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Dual inoculation with AM fungus *Funneliformis mosseae* and PGPR *Bacillus sonorensis* enhances growth of brinjal seedlings raised in pro trays

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ABSTRACT

Investigation was conducted in pro trays to evaluate the effect of dual inoculation with the AM fungus *Funneliformis mosseae* + PGPR *Bacillus sonorensis* in enhancing the growth of brinjal seedlings. Different growth parameters like shoot and root length, total seedling length, stem diameter, fresh and dry weight of seedlings, biovolume index, plant strength, vigour index, NPK uptake and mycorrhizal root colonization were monitored. The results brought out that the seedlings treated with the microbial consortium showed significantly improved growth compared to uninoculated seedlings. The increase in biovolume index and dry weight of inoculated seedlings was 110% and 300% more, respectively, compared to uninoculated seedlings. The NPK uptake in inoculated seedlings were 98, 83 and 10% more, respectively, than the uninoculated seedlings

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

INTRODUCTION

Sustainable agriculture aims to reduce chemical fertilizers and increase organic inputs for long-term crop production, which benefits the environment and provides a healthy and natural atmosphere for diversified organisms to live in (Kuila and Ghosh, 2022). This can be achieved by introducing beneficial microorganisms that provide sustainable plant productivity, improve plant nutrient availability, and maintain soil health (Laranjeira *et al.,* 2022; Igiehon and Babalola, 2021).

To meet the growing food demand, producing good quality seedlings is essential for getting good quality crops when planted in the field. Hence, the production and timely distribution of quality seedlings have a greater scope to meet the growing demand. The pro tray nursery is now becoming popular among nurserymen/women to produce quality vegetable seedlings, especially from expensive hybrid seeds under shade net or polyhouse. This technique provides an independent area for each growing seedling and improves seed germination and root development with minimum seedling mortality. Thus it offers healthy, uniform seedlings in a shorter period, easier handling with cheaper transportation and better establishment when transplanted to the field (Bharathi *et al.,* 2014).

Further, this pro tray method helps increase the income of the nurserymen/women and, in turn, the farmer (TNAU, 2014). Substrates like cocopeat, vermiculite, perlite or their mix are commonly used for raising seedlings in pro trays. Inoculating the substrate or seed with beneficial microorganisms is a biotechnological approach for producing healthy and vigorously growing seedlings (Jayashree *et al.,* 2017).

Brinjal or eggplant (*Solanum melongena* L.) is one of India's most popular vegetable crops cultivated on 0.32 million hectares. Traditionally the brinjal seedlings are produced by farmers on raised nursery beds. Still, alternatives are sought to overcome these problems due to expensive hybrid seed material, non-uniform growth of seedlings, higher mortality and incidence of pests and diseases. Thus pro tray nursery technology is now becoming popular.

Arbuscular mycorrhizal (AM) fungi on the growth and phosphate nutrition of various plants, including vegetables, have been studied extensively (Rodrigues and Rodrigues, 2019, Bagyaraj *et al.,* 2022a). All AM fungi are obligate biotrophs, and they benefit plants by increasing uptake of diffusion-limited nutrients like P, Zn, and Cu, protection from pathogens, tolerance to drought, pathogen protection, beneficial alterations of plant growth regulators and synergistic interactions with beneficial soil microorganisms (Raghavendra *et al.,* 2018; Rodrigues and Rodrigues, 2019). Mycorrhizal plants develop extensive root systems compared to non-mycorrhizal plants, which ensures the plant has increased availability of water and nutrients, thereby helping better plant growth and development (Bagyaraj, 2014; Mathimaran *et al.,* 2017). Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that improve plant growth by facilitating the uptake of nutrients, producing phytohormones and by preventing the deleterious effect of phytopathogenic organisms. Co-inoculation of AM fungi with PGPR is more useful in improving plant growth than single inoculation with either because of synergistic interaction, and many studies confirm the same (Giovannini *et al.,* 2020; Laranjeira *et al.,* 2022). This suggests the need to develop a suitable microbial consortium for inoculating different crop plants (Bagyaraj, 2014; Bagyaraj *et al.,* 2022a).

