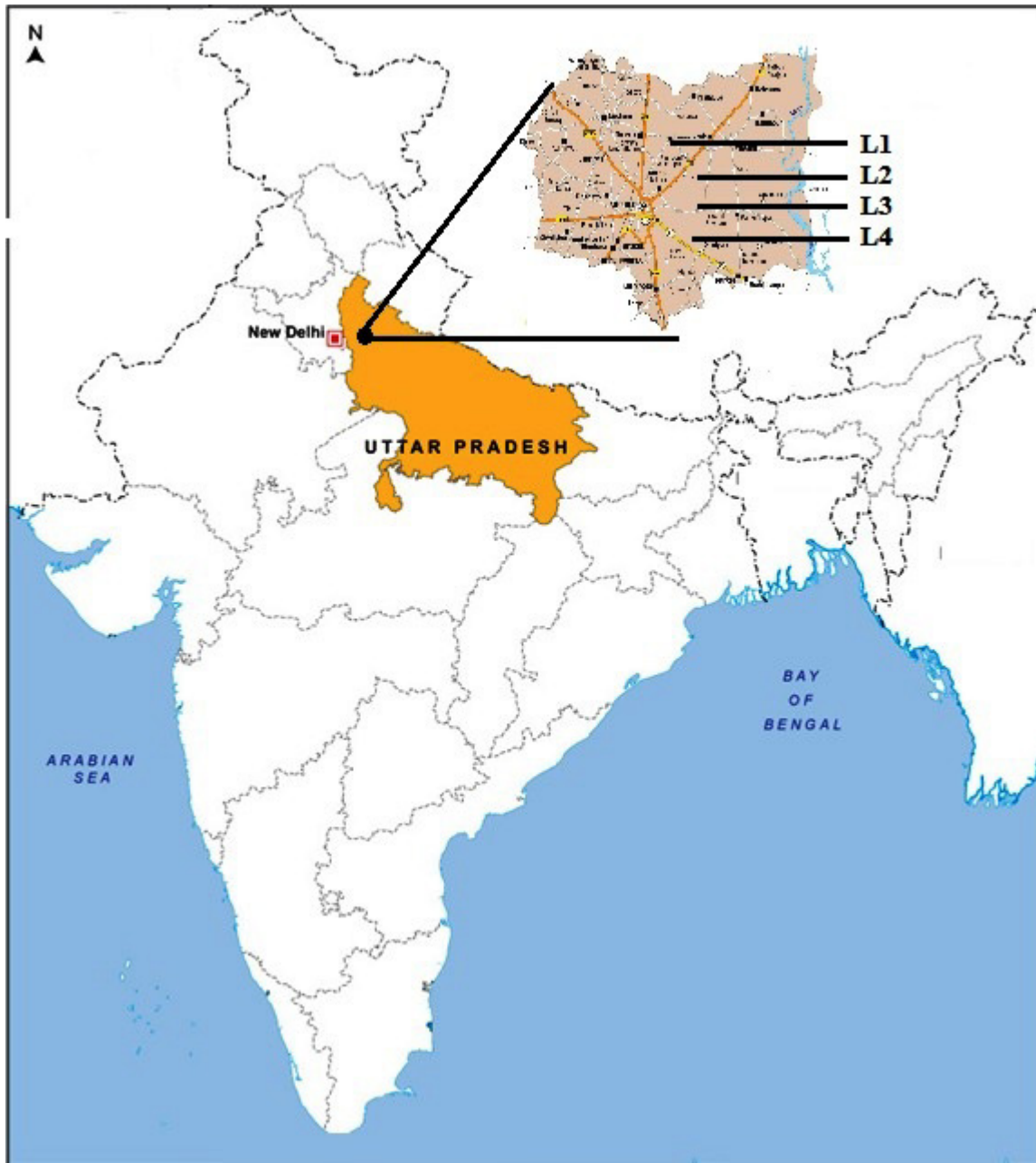


Figure 1. The map shows the locations of the experiment conduction

transplant of the *Capsicum* seedlings from the nursery, the soil was completely cleared off the floor vegetation, so that the floor vegetation microflora does not interfere with the inoculated microbes of the *Capsicum* plants.

AM fungi

The AM fungi (*Glomus fasciculatum*) employed

for the study were procured from the localized vegetable marketplace, sold by a government-endorsed dealer.

