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Influence of am fungus *funneliformis mosseae* and k solubilizing bacterium *bacillus mucilaginosus* on the growth of tomato seedlings raised in pro trays

Running head: influence of am fungi and ksb on the growth of tomato seedlings.

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Abstract

Raising vegetable seedlings in pro trays is becoming an innovative approach to produce quality seedlings in horticulture. The present investigation was conducted to evaluate the effect of AMF *Funneliformis mosseae* and K solubilizing bacterium *Bacillus mucilaginosus* singly and together in enhancing the growth of tomato seedlings raised in pro trays under polyhouse conditions. Different growth parameters like shoot and root length, total seedling length, stem diameter, dry weight of seedlings, biovolume index, plant strength, vigour index, macro and micro nutrient uptake, mycorrhizal root colonization and the population of *B. mucilaginosus* in the root zone soil were monitored. Significantly higher shoot length, root length, stem diameter and biovolume index were recorded in the treatments inoculated with *F. mosseae* alone followed by *B. mucilaginosus* alone. Most of the plant growth parameters were significantly less in the dual inoculation treatment with *F. mosseae* + *B. mucilaginosus* compared to single inoculation with either of them. This brings out the negative influence of the two inoculants on each other leading to a reduced effect on plant growth.

Keywords: *Bacillus sonorensis, Dual inoculation, Funneliformis mosseae, Nursery Technology*

Introduction

Sustainable agriculture focuses on productivity of crops along with benefiting the environment and increasing soil biodiversity by improving natural and healthy environment [1]. The use of chemical fertilizers can be reduced by applying beneficial soil microorganisms like nitrogen fixers, mycorrhizal fungi, phosphate solubilizers and plant growth promoting rhizomicroorganisms (PGPR) to improve and sustain plant productivity, nutrient availability and maintain soil health. The production of good quality seedlings is essential for quality and higher yield of crops and thereby producing and timely distribution of inoculated seedlings have a greater scope to meet the growing demand. The pro tray technology used in nurseries for producing seedlings under shade net by nurserymen is becoming more common in India. Seedlings grown in pro trays provide independent area, improves seed germination, better root development and minimize seedling mortality which leads to production of healthy uniform seedlings in shorter duration. Further this technology helps in easy handling, cheaper transportation and better establishment of the seedlings when transplanted into field. Also, this technology reduces the production cost of seedlings as hybrid seeds are expensive [2]. Soil-less media like vermiculite, cocopeat and perlite are commonly used substrates for raising seedlings in pro trays. Inoculation of the planting medium or seed with the beneficial microbial

consortium is a biotechnological approach for producing healthy, vigorously growing seedlings [3-4].

Tomato (*Lycopersicon esculentum Mill.*) belongs to the family Solanaceae and is the most popular vegetable crop grown worldwide with production of 187 million tonnes per annum. Its annual production in India is 20.6m tonnes [5] and is playing a vital role in the upliftment of the farming community through its prolific yield potential and hence farmers depend on these crops for cash income. Tomato is mainly propagated by seeds and traditionally these seedlings are produced on raised nursery beds. Due to expensive seed material, high incidence of pests and diseases, high rate of mortality and non-uniform growth of seedlings this method is not being followed by farmers often. On the other side nowadays, nurseries are establishing well on an entrepreneurial mode due to government intervention and technological support from public and private organizations. These nurseries are raised in protected structures which results in easy monitoring and uniform growth of seedlings, fewer incidence of pests and diseases and a lower rate of mortality. Hence, the majority of farmers rely on commercial nurseries for tomato seedlings where commercial nursery seedlings advance tomato cultivation by 21 days thereby avoiding the risk of managing a nursery [6].

PGPR are beneficial bacteria inhabiting the soil ecosystem [7] that improve plant growth by facilitating the uptake of nutrients, regulating the production of phytohormones and by preventing the deleterious effect of phytopathogenic organisms by producing siderophores, antibiotics, lytic enzymes, HCN and ammonia [8]. Bacillus mucilaginosus has been reported to increase plant growth and yield of a few crop plants [9].