Individual inoculation with AM fungus *Funneliformis mosseae* and PGPR *Bacillus sonorensis* increases the growth of chilli seedlings, and when co-inoculated, further enhancing plant growth has been reported (Thilagar *et al.,* 2016a). The dual inoculation with these two organisms has also been reported to improve the growth and quality of seedlings of a few vegetable crops grown in pro trays (Desai *et al.,* 2020; Bagyaraj *et al.,* 2022b). There are numerous methods for applying microbial inoculants to improve plant growth (Glick, 2012). The pro tray nursery is a recent technology widely gaining popularity for quality seedling production due to better crop establishment when transplanted to the main field (Bisen *et al.,* 2015; Mahmood *et al.,* 2016).

As we know, no information on the production of brinjal seedlings in pro trays with microbial inoculation is available. Hence, it was contemplated to evaluate the ability of the selected AM fungi co-inoculated with PGPR in enhancing the growth of brinjal seedlings raised in pro trays under poly house conditions.

MATERIAL AND METHODS

The present study was conducted at the Centre for Natural Biological Resources and Community Development (CNBRCD), Bengaluru. The brinjal seeds of the variety Suvarna Hybrid were used in the study. *F. mosseae* used in the study was maintained at CNBRCD culture collection in poly house using *Chloris gayana* (Rhodes grass) as the host using vermiculite, soilrite and perlite as the substrate, in the ratio 3:1:1 (v/v/v). Seventy-five days after sowing, the grass was harvested, roots finely chopped, mixed with the substrate and air-dried. This air-dried product served as mycorrhizal inoculum. The PGPR *B. sonorensis* was grown in 200 ml of Luria - Bertani (LB) broth in a conical flask, incubated at 37°C for 24 hours and used for inoculating seedlings in pro trays.

The brinjal seedlings were raised in pro trays with 50 cells. Each cell was filled with 18.5g of the substrate and 1.5 g of vermicompost and mixed well. The experiment had two treatments: (i) uninoculated and (ii) inoculated with the *F. mosseae* + *B. sonorensis*. Three pro trays each with 50 cells, were kept for uninoculated and another three for microbial consortium inoculation. A seeding hole was made in the substrate of each cell, and in each seeding hole, 1g of *F. mosseae* inoculum con-taining 7000 IP/g and 1 ml of *B. sonorensis* containing 14 x 10^8 cfu/ml were added. Two brinjal seeds were sown to each cell of the respective pro trays and thinned to one after germination. Five ml of Ruakura nutrient solution (Smith *et al.,* 1983) with P was applied to each cell 15 days after sowing, and after that, 5ml of the nutrient solution without P was applied once in 7 days to all the cells. The plants were maintained in a polyhouse and watered as and when necessary.

The seedlings were harvested 40 days after sowing. Various plant parameters such as shoot length, root length, seedling length, stem diameter, plant strength, vigour index and biovolume index were determined using appropriate methods outlined by Fernandez *et al.* (2021). The dry weight of the seedlings was determined after drying in an oven at 60°C. The dried seedlings were then powdered, and the concentrations of N, P and K were determined following the standard procedures (Jackson, 1973). Roots were cut into 1cm size and stained using Trypan blue, and the per cent mycorrhizal root colonization was estimated as described by Philips and Hayman (1970). Data were subjected to Students T-test using Assistat 7.7 beta statistical software to determine the significant difference between the treatments at P≤0.05.

RESULTS

The results of the present experiment showed that the germination percentage was 85% in inoculated and 72% in uninoculated treatments. The seedling length and the vigour index of the inoculated seedlings with microbial consortium were significantly higher when compared to the uninoculated seedlings, indicating that the microbial consortium influenced the seedling emergence rate and thus the seedling growth (**Table 1; Fig. 1 & 2**). The height of the inoculated seedlings was significantly higher than the uninoculated seedlings. Unlike uninoculated control, dual inoculation resulted in significantly higher root length and stem diameter. The increase in biovolume index was 110% more in inoculated seedlings than uninoculated seedlings. Inoculated seedlings with *F. mosseae* + *B. sonorensis* had higher fresh and dry weights when compared to the uninoculated seedlings. The increase in dry weight of inoculated seedlings was 300% more compared to uninoculated seedlings. Mycorrhizal root colonization was 80% in inoculated and only 5% in uninoculated seedlings. Inoculation with microbial consortium resulted in 98%, 83%, and 10% increased N, P and K uptake compared to the uninoculated seedlings (T**able 1**).

Table 1: Influence of dual inoculation with *Funneliformis mosseae* + *Bacillus sonorensis* on the growth parameters of brinjal seedlings raised in pro trays 40 days after sowing.