Bacterial endophytes

Bacterial endophytic strains used in this study were purified from nagphani plants (*Opuntia ficus-indica*) procured from sand dunes and

deserts around Jaisalmer, Rajasthan, India. Out of 177 bacterial strains, three endophytic strains (one *Bacillus subtilis* (BS 9), two *Pseudomonas* sp. (P 4, P 123) were opted, considering their plant growth enhancing abilities such as phosphate solubilization, siderophore synthesis, ACC deaminase activity, indole acetic acid (IAA) generation³⁵. These endophytes have been selected for this study because of their PGPR and osmotic tolerance attributes, as these strains have already shown their potential in managing salinity and drought along with supporting improved growth kinetics of tomato plants³⁵.

Microbial treatment preparation

Different combinations of AM Fungi and endophytes (BS9, P4 and P123) were taken into consideration for assessing their potential for growth and nutrient acquisition by *C. frutescens* L. as opposed to uninoculated control. Various treatments were employed: (i) uninoculated (control), (ii) BS 9 (iii) P 4 (iv) P 123 (v) AM Fungi (vi) BS 9+AM Fungi (vii) P 4+AM Fungi (viii) P 123+AM Fungi, respectively. The seeds of *C. frutescens* were sprayed with 20% jaggery solution and subsequently coated with endophytic bacteria which had already been mixed with charcoal powder (10^8 g⁻¹). Further, the seeds were dried in air for a duration of 30 min under shade (ambient temperature 25-28°C) and propagated in biodegradable peat pots. The AM fungi granules @ 1% were mixed with 100 g of peat moss pots before sowing the seeds; this was done in order to make fungal inoculum active and infective at the time of seed sowing¹⁷. The control plants were not given any microbial treatment. The biodegradable pots were filled with peat moss, and in each small pots three to four bacterial treated seeds were sown. The pots were watered as and when required. After 35 days, when the seedlings attained a height of around 6-7 inches, they were transplanted into main field plots, maintaining the spacing of 45 cm × 30 cm. The growth of the plants in the respective plots was recorded and the log register was maintained.

Post harvest measurement

Pepper crop was harvested carefully after a

period of approximately 19 weeks of sowing. Subsequently, diverse plant growth factors such as length (cm) and dry weight (g) of shoot and root were evaluated. Likewise, the number of fruits borne by the plants, along with fresh weight (g) and length of the fruits (cm), were also determined. Entire plant biomass samples, viz shoots (including foliage and stalk) and roots were stored in distinctly marked paper bags and dehydrated using an oven at 77°C for 50 h to determine dry weights. Oven-dehydrated stalks and roots were analyzed for tissue N, P, and K contents. The Nitrogen levels in stalk and root specimens were estimated by employing the micro-Kjeldahl digestion method, whereas overall P was estimated by the molybdenum blue procedure following the triple acid digestion step (conc. nitric acid [HNO₃], sulphuric acid [H₂SO₄] and 60% perchloric acid [HClO₄])³⁶. The concentration of K was determined by the flame photometry method³⁷. The data presented in the manuscript is the mean of all four locations of *Capsicum* crop cultivation.

Statistical analysis

To estimate the influence of endophytes and AM Fungi inoculation on chili crops, a completely randomized block design (CRBD) was employed for each and every experimental group. To ascertain the repeatability of the findings, experiments were performed in triplicate. Mean value deviations were determined for the different growth parameters by employing Duncan's multiple range tests (DMRTs) at P < 0.05.

Results and discussion

The physicochemical characteristics of soil samples acquired from the rhizosphere of *C. frutescens* from various areas of district Meerut, India have been listed in Table 1.

Soil consistency varied from sandy to sandy-loam with pH ranging from 7.64 to 7.82. The organic carbon concentration in the soil varied between 9.41 and 13.38 g Kg⁻¹. The N content ranges from 215-232 Kg h⁻¹, P concentration ranged between 24.2-29.1 Kg h⁻¹ and the K content varied from 198 to 228 Kg h⁻¹. The electrical

Table 1. Physicochemical characteristics of different soils of four experimental sites in Meerut District

Experimental sites	pH	EC (dSm ⁻¹)	OC (g Kg ⁻¹)	N (Kg h ⁻¹)	P (Kg h ⁻¹)	K (Kg h ⁻¹)
L1	7.78	0.27±0.021	12.42±0.29	215±13.7	25.7±2.32	198±9.71
L2	7.82	0.27±0.019	9.41±0.27	232±17.5	29.1±2.68	228±9.85
L3	7.64	0.31±0.028	13.38±0.35	226±14.6	27.4±3.12	209±10.23
L4	7.75	0.30±0.029	11.37±0.28	219±12.3	24.2±2.47	204±9.87

conductivity (EC) ranges from 0.27 to 0.31 (dSm⁻¹). Usually, soils are neutral with respect to pH and have a good amount of N and K contents. On the basis of soil characteristics, soil fertility and suitability for cultivation of *C. frutescens* can be determined. Soil too contains a sufficient amount of carbon content, which is necessary for soil microbiota survival and proliferation. The current findings revealed notable improvements in various assessed growth parameters and fruit productivity in contrast to the control group of *Capsicum* plants. At the time of final harvest (19-20 weeks), *Capsicum* saplings that were treated with microbial inoculants exhibited significantly increased length of shoot and root biomass, as evaluated against the untreated control seedlings. The *Capsicum* plants had their longest shoot when the three microbes, namely AMF+Bacillus sp.+Pseudomonas sp. (P 123), were used together. Arbuscular mycorrhizae are prevalent in various ecosystems and are associated with almost most of the angiosperms, positively impacting plant maturation and nutrient absorption³⁵. Recently in 2024, Matthews *et al*³⁸. examined the effect of nonnative bacterial endophytes on the growth parameters of *Capsicum annum*, while³⁹ studying the associated diversity of AMF with *C. annum* rhizosphere. Owing to the beneficial potential of AM fungi in agro-ecosystem, these have been employed to enhance the growth of host plants, leading to the conduct of several surveys aimed at listing and evaluating AM fungal species and their ability to colonize host plants in various geographical territories of the nation^{40,41}.

The inoculation of bioinoculants enhanced the *Capsicum* shoot and root length along with the shoot and root fresh and dry weight (Figure

2). This enhancement was owing to the plant growth augmenting properties of the bacterial endophytes and AM fungi. Further, inoculation of efficient bacterial endophytes (BS9, P4 and P123) also helped in enhancing the plant growth by secreting plant growth regulators, such as indole acetic acid, low molecular weight organic chelates siderophores and phosphate and potassium solubilization potential, along with biocontrol aspects. Our results are in accordance with the^{22,42}, who also demonstrated the effect of inoculated microbes as an efficient biocontrol agent, phytohormone producer and P solublizer, which positively influenced the inoculated plant growth. All the above mentioned microbial parameters are crucial in enhancing the host plant growth, including structural modifications in root structure, which absorb more nutrition from the soil.

Treatment P123+AMF resulted in improved plant growth parameters, followed by P4+AMF and BS9+AMF. The results showed that dual inoculation of bacteria and AM fungi resulted in higher growth parameters compared to single microbial inoculation. The effect of all the microbial inoculations, whether alone or in consortium, exhibited higher plant growth parameters than the uninoculated control (Figure 2). Other important parameters of the *Capsicum* crop were fruit weight, fruits per plant and fruit length. All these parameters were also positively influenced by the bioinoculants, and the parameters were significantly higher than the non-inoculated treatment (Figure 3). Maximum fruit weight of 117.7, 115.4 and 111.3 g was obtained in treatments P123+AMF, P4+AMF and BS9+AMF, respectively, while the control

