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Co-inoculation effect of AM fungi and phosphate solubilizing bacteria in growth and morphological character of chilli (*Capsicum annuum* L.)

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Abstract
Chilli plants are considered as good nutrition source, which can be consumed by humans, animals, and used as a medicinal purpose. In non-sterile soil that included glucose and hydroxyapatite, the effects of phosphate-solubilizing bacteria (PSB) and vesicular-arbuscular mycorrhizae (VAM) on plant growth, soil microbial activity, and the synthesis of organic acids were examined. The tripartite interaction between PSB, mycorrhizal fungi and chilli crops are considered to be a beneficial effect on nutrient uptake of chilli crops, disease resistance and plant growth development. This process makes a secondary root system that improves nutrient status of the host plants, water absorption, growth and disease resistance and influencing mineral nutrients. Emerging advancements in improving precision Agriculture plays an important role in betterment of crops and their components. Dual inoculation of PSB and AM fungi leads to high yield of the crop and increase their nutrient concentration. This bacterial and fungal colonization alter the quality and abundance of the soil microflora and can modify the overall rhizosphere microbial activity. In this study, the combination of PSB and mycorrhizal fungi shows more benefits than their use alone in the chilli plants. On co-inoculation, they influence root system architecture and yield of plants. Treatments were made according to the co-inoculation and plant growth was analyzed in terms of their pod length, number of seeds per pod, number of pods per plants and yield. It has been concluded that application of Nitrogen and Phosphorus along with mycorrhiza and PSB was found to be most effective in comparison with other treatments.

Keywords: Arbuscular mycorrhizal fungi, PSB, *Capsicum annuum* L., Dual inoculation, crop growth

Introduction

As a vegetable, chilli (*Capsicum annuum* L.), a member of the Solananceae family, is eaten both fresh and dehydrated (Bosland and Vostava, 2000) [4]. Vitamins A, C, E, B1 and B2, potassium, phosphorus, and calcium are all found in pepper. They can boost plant growth by increasing the uptake of nutrients by plants, particularly very immobile elements like P, Zn, and Cu (Ryan & Anguus, 2003) [10]. This will increase the biomass of roots and shoots. AMF inoculation has been incorporated into horticultural methods because it can help vegetable crops that need a nursery stage. There are numerous challenges to the environment that chilli plants must overcome, such as bacterial, fungal, and viral infections, as well as physical harm from insects that constantly consume their leaves. The availability of vital nutrients required for healthy plant growth and the development of reproductive organs like the flowers and fruit is another significant issue for tomato plants. These deficits can result from both the absence of nutrients in the media and from the fact that the nutrients required for plant growth are greater than the roots' capacity to absorb available nutrients during periods of rapid growth.

One of the most significant commercial crops is chilli, the all-purpose spice known as the "wonder spice." Numerous types of chilli are grown and utilised in everyday life as vegetables, pickles, spices, and condiments. The most essential component of many international cuisines is chilli. It enhances the foods' flavour, colour, pungency, texture, and scent. Indian chiles are well-known for their hue and degree of heat. Even before the crop was employed in cooking, people most likely used it as a medicinal plant because of its rich, varied, and abundant nutrients. Since ancient times, the people have been able to survive thanks to their knowledge of how to exploit these plants.

By producing antibiotics or causing resistance in the host plants, the AM fungus symbiotic relationship competes with diseases for resources and space. These microorganisms have also been used for pathogen biocontrol (Berg *et al*., 2007) [3]. By compensating for the loss of root biomass or capacity brought on by diseases like nematodes and parasites, AM fungi have been suggested to improve host resilience to pathogen attack (Cordier *et al*., 2017) [5]. According to the majority of research, colonisation by AM fungus alters the host plant's root engineering (Gutjahr *et al*., 2013) [8]. Mycorrhizal plants frequently exhibit increased root development and spreading in addition to an enlarged nutritional status (Gamalero *et al*., 2010) [7]. The current study was designed to examine the effects of co-inoculation of PSB and AM fungus in chilli, which aids in plant growth and development, taking into account the previously mentioned factors.

Materials and Methods

AM fungi culture was purchased from CMCC (Centre for Mycorrhizal Culture Collection). These mycorrhizal cultures are then sterilized and brought into in-vitro condition for its propagation and preservation. PSB was collected from the microbiology lab and sub culturing was maintained and preserved in deep freezer at -20 °C. A field was selected in Salem and was grown in well-drained soils and optimum pH of 6.0-7.0, temperature 25-30 °C was maintained for their production. Chillies are a subtropical crop that grow best in warm, humid climates with annual rainfall of 75- 100 cm.