Parameters	Treatment		T-test value*
	Uninoculated control	Inoculated with Bs + Fm	
Shoot length (cm/ seedling) (SL)	5.29	8.22	19.25
Root length (cm/ seedling) (RL)	5.64	11.24	9.08
Seedling length (cm/ seedling) (SL+RL)	10.93	19.46	13.13
Stem girth (mm/ seedling)	1.12	1.51	8.87
Fresh weight (g/ seedling)	0.17	0.70	19.55
Dry weight (g/ seedling)	0.02	0.08	18.22
Biovolume index	5.90	12.37	17.09
Seedling strength (g/ cm^2)	0.003	0.010	13.12
Seedling vigour index	251.57	681.24	22.22
N concentration (%)	1.31	2.59	15.68
P concentration (%)	0.06	0.11	3.06
K concentration (%)	2.11	2.31	6.93
Per cent mycorrhizal root colonization	05	80	17.21

Bs *Bacillus sonorensis*, Fm *Funneliformis mosseae*; *significance level at P≤0.05; DAS days after sowing

DISCUSSION

Increased germination due to inoculation with microbial inoculants has been reported earlier (Paredes-Pa´liz *et al.,* 2016). In the present study, seedlings inoculated with *F. mosseae* + *B. sonorensis* significantly had higher height compared to uninoculated seedlings supporting the observations made in other plants like chilli, tomato, ashwagandha and Kalmegh (Pagano *et al.,* 2007; Dag *et al.,* 2009; Thilagar *et al.,* 2014 and 2016b; Desai *et al.,* 2020). Increased root length, stem diameter and biovolume index have been reported because of dual inoculation with AMF + PGPR in other plants (Arpana and Bagyaraj, 2007; Anuroopa *et al.,* 2017) and also seedlings grown in pro trays (Sukeerthi *et al.,* 2020, Fernandez *et al.,* 2021).

The significant increase in the dry weight of seedlings

Fig. 1: Difference between the growth of uninoculated and inoculated (*F. mosseae* + *B. sonorensis*) brinjal seedlings raised in pro trays 40 days after sowing

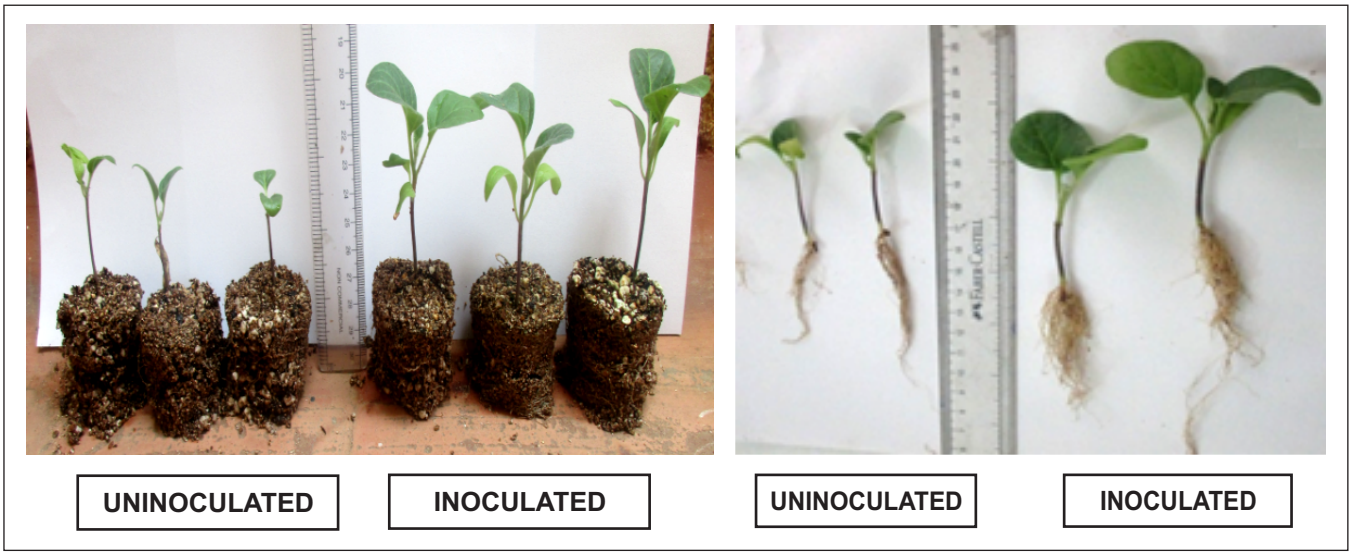

Fig. 2: Comparison of uninoculated and inoculated (*F. mosseae* + *B. sonorensis*) brinjal seedlings.