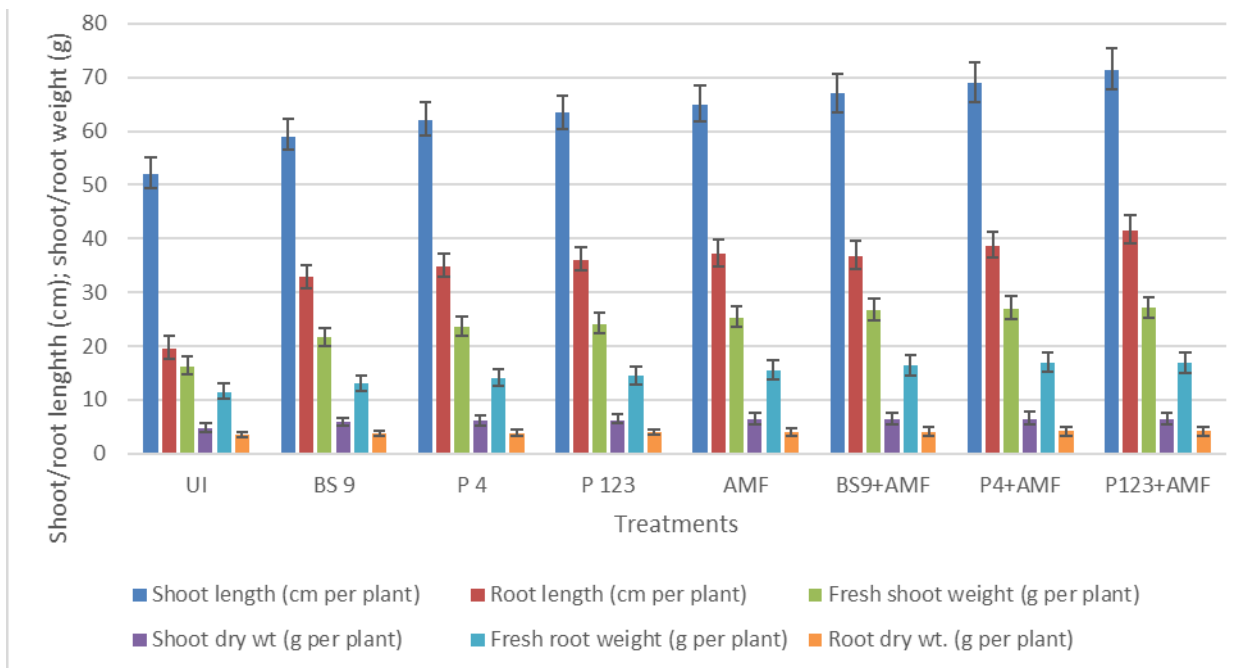


Figure 2. Capsicum plant growth parameters influenced by microbial inoculation

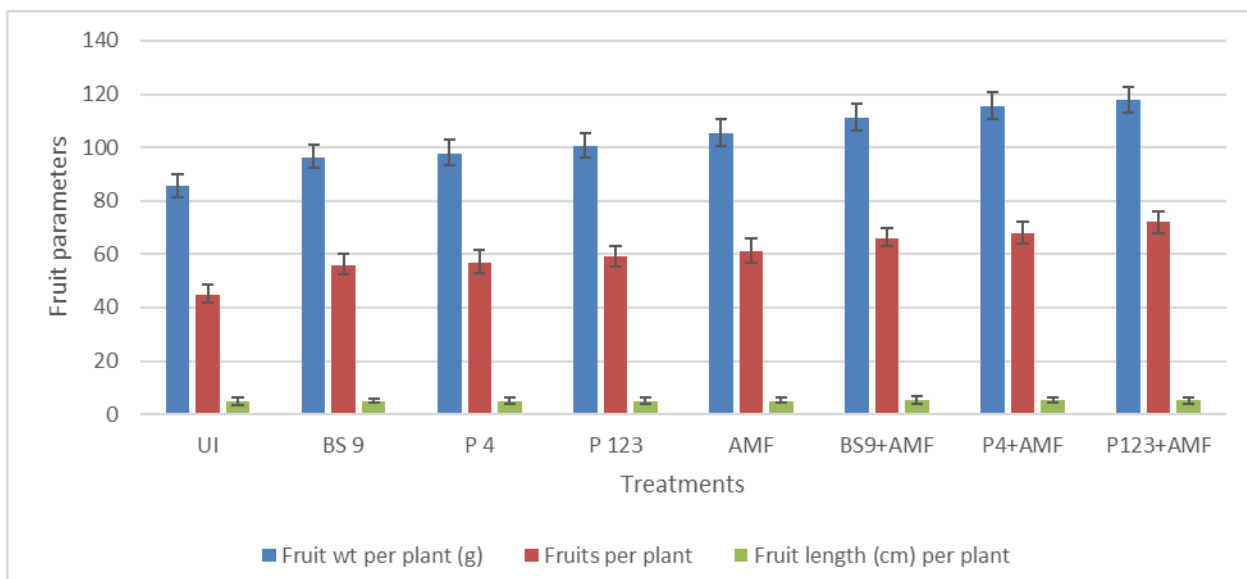


Figure 3. Capsicum fruit growth parameters influenced by microbial inoculation

treatment resulted in fruit weight of 85.5 g. The highest number of fruits per plant were observed in treatment P123+AMF⁷², followed by P9+AMF⁶⁸ and BS9+AMF⁶⁶, while in control treatment, 45 fruits per plant were noted. The maximum length of fruit, 5.15 cm was seen in treatment P123+AMF, followed by 5.13 cm