However, too much rainfall can be detrimental to chillies, causing poor fruit set and rooting of fruits if it's accompanied by high humidity. The ground needs to be fine-tuned before planting. Optimum microbial population after fertilizer treatment is 2.3 x 10 in soil. Phosphorus nutrition has a unique importance in chilli cultivation. Other than its role in metabolic procedure and vitality changes, P extensively impacts root division which are seat of natural N fixation and encourages plants to draw nutrients from lower layers and thusly flourish under moist stress conditions. Phosphorus accessibility is a restricting element for plant production in numerous rural soils.

An enormous portion of applied phosphorus fertilizer might be fixed to iron and aluminum oxides and then not available to plant take-up. Inoculation with PSB, VAM strains, nitrogen levels and their interactions significantly increased the mean dry weight and rhizobial counts in the rhizosphere of chilli. It is evident from the data that availability of nutrients in soil such as N, P and K significantly improved due to inoculation of PSB with chilli. The effects on nitrogen availability in soil were due to the presence of large amount of PSB in the rhizosphere of chilli that fixes atmospheric nitrogen and enriches the soil after the degeneration of root nodules. The increase in phosphorus and potassium availability might be due to synergistic effect of N with phosphorus and potassium which increased the availability of P and K in the soil.

Land preparation and fertilizer application

The experimental area was thoroughly prepared by ploughing three times and well decomposed farm yard manure (FYM) was applied at the rate of 25 t/ha followed by the preparation of plots. Fertilizers were applied to the plots as per the treatments as shown in table-1. N was applied to the plots in two doses, one at the time of transplanting and second in the month of June, whereas the entire N and P was applied in a single dose at the time of transplanting as per the designed treatments.

Nitrogen Estimation

Sensing technologies like Artificial Intelligence and Machine Learning are in rapidly advancement since last decade and hence became cost effective. This approach has provided manageable algorithmic solution in precision agriculture. Since, Nitrogen is directly involved in Photosynthesis and many their biological processes, so their economic impact must be considered. Nitrogen estimation can be done with two methods i.e. destructive and non-destructive methods. Most commonly used is destructive method which is a traditional chemical based analysis. Non-destructive method is based on remote sensing in which there is canopy reflectance in visible-NIR wavelengths (400-900 nm) and spectral indices of the Nitrogen estimation is extracted from hyper spectral data.

Another method is through Spectral Reflectance with digital camera which can be estimate the amount of nitrogen. In this method, images is taken with autofocus, White Balancing, and Exposure time in a complementary metal oxide semiconductor (CMOS) Camera built into an O_2 Atom handheld computer combined with a GSM mobile telephone. Image geometry must be considerate while experimenting such as height of the image is set up such that edges are midway in the crop. Hence, Calibration is done from the recorded values (Li *et al*., 2010) [12].

Nitrogen estimation can be done through Chlorophyll content per leaf area. Chlorophyll, one of the important component during photosynthesis, capable of converting light energy into chemical energy. Hence, it's a reliable indicator of Nitrogen. SPAD meter is used to estimate chlorophyll content which can estimate Nitrogen content (Xiong *et al*., 2015) [11].

Phosphorus Estimation

Phosphorus can be estimated by phosphomolybdate blue and Phospho molybdate-vandate-yellow color method with the help of ammonium hydroxide. Sample is washed and dried at 72 °C and then sieved through 2mm sieve. The sample is then treated with sulfuric acid, nitric acid, and perchloric acid. Phosphorus standards were prepared to give 0.38\ig P/mL for the Mo-blue color determination and 4.0 fig P/mL for vanadate-yellow color method determination with 0 to 20 Jig Mn/mL in the final dilution (Gupta *et al*., 2008) [2].

Another method includes the use of acetic acid which is time effective and easy to perform. In this method, the sample is treated with 2.5% of acetic acid and then shake well and the mixture is kept still for 3-4 hours until the supernatant separates. Then color developing reagent with distilled water is added, mixed well. The solution is left for 15-25 minutes and blue color is developed. The intensity of blue color determines the amount of phosphorus (Silva *et al*., 2015) [6].