Arbuscular mycorrhizal (AM) fungi are obligate symbionts belonging to phylum Glomeromycota [10] which establishes a mutual relationship with the roots of host plants that involves the bi-directional transfer of nutrients. Finely branched arbuscules and swollen vesicles are developed in the roots of the host plant, where arbuscules act as the site of nutrient exchange [11]. Mycelium developed from the root system helps the host plant to acquire nutrients from inaccessible soil regions. AM symbiosis helps plants to trap diffusion-limited nutrients like P, Zn, Cu, etc., especially in nutrient-deficient soils [10]. AM symbiosis is also known to improve soil texture, drought and salinity tolerance, and disease resistance [12] in the host plant. AM fungi were found to be effective in enhancing the growth and yield of tomato, chilli and capsicum by few workers [13-14].

AM fungi interacting synergistically with PGPR like N-fixers, P-solubilizers in soil and enhancing plant growth has been reported by earlier workers in many crop plants [15-16]. The objective of the current work was to evaluate the effect of a microbial consortium consisting of the AM fungus Funneliformis mosseae and the K solubilizing bacterium *Bacillus mucilaginosus* on the growth of tomato seedlings raised in pro trays under poly house conditions.

Materials and Methods

The experiment was conducted at the Centre for Natural Biological Resources and Community Development (CNBRCD), Bengaluru, India. The seeds of Zinnia and Balsam used in the study were procured from the University of Agricultural Sciences, GKVK Campus, Bengaluru, India.

Inoculum Preparation

Sub-culturing of *B. mucilaginosus* was done on *Glucose Yeast extract Calcium* (**GYCa)** agar plates and incubated at 37°C for 24 hours. A single colony from the sub-cultured plate was inoculated into 500ml of *GYCa* broth and incubated on a shaker for 4 days. The culture suspension was centrifuged under the refrigerated condition at a speed of 10,000 rpm for 10 min. at the temperature of 10 ℃. After centrifugation, the supernatant was discarded and to the pellet phosphate buffer was added and mixed thoroughly. This was used for inoculation of the substrate in pro-trays. The bacterial population was enumerated by performing a serial dilution of the culture and plating onto *GYCa* agar [17]. *F. mosseae* culture was maintained in a polyhouse, using *Chloris gayana* (Rhodes grass) as the host and vermiculite: perlite: soilrite in the ratio of 3:1:1 by volume + 8% sterilized soil as substrate. The plants were harvested 75 days after sowing (DAS) and finely chopped roots along with the substrate which contained spores and hyphae were air dried and used as inoculum. The number of infective propagules was determined using the MPN method with 10-fold dilution [18].

Experimental Setup

The cells of the pro trays were filled with 20g of the substrate described above for maintaing the AM fungus. There were four treatments viz. uninoculated, inoculated with *F. mosseae* alone, *B. mucilaginosus* alone and inoculated with both the organisms *F. mosseae* + *B. mucilaginosus*. One hundred cells of two pro trays (each with 50 cells) served as uninoculated control and 100 cells of two pro trays

served for each of the inoculated treatment. A planting hole was made in the substrate and 1g of *F. mosseae* inoculum (containing 2.2×10^3 IP/g) and 1ml of *B. mucilaginosus inoculum* (containing 1.9×10^7 CFU/ml) was added. Three seeds were sown in each cell. The seedlings were thinned to one per cell after a few days. The seedlings were maintained in a polyhouse and watered as and when necessary. Five ml of Ruakura nutrient solution without P was added to all the cells once in 10 days starting from 20 days after sowing [19].

Parameters Evaluated

Just before harvest, 60 DAS, plant growth parameters such as shoot length and stem diameter were determined. Shoot length was measured from the substrate surface to the tip of the plant. The stem diameter was measured 1cm above the substrate. The root length of the plants was determined. The bio-volume index was calculated using the formula given by [20]. The seedling vigour was calculated using the standard formula [21]. The plant strength was calculated using the formula given by [22]. The samples were dried in a hot air oven at 60ºC after which the dry weight was determined. The samples were then powdered and the nitrogen concentration was determined by the Micro Kjelhdahl method [23]. Phosphorus concentration was estimated by vanadomolybdate phosphoric yellow colour method [24]. Potassium concentration was determined by the Flame photometer method [25]. The micronutrient analysis of the samples was performed using atomic absorption spectrophotometer with a hallow cathode lamp set to standard wavelengths [26]. The roots were washed and cut into 1cm bits and subjected to trypan blue staining and the percent mycorrhizal root colonization was determined following the procedure of [27]. The *B. mucilaginosus* population in the substrate was enumerated by serial dilution and plating onto *GYCa* agar plates [28]. Raw data of each parameter were subjected to analysis of variance (ANOVA) at significance level ($p\leq0.05$) and means were compared by Duncan's multiple range test (DMRT) using Ag Res Statistical software (ver. 3.01) by Pascal Int. a software solution [29].