and 5.11 cm in treatments P9+AMF and BS9+AMF, which was more than the control treatment (4.91cm) (Figure 3).

N, P and K are very important nutrients in any crop plant; microbes play a significant role in mobilizing the macro as well as micronutrients from rhizospheric soil to the inner plants part.

Plants are able to absorb sufficient amounts of nutrients themselves. Moreover, plants with the help of their associated microbes flourish better and uptake more nutrients compared to those plants, which are associated with non-competent microbes⁴³. With regard to nutrient uptake by *Capsicum*, treatment P123+AMF exhibited maximum uptake of N, P, and K in root and shoot (3.73/2.89, 0.39/0.34 and 5.56/4.34%) respectively, followed by the treatments P4+AMF and BS9+AMF and the least amount of uptake was observed in control treatments (1.67/1.25, 0.19/0.13 and 2.47/1.36%) in shoot/root, respectively (Figure 4). AM fungi help in mobilizing N and P from the soil and⁴⁴, enhanced K content in sweet potato has been reported⁴⁵. Further, Liu *et al.*⁴⁶ also reported enhancing nutrient availability in *Capsicum annum L.* by employing *Trichoderma guizhouense*. The workers reported enhanced plant growth along with improved nutrients in *C. annum L.*

The use of bio-inoculums also led to a

substantial increase in fruit productivity in plants compared to plants that were not inoculated. The outcomes of this work evidently demonstrated that mycorrhizal symbiotic relationships significantly boosted the growth of *Capsicum frutescens L.*¹ and mostly the grouping of efficient bacterial strains with arbuscular mycorrhiza accelerated plant development and better fruit yield². It is widely recognized that many trees and shrubs that fix nitrogen rely on mycorrhiza to take in mineral elements necessary for plant growth and effective nitrogen fixation⁴⁷.

The findings indicated that plant growth-promoting endophytes influenced both plant growth and fruit yield. Researchers are exploring the effects of introducing favorable rhizospheric and endophytic microorganisms to the roots on certain quality parameters^{48,49}. Augmenting plant nutrition may be the way in which plant growth-promoting endophytes boost *capsicum* crop productivity, as it enhances the nutritional status of plants by augmenting nutrient availability from