inoculated with AMF + PGPR is in agreement with earlier reports in crops like capsicum and tomato (Desai *et al.,* 2020) grown in pro trays and also in poly bags like French bean (Hemlata and Bagyaraj, 2015) and basil (Hemavathi *et al.,* 2006). Significantly higher mycorrhizal root colonization of inoculated seedlings indicates the better proliferating ability of *F. mosseae* with brinjal as the host, as reported earlier in China aster and gaillardia (Fernandez *et al.,* 2021). *Funneliformis mosseae* enhancing AM colonization in the French bean and chilli has been reported earlier by Thilagar *et al.* (2014) and Hemlata and Bagyaraj (2015).

B. sonorensis population in the inoculated substrate was 12 x 10^9 cfu/ml. *B. sonorensis* is a PGPR producing phytohormones and protects plants against pathogens (Thilagar *et al.,* 2016a). In the present study, dual inoculation resulted in significant increase in N, P and K uptake compared to the uninoculated seedlings. Few earlier workers have observed similar results of enhanced uptake of nutrients because of inoculation with *F. mosseae* + *B. sonorensis* (Desai *et al.,* 2020). This is perhaps due to the activity of *B. sonorensis,* which increases the root surface area by the production of phytohormones and, in turn, better mycorrhizal root colonization, which improves the uptake of nutrients (Hemlata *et al.,* 2015). AM fungi being obligate symbionts benefit plants by increasing uptake of diffusion-limited nutrients like P, Zn, and Cu, protection from pathogens, tolerance to drought, pathogen protection, beneficial alterations of plant growth regulators and synergistic interactions with beneficial soil microorganisms (Bagyaraj, 2014; Raghavendra *et al.,* 2018; Rodrigues and Rodrigues, 2019). Mycorrhizal plants develop an extensive root system compared to non-mycorrhizal plants, ensuring the plant has increased availability of water and nutrients, thereby helping better plant growth and development (Bagyaraj, 2014; Mathimaran *et al.,* 2017). Dual inoculation of AMF + PGPR promoting nutrient uptake and growth of plants compared to inoculation with either of them is well documented (Hemlata

and Bagyaraj, 2015; Thilagar *et al.,* 2016b; Anuroopa *et al.,* 2017).

In an earlier study, it was found that dual inoculation used in the present study (*F. mosseae* + *B. sonorensis*) applied to the field at the time of transplanting not only enhanced plant growth and yield of chilli but also reduced the application of chemical fertilizer by 50%, which improved soil health and reduced environmental pollution (Thilagar *et al.,* 2016b). In the present study, dual inoculation with *F. mosseae* + *B. sonorensis* was done during sowing in pro trays to produce healthy, vigorously growing seedlings. Recently agricultural scientists/nursery growers have accepted the benefit of raising seedlings of vegetable crops in pro trays rather than in nursery beds. The advantages of this pro tray technology are improved crop uniformity, shorter growing season, efficient use of expensive hybrid seeds, enhanced yield and thus increased net income (Bharathi *et al.,* 2014; TNAU, 2014). This pro tray nursery technology has also offered employment opportunities to landless labourers, especially women (Naresh *et al.,* 2015). Inoculating the substrate with the selected AMF + PGPR has been reported earlier to produce quality vegetable seedlings like tomato and capsicum (Desai *et al.,* 2020).

As far as we know, this is the first report of using this microbial consortium to produce quality brinjal seedlings in the nursery, an important vegetable crop of India. Adding a selected microbial consortium will significantly increase the quality of seedlings raised in pro trays. This simple technology can quickly be followed by the nursery growers for raising healthy, vigorously growing brinjal seedlings which will perform better when transplanted to the field with reduced fertilizer application, which is an approach towards sustainable agriculture. This simple pro tray technology with *F. mosseae* + *B. sonorensis* can be disseminated through training programmes and demonstrations in future.

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CONFLICT OF INTEREST

All the authors of the manuscript declare that they do not have any conflict of interest.

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