Results and discussions

The seedling shoot length and stem diameter showed a significant increase due to inoculation with *F. mosseae* and *B. mucilaginosus* singly, and together; the highest being in seedlings inoculated with *F. mosseae* followed by single inoculation with *B. mucilaginosus* and also dual inoculation with both the organisms. Root length was significantly more in seedlings singly inoculated with *F. mosseae* or *B. mucilaginosus* (Table-1). The dry weight was highest in *F. mosseae* inoculated seedlings which was statistically on par with the treatment *B. mucilaginosus*. The dry weight of dual inoculated seedlings did not differ significantly from the uninoculated treatment. Inoculation with the two inoculants singly and together enhanced significantly the BI compared to uninoculated seedlings. The vigour index followed more or less a similar trend. Compared to the uninoculated treatment plant strength was significantly more in the treatments *F. mosseae* alone and *B. mucilaginosus* alone. The vigour index of dually inoculated seedlings was statistically on par with the uninoculated seedlings (Table 2). Enhanced seedling length, stem diameter, bio-volume index and dry weight due to inoculation with AM fungi have been reported earlier in vegetable seedlings raised in pro trays [10]. Increased plant growth observed in the present study because of potash solubilizing bacterial inoculation is in conformity with the studies made earlier by other workers [9]. Among the inoculation treatments studied, *G. mosseae* alone significantly improved most of the plant growth parameters studied. This was followed by inoculation with the potash solubilizing bacterium, *B. mucilaginosus*. There are several earlier reports that co-inoculation with *N-fixers, P- solubilizers* and other *PGPR* improve plant growth much more then inoculation with single inoculum [30]. Negative intraction between AM fungi and beificial soil microorganisms is very rare and unusual [31].

The nitrogen concentration was highest in B. mucilaginosus inoculated treatment followed by *F. mosseae* and *B. mucilaginosus* + *F. mosseae* treatments. Regarding P concentration it was significantly more in *F. mosseae* treatment followed by the other two inoculated treatments. Potassium concentration was the highest in B.

mucilaginosus treated seedlings but statistically not differing from the seedlings treated with *G. mosseae* alone and *B. mucilaginosus* + *F. mosseae*. The *NPK* concentration was the least in the uninoculated seedlings. Ca and Mg concentrations did not differ significantly among the treatments studied. Regarding the micronutrient uptake, inoculation with B. mucilaginosus increased only the uptake of *B. F. mosseae* treatment significantly increased the uptake of all the micronutrients studied except Fe. Dual inoculation with *B. mucilaginosus* + *F. mosseae* increased the uptake of Cu, Mn and Fe (Table-3). The highest P concentration in the treatment of *F.*

mosseae alone is because AM fungi produce extra-radical hyphae exploring greater volume of soil that takes up diffusion-limited nutrients like P, Cu, Zn etc [32]. The mechanisms suggested for this increased P uptake are the external hyphae exploring greater volume of soil for P away from the root. Effective P acquisition by external hyphae is by the production of phosphatases and Pi transporters and smaller radii of absorptive systems [33].

The inoculation treatments with *F. mosseae* alone and when co-inoculated with *B. mucilaginosus* increased the percent mycorrhizal root colonization. The CFU of *B. mucilaginosus* was maximum in the treatment *B. mucilaginosus* alone followed by the treatment *B. mucilaginosus* + *F. mosseae* but differing significantly. In the uninoculated and *F. mosseae* alone treatments, the population of B. mucilaginosus could not be encountered (Table-4). Significantly higher mycorrhizal root colonization in *F. mosseae* alone and *B. mucilaginosus* + *F. mosseae* treatments compared to uninoculated plants indicate the better proliferating ability of *F. mosseae* with tomato as host upholding the earlier reports [10; 34]. Higher CFU of B. mucilaginosus in the rhizosphere of tomato inoculated with *B. mucilaginosus* brings out the ability of the bacterium to establish in the rhizosphere.

