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Inoculation with Selected Microbial Consortia Promotes Growth of Chilli and Basil Seedlings Raised In Pro Trays

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Abstract

Chilli is an important commercial crop with high economic importance and basil is an important medical plant used in ayurvedic medicine with many pharmacological uses. Inoculation of the planting medium with beneficial microbial consortia is an innovative approach to produce healthy and quality seedlings in horticulture. The present investigation was carried out to determine the influence of the two microbial consortia *Bacillus sonorensis* + *Funneliformis mosseae* on chilli and *Pantoea dispersa* + *Funneliformis monosporus* on basil seedlings (based on earlier studies) grown in pro trays under polyhouse conditions. The plant growth parameters such as length of shoot, stem diameter, biovolume index, plant strength, vigour index, dry weight and nutrient uptake were analyzed 60 days after sowing. The results brought out that the growth of inoculated seedlings was significantly more compared to uninoculated seedlings. It was concluded that inoculating the substrate in pro trays with selected microbial consortia results in producing healthy, vigorously growing chilli and basil seedlings.

Keywords: *Bacillus sonorensis; Capsicum annuum; Funneliformis monosporus; Funneliformis mosseae; Ocimum basilicum; Pantoea dispersa*

Abbreviations: PGPR: Plant Growth Promoting Rhizobacteria; AMF: Arbuscular Mycorrhizal Fungi; CNBRCD: Centre for Natural Biological Resources and Community Development; LB: Luria Bertani; MNG: Modified Nutrient Glucose; IP: Infective Propagule; MPN: Most Probable Number; DAS: Days After Sowing.

Introduction

Capsicum annuum L. (chilli) is one of the commercial spice crops belonging to the family Solanaceae and is grown globally with a production of 45.0 million tons annually [1]. India is one of the global leader in chilli production and its chilli is famous for two important commercial qualities viz. its colour and pungency level [2,3]. Recently it is gaining importance in the global trade market because of its value-added products like chilli powder, oleoresin, capsanthin and many others [4]. *Ocimum basilicum* L. (sweet basil) belongs to the family Lamiaceae (mint family) and is cultivated throughout South-East Asian tropics. Basil is one of the most important medicinal plants as the leaves, stem and roots are used in ayurvedic preparations and the essential oil 'eugenol' extracted from basil exhibits antimicrobial, anti-inflammatory and anticancer activities [5,6]. In India it is cultivated over an area of 25,000 hectares. Sustainable agriculture aims at maintaining soil fertility for a long time and achieving optimized yield using low input [7]. It focuses on increasing the soil biodiversity by providing a healthy environment for the organisms to live [8]. Microbial diversity in soil include variety of bacteria, fungi and algae including N-fixing and P-solubilizing organisms, pesticide degraders, mycorrhizal fungi, etc. [9,10]pushed by the need for high

productivity, have stimulated the intensive use of pesticides and fertilizers. Unfortunately, negative effects on water, soil, and human and animal health have appeared as a consequence of this indiscriminate practice. Biofertilizers are beneficial microorganisms which are introduced to soil to promote better plant growth [11]. Addition of organisms such as N-fixers, P and K-solubilizers in addition to promoting plant growth also reduces the application of chemical fertilizers [12,13]. Many studies have reported that plant growth promoting rhizobacteria (PGPR) promotes plant growth by solubilizing the phosphorus and potassium, fixing and enhancing uptake of nitrogen, producing phytohormones, antibiotics, siderophores, HCN, etc. thus protecting plants against pathogens [14]. Arbuscular mycorrhizal fungi (AMF) produce and form network of hyphae which draw diffusion limited nutrients from the soil and supplies it to the plants and also protect plants from soil-borne diseases and adverse environmental conditions [15]. The AMF and PGPR mostly have synergistic interaction and thus dual inoculations with both of them promote plant growth better than single inoculation with either of them. Therefore a microbial consortium is recommended now-a-days for inoculating crop plants which not only improves plant growth but also the soil quality [16].

There are numerous methods for the application of these microbial consortia for improving plant growth [17]. 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 to the main field [18,19]. Few studies brought out that inoculating the substrate in pro trays with selected microbial consortia resulted in healthy vigorously growing quality seedlings [20,21]. Our earlier studies have shown that using the selected microbial consortia *Bacillus sonorensis* + *Funneliformis mosseae* is the best for promoting the growth and yield of chilli [22] and *Pantoeae dispersa* + *Funneliformis monosporus* is the best for inoculating basil [23] under field conditions. The objective of the current work is to evaluate the effect of the selected microbial consortia for chilli and basil on the growth of these seedlings raised in pro trays under polyhouse conditions so that it becomes a nursery technology in future.

Material and Methods

The experiment was conducted at Centre for Natural Biological Resources and Community Development (CNBRCD), Bengaluru, India. The chilli seeds (*Capsicum annuum* L. variety Arka Gagan) used in the study was procured from the Indian Institute of Horticulture Research, Bengaluru and seeds of basil (*Ocimum basilicