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Research Article

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Microbial consortium promotes growth of Zinnia and Balsam seedlings raised in pro trays

D. Sukeerthi[1,2], N. Nikhil Sai[1], R. Ashwin[1], D.J. Bagyaraj[1]*

[1]Centre for Natural Biological Resources and Community Development (CNBRCD), 41 RBI Colony, Anand Nagar, Bengaluru - 560 024, Karnataka, India, [2]St Joseph's College (Autonomous), 36, Lalbagh Road, Bengaluru - 560 027, Karnataka, India

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***Corresponding Author:**
D.J. Bagyaraj
Email: djbagyaraj@gmail.com

ABSTRACT

Zinnia and Balsam are flowering plants with high economic importance in floriculture. Inoculation of the planting medium with a beneficial microbial consortium is an innovative approach to produce quality and healthy seedlings in floriculture. In the present study the influence of a microbial consortium of the arbuscular mycorrhizal fungus (AMF) *Funneliformis mosseae* and a plant growth promoting rhizobacterium (PGPR) *Bacillus sonorensis* on flowering plants Zinnia and Balsam in pro-trays under poly house conditions was investigated. Estimation of various plant growth parameters such as plant height, stem diameter, bio-volume index, vigour index, plant strength, fresh weight, dry weight and nutrient uptake was carried out to analyse the ability of the consortium to improve seedling growth. Microbial parameters such as mycorrhizal root colonization and spore count, and population of PGPR in substrate was also studied. The results suggested that inoculating the substrate in pro trays before sowing the seeds with the consortium increased plant growth significantly compared to the uninoculated plants.

KEYWORDS: *Bacillus sonorensis*, Balsam, *Glomus mosseae*, Pro trays, Zinnia

INTRODUCTION

Sustainable agriculture aims at maintaining soil fertility for a long time and achieving optimized yield using low input [1]. It focuses on producing long term crops and increases the biodiversity by providing a healthy environment for the organisms to live [2]. Biofertilizers are beneficial microorganisms which are introduced to soil to promote better plant growth [3]. Addition of beneficial microorganisms such as nitrogen fixers, phosphate solubilizers, PGPR, AMF etc. is beneficial to the plants and reduces the use of chemical fertilizers [4,5]. There are numerous methods for the application of these beneficial microbes for improving plant growth [6]. The Pro tray nursery is a recent technology widely gaining popularity for quality seedling production. Such seedlings have an independent area for each seedling; hence improved seed germination, better root development, easy handling, cheaper transportation and better establishment of the crop when transplanted in the main field [7,8].

Zinnia belongs to the family Asteraceae. It is used as cut flowers, bedding plants and as companion plants as it attracts wasps and hummingbirds that deter cucumber pests and whiteflies respectively. Balsam belongs to the family Balsaminaceae. It produces beautiful flowers. Further it has useful medicinal properties, such as, its leaves and flowers are used to treat snake bites and skin burns respectively. It is also used to treat gastritis, constipation and also as a hair growth stimulant [9].

Soil harbours a large population of microorganisms. The highest concentration of microbial population is around the roots i.e. the rhizosphere region [10]. This particularly is due to the presence of sugars, amino acids, organic acids etc. in the root exudates [11,12,13]. PGPR are beneficial bacteria in the rhizosphere promoting plant growth by various mechanisms. [14] Direct mechanisms include nitrogen fixation, phosphate solubilisation, production of growth hormones, iron sequestration, etc. Indirect mechanisms include inhibition of plant pathogens through production of antibiotics and siderophores [6]. The PGPR *Bacillus sonorensis* is a Gram-positive motile rod, forming endospores. It forms characteristic brown colonies on tyrosine agar [15].

AMF form symbiotic association with nearly 80% of the plants [16]. They facilitate uptake of diffusion limited nutrients and protect the plants against biotic and abiotic stresses in exchange for photosynthates of the plant [17,18]. Inoculation with AMF improving plant growth is well documented. Dual inoculation with AMF and PGPR significantly improving plant

growth compared to single inoculation with either of them has been reported earlier in other plants [19]. Some workers have pointed out that the effect of the microbial consortium consisting of two or more microorganisms may or may not be greater than their individual effects [20]. The objective of the current work was to evaluate the effect of a microbial consortium consisting of AMF *Funneliformis mosseae* and the PGPR *Bacillus sonorensis* on the growth of flowering plants Zinnia and Balsam raised in pro trays under poly house conditions.

MATERIALS AND METHODS

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

Inoculum Preparation

Sub-culturing of *B. sonorensis* was done on Luria-Bertani (LB) agar plates and incubated at 37°C for 24 hours. A single colony from the sub-cultured plate was inoculated into 500ml of LB broth and incubated at 37°C for 24 hours. This was used for inoculation of the substrate in pro-trays. The bacterial population was enumerated by performing serial dilution of the culture and plating onto LB agar. *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 MPN method with 10-fold dilution [21,22].

Experimental Setup

The cells of the pro trays were filled with 20g of the substrate described above. There were two treatments for each plant type, 100 cells of two pro trays (each with 50 cells) served as uninoculated control and 100 cells of two pro trays served as inoculated treatment. A planting hole was made in the substrate and 1g of *F. mosseae* inoculum (containing 2.2×10^3 IP/g) and 2ml of *B. sonorensis* inoculum (containing 1.9×10^5 cfu/ml) was added. The seeds were sown and watered regularly. Five ml of Ruakura nutrient solution without P was added to all the cells once in 10 days starting from 20 days after sowing [23].

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. Stem diameter was measured 1cm above the substrate. Root length and fresh weight of the plants were determined. The bio-volume index was calculated using the formula given by Hatchell [24]. The seed vigour was calculated using the standard formula [25]. The plant strength was calculated using a formula given by Maskina [26]. The samples were dried in a hot air oven at 60°C after which the dry weight was determined. The samples were then powdered and the nitrogen concentration was determined by Micro Kjelhdahl method [27]. Phosphorus concentration was estimated by vanadomolybdate phosphoric yellow colour method [28]. Potassium concentration was determined by Flame photometer method [29]. The

Table 1: Influence of microbial consortium on plant growth parameters of Zinnia and Balsam raised in pro trays 60 DAS

Growth parameters	Zinnia			Balsam		
	Uninoculated control	Inoculated	T-test value	Uninoculated control	Inoculated	T-test value
Shoot length (cm)	12.66	24.19	5.43**	9.58	15.33	12.66**
Root length (cm)	10.02	13.39	4.61**	6.84	13.91	16.39**
Stem diameter (mm)	1.74	2.19	6.19**	2.61	4.01	10.18**
Bio-volume index	39.68	82.25	11.80**	43.71	117.66	13.19**
Plant strength	0.006	0.009	2.08*	0.005	0.007	6.39**
Vigour index	1100.71	2465.65	9.86**	1444.5	2694.8	9.07**
Number of flowers	18	96	5.71**	-	-	-
Fresh weight (g)	0.49	1.77	4.57**	0.94	2.77	12.22**
Dry weight (g)	0.12	0.33	12.07**	0.08	0.20	10.84**

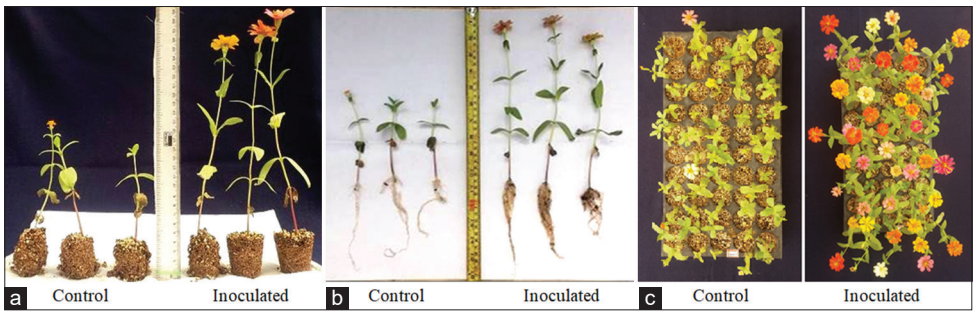

Figure 1: Effect of the microbial consortium on (a) shoot length, (b) root length and (c) flowering of Zinnia 60 DAS

micronutrient analysis of the samples was performed using atomic absorption spectrophotometer with a hallow cathode lamp set to standard wavelengths [30]. 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 Philips and Hayman [31]. The AM spore number in the substrate was determined by wet-sieving and decantation method [32]. The *B. sonorensis* population in the substrate was enumerated by serial dilution and plating onto LB agar plates. [33] Data was subjected to T-test at a significance level ($P \leq 0.05$).