Treatment

T_0	Control
T_1	PSB alone
T_2	VAM alone
T_3	PSB + VAM
T_4	PSB + VAM + Phosphorus 50%
T_6	PSB + VAM + Nitrogen 25%
T_7	PSB + VAM + Phosphorus 50% + Nitrogen 25%

Results and Discussion

Pod length (cm)

Pod length of chilli was measured to compare the treatments and found that combined application of VAM, PSB along with nutrients showed highest. Maximum pod length was observed in T_7 (PSB + VAM + Phosphorus50% + Nitrogen 25%) followed by T_2 (VAM alone) and the minimum length of pods was observed in T_0 (control). The maximum length of pod was 8.48 cm and the minimum length was 4.30cm in uninoculated control.

Number of seeds per pod

Number of seeds per pod were measured among all the treatments to compare the single inoculation and combined inoculation Maximum seeds per pod were observed in the T_7 (13 seeds) (PSB + VAM + Phosphorus 50% + Nitrogen 25%) followed by T_4 (PSB + VAM + Phosphorus 50%) with 40 seeds per pod and minimum number of seeds were observed in uninoculated control (T_0) with 22 seeds per pod.

Number of pods per plant

The combined application of the PSB and mycorrhiza also influenced number of pods per plant. Number of pods per plant was observed and maximum number of pods showed in T_7 (PSB + VAM + Phosphorus 50% + Nitrogen 25%) with 35 pods, followed by T_4 (PSB + VAM + Phosphorus 50%) with 32 pods. Minimum number of pods were observed in T_0 (control) with 20 pods per plant.

Nutrient Content and Uptake

Data presented in Table 2 reveal that nutrient (NPK) contents and their uptake in chilli were enhanced with the sole application of either AM fungi or PSB inoculation over control. However, combined application of the PSB and mycorrhiza also influenced nutrient content (NPK) and uptake in chilli. The P and nutrient content was observed and maximum number of plants showed in T_7 (PSB + VAM + Phosphorus 50% + Nitrogen 25%) 1.60% P with, followed by T_4 (PSB + VAM + Phosphorus 50%) with 1.59% P. Minimum number of pods were observed in T_0 (control) with 1.42% P per plant, but the values tended to be close to each other, which indicates that these biofertilizers have some role in nutrient dynamics in the soil plant system (Narsian and Patel 2009) [9].

Table 1: Measurement of Length of pod, Number of seeds per pod and Number of pods per plant provided with different treatments

Treatment	Length of pod (cm)	Number of seeds per pod	Number of pods per plant
T_0	4.30	22	20
T_1	7.00	28	26
T_2	7.30	25	30
T_3	6.00	30	26
T_4	6.02	35	32
T_5	6.05	32	31
T_6	6.66	30	26
T_7	8.48	40	35