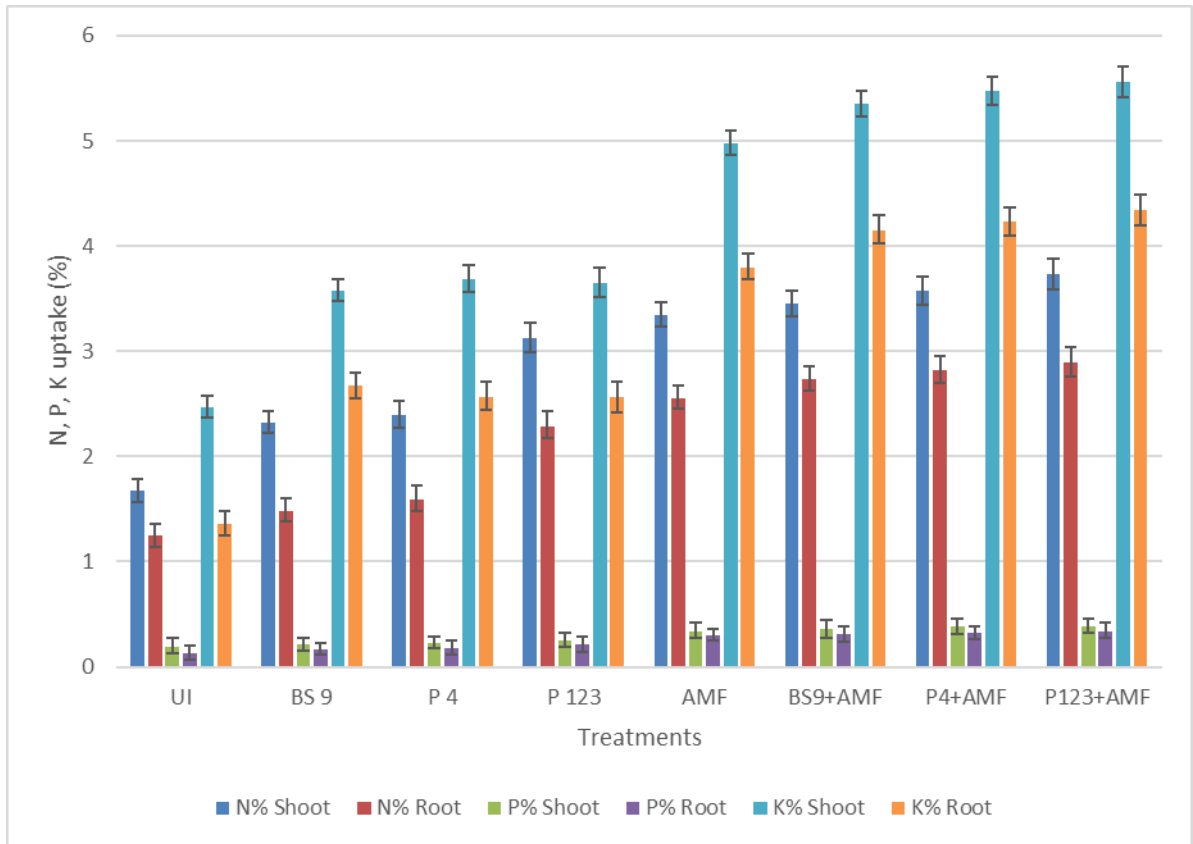


Figure 4. N, P and K (%) uptake in *Capsicum* plant shoot and root

the rhizosphere^{17,50}. Phytohormones generated by plant growth-promoting endophytes are believed to modify the distribution of nutrients in plants, change root growth and influence fruit formation and development⁵¹. The study unequivocally demonstrates that introducing efficient endophytes along with AM fungi into the *capsicum* roots positively impacted growth in field conditions. It is important to continue conducting field experiments to ensure that the positive effects observed are sustained in typical production approaches. *Bacillus* spp. and *Pseudomonas* spp. are classified as good biofertilizers, that directly enable the host plants with nutritional elements, indirectly impact the root's growth and its morphological features, and contribute to other favorable symbiotic associations⁵². An evaluation of bacterial endophytes and AMF influence on productivity demonstrated improved quality of *capsicum* fruit, possibly through increased mineral levels in treated plants⁵³. Treatment with plant growth-stimulating bacteria enables better absorption of nutritional elements by the plants owing to the generation of growth regulators in and around the root interface. These growth factors promoted root development leading to enhanced water and nutrient absorption^{54,55,56}. A noteworthy enhancement in phosphorus and nitrogen levels in *Capsicum frutescens* L. plants was noticed when they were dual-inoculated, as opposed to when they were single or un-inoculated though inoculation of individual bacteria also improved N, P, and K content as compared to untreated plants.

Individually, these microorganisms increased fruit yield, productivity and plant biomass. However, the synergistic effect of the combined inoculation showed even greater significance. The interrelationship between the microorganisms facilitates the production of beneficial compounds, which in turn enhances microbial growth⁵⁷. Our research has conclusively demonstrated the beneficial effects of bio-inoculation with all the treatments. Furthermore, our findings undeniably indicate that microbial inoculation has led to significant and positive changes in host plant growth and fruit production. Hence, the

combined utilization of Arbuscular Mycorrhizal Fungi (AMF) and beneficial endophytes (*Bacillus* and *Pseudomonas*) should be considered as promising inoculants for enhancing the growth and yield status of *C. frutescens* L. under field conditions of tropical regions.

Competing interests

The authors declare that they have no competing interests.

Author contributions

Conceptualization, writing original draft preparation, MK, CS, formal analysis; Viv K, data curation; Vij K; Formal analysis; review and editing; VR, MK, Viv K. All authors have read and agreed to the published version of the manuscript.

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