RESULTS AND DISCUSSIONS

There was an increase in shoot length, root length and stem diameter of zinnia and balsam when treated with the microbial consortium (Table 1). The plants treated with the microbial consortium showed a significant increase in the bio-volume index, plant strength and vigour index when compared to the uninoculated plants indicating that they grow more vigorously (Table 1). There was a significant increase in the fresh weight and dry weight of zinnia and balsam treated with the microbial consortium when compared to the control (Table 1 and Figures 1 and 2). Microbial consortium of *F. mosseae* + *B. sonorensis* added to the substrate in pro-trays enhancing seedling height, stem diameter, biovolume index, plant strength, vigour index and dry weight has been reported earlier in crops like tomato and capsicum [34]. There was also an increase in the number of flowers of zinnia in the inoculated plants compared to uninoculated plants in a span of 60 days (Table 1 and Figure 1). This indicated that the microbial consortium also induced flowering in zinnia. There are earlier reports that AMF and PGPR inoculation induced early flowering in some plants [35-36]. In balsam there was no flowering on 60 DAS. Inoculation of zinnia raised in pots with other AMF or PGPR have been reported to improve plant growth by earlier workers [37-39]. There was a significant difference between inoculated and uninoculated plants in the uptake of macro and micro nutrients in both zinnia and balsam (Table 2). The inoculated plants had 99% mycorrhizal root colonization while the uninoculated zinnia and balsam had only 6 and 4% colonization respectively. The mycorrhizal spore numbers were absent in the uninoculated treatment while it was 21 and 35 per 50g of substrate in zinnia and balsam respectively (Table 3). The population of *B. sonorensis* in the substrate was encountered only in the inoculated treatments (Table 3.). It can be concluded that the inoculation with microbial consortium consisting of AMF + PGPR resulted in improved growth of zinnia and balsam. This supports the work done by earlier workers in other plants under pot culture or field conditions [40-42]. There are very few reports on using microbial consortium in pro trays for enhancing seedling growth of vegetable crops [34, 43-48]. Perhaps this is the first report on plants important in floriculture. Recently nurserymen, scientists and farmers have

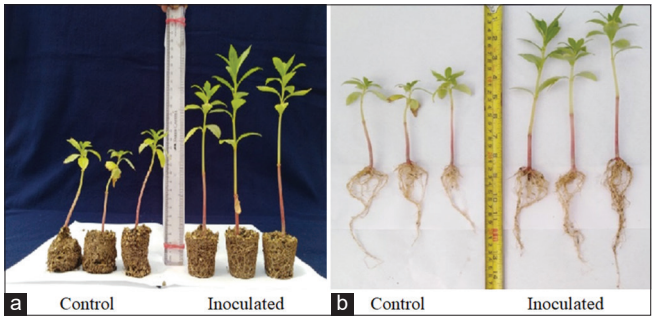

Figure 2: Effect of the microbial consortium on (a) shoot length and (b) root length of Balsam 60 DAS

Table 2: Effect of microbial consortium on the nutrient uptake of Zinnia and Balsam raised in pro trays 60 DAS

Nutrients	Zinnia			Balsam		
	Uninoculated control	Inoculated	T-test value	Uninoculated control	Inoculated	T-test value
Nitrogen (N) (%)	1.57	1.61	7.21**	1.74	1.84	6.54**
Phosphorus (P_2O_5) (%)	0.15	0.23	9.83**	0.04	0.14	7.32**
Potassium (K_2O) (%)	1.93	3.88	9.65**	1.57	3.60	10.54**
Calcium (Ca) (%)	1.78	2.74	3.32**	2.51	3.27	5.67**
Magnesium (Mg) (%)	0.99	1.69	7.17**	1.23	1.74	5.15**
Zinc (Zn) (ppm)	54.64	70.06	8.44**	114.4	125.4	3.72**
Copper (Cu) (ppm)	44.88	60.44	13.67**	66.62	79.07	6.56**
Manganese (Mn) (ppm)	68.54	329	11.33**	95.5	109.8	4.91**
Boron (B) (ppm)	68.03	113.5	13.86**	69.63	73.68	13.81**
Molybdenum (Mo) (ppm)	70.69	74.71	6.12**	85.76	94.66	12.80**
Iron (Fe) (ppm)	2689	2994	6.84**	3526	3984	12.74**

Table 3: Effect of microbial consortium on the mycorrhizal root colonization and spore count, and *B. sonorensis* population in the substrate of Zinnia and Balsam raised in pro trays 60 DAS

	Zinnia			Balsam		
	Uninoculated control	Inoculated	T-test value	Uninoculated control	Inoculated	T-test value
Percent mycorrhizal colonization	6	99.99	4.74**	4	99.99	3.52**
Spore number/ 50g of substrate)	0	21	3.28**	0	35	2.84**
Bacillus sonorensis (CFU/ g of substrate)	0	1.95×10^4	3.12**	0	1.5×10^4	3.83**

accepted the benefits of pro tray technology for raising crops like tomato, chilly, capsicum etc. The present study brings out that this technology can be extended to crops important in floriculture also.

CONCLUSION

The results obtained from this experiment brought out that the microbial consortium of *F. mosseae* + *B. sonorensis* is beneficial to the growth of flowering plants zinnia and balsam raised in pro trays. The results indicated that inoculation with microbial consortium helped to enhance germination, vigour index, plant strength, plant growth and nutrient uptake. The inoculated AMF and PGPR showed rhizosphere competence as evidenced by enhanced numbers in the substrate. Inoculation of the planting medium with beneficial microbial consortium is a biotechnological approach for producing healthy, vigorously growing seedlings. This technology can be used in floriculture nurseries for production of quality seedlings. It will also fetch higher income to the nurserymen.

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