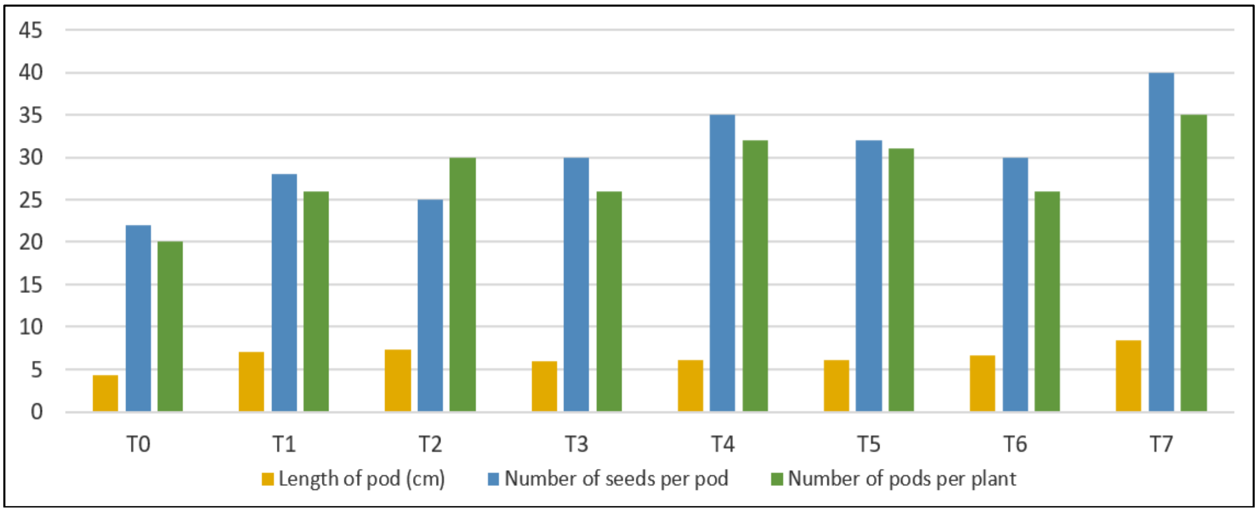

Fig 1: Measurement of length of pod, Number of seeds per pod and Number of pods per plant provided with different treatments

Table 2: Effect of co-inoculation of AM fungi, PSB, and applied P on available nutrient status (mg kg-1 soil) of soil

Treatments	Nutrient content (%)		
	N	P_2O_5	K_2O
T_0	1.42	0.24	0.32
T_1	1.43	0.25	0.33
T_2	1.44	0.26	0.34
T_3	1.45	0.25	0.34
T_4	1.59	0.36	0.38
T_5	1.47	0.27	0.36
T_6	1.48	0.28	0.37
T_7	1.60	0.63	0.44

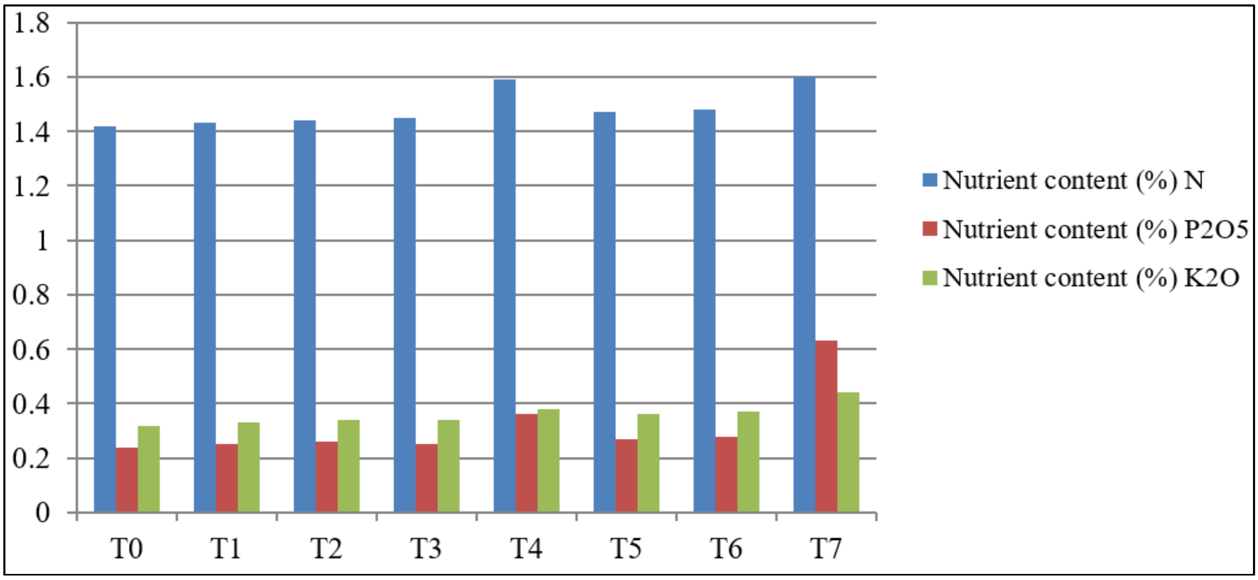

Fig 2: Effect of co-inoculation of AM fungi, PSB, and applied P on available nutrient status (mg kg-1 soil) of soil

Conclusion

From the observed results, treatment T_7 PSB+ Mycorrhiza + Nitrogen 25% + Phosphorus 50% application was found to be most effective in maximizing the length of pods, number of seeds per pod, number of pods per plant. From this analysis, it has been concluded that co-inoculation of mycorrhiza, PSB along with nitrogen and phosphorus was found to be most effective in comparison to other treatments. Present investigation observed that chilli solublizes more amount of phosphorous as well as nitrogen by this dual inoculation. It is therefore suggested and recommended that dual inoculation of PSB and AM fungi should be adopted by farmers to produce more yield of *Capsicum annuum* L. and to sustain soil health.

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