The results of the present study bring out that inoculation with *F. mosseae* alone or *B. mucilaginosus* alone promoted plant growth better compared to dual inoculation with F. mosseae + B. mucilaginosus. This brings out the negative effect of the two inoculants on each other leading to a reduced effect on plant growth. Such incompatibility between the AM fungus Claroideoglomus etunicatum and *PGPR* like Pantoea dispersa and *P. agglomerans* has also been observed earlier [31]. In conclusion, it can be said that AM fungus *F. mosseae* and *B. mucilaginosus* used in the present study are also not compatible. The mechanisms responsible for such interactions need further study.

Table 1: Influence of microbial inoculation on shoot length, root length and stem diameter of tomato seedlings raised in pro trays 60 DAS.

Treatments	Shoot length (cm/seedling)	Root length (cm/seedling)	Stem diameter (mm/seedling)
T1: Uninoculated (U)	15.62^{c}	12.0^{b}	1.54^{c}
T2: Bacillus mucilaginosus (Bm)	18.31^{ab}	13.4^{b}	1.58^{b}
T3: Funneliformis mosseae (AMF)	19.37^{a}	18.0^{a}	1.60^{a}
T4: Bm + AMF	17.84^{b}	12.9^{b}	1.57^{b}
SEd	0.57	2.11	0.01
CD (0.05)	1.11	4.46	0.02

Note: Means in each column with same alphabets are not significantly different at P ≤ 0.05

Table 2: Influence of microbial inoculation on dry weight, biovolume index, plant strength and vigour index of tomato seedlings raised in pro trays 60 DAS

Treatments	Dry weight (g/seedling)	Biovolume Index	Plant strength	Vigour index
T1: Uninoculated (U)	0.29^{b}	24.69^{c}	0.0108^{c}	1852.5^{b}
T2: Bacillus mucilaginosus (Bm)	0.43^{ab}	28.91^{ab}	0.0213^{b}	2355^{a}
T3: Funneliformis mosseae (AMF)	0.49^{a}	30.55^{a}	0.0361^{a}	2527.5^{a}
T4: Bm + AMF	0.34^{b}	28.61^{b}	0.0136^{bc}	2160^{ab}
SEd	0.05	0.94	0.0029	174.8772
CD (0.05)	0.10	1.86	0.0061	370.7281

Note: Means in each column with same alphabets are not significantly different at P ≤ 0.05

Table 3: Influence of microbial inoculum on nutrient uptake of tomato seedlings raised in pro trays 60 DAS.

Treatments	Macro/Secondary nutrients (%)					Micronutrients (ppm)					
	N	P_2O_5	K_2O	Ca	Mg	Zn	Cu	Mn	B	Mo	Fe
T1: Uninoculated (U)	1.97^{c}	0.78^{c}	2.33^{b}	1.69	0.31	51.8^{b}	17.61^{b}	43.13^{c}	41.52^{c}	60.33^{b}	2511^{b}
T2: Bacillus mucilaginosus (Bm)	2.95^{a}	0.84^{b}	2.79^{a}	1.22	0.35	53.1^{b}	20.21^{b}	44.04^{c}	47.60^{b}	62.23^{b}	2356^{b}
T3: Funneliformis mosseae (AMF)	2.55^{b}	0.91^{a}	2.58^{ab}	1.18	0.34	63.2^{a}	23.48^{a}	54.34^{b}	56.76^{a}	90.68^{a}	2538^{b}
T4: Bm + AMF	2.64^{b}	0.80^{bc}	2.58^{ab}	2.01	0.32	52.4^{b}	12.85^{c}	64.70^{a}	43.26^{c}	61.45^{b}	3429^{a}
SEd	0.07	0.02	0.13	NS	NS	1.93	1.32	1.70	1.68	1.75	87.84
CD (0.05)	0.16	0.05	0.29	NS	NS	4.46	3.03	3.93	3.87	4.04	202.56

Note: Means in each column with same alphabets are not significantly different at P ≤ 0.05

Table 4: Influence of microbial inoculation on percent mycorrhizal root colonization and B. mucilaginosus population in the substrate of tomato seedlings raised in pro trays 60 DAS

Treatments	Percent root Colonization (%)	CFU/g $\times 10^5$
T1: Uninoculated (U)	45^b	0
T2: Bacillus mucilaginosus (Bm)	58^b	15.8^a
T3: Funneliformis mosseae (AMF)	98^a	0
T4: Bm + AMF	84^a	6.4^b
SEd	7.92	4.92
CD (0.05)	16.78	10.76

Note: Means in each column with same alphabets are not significantly different at P ≤ 0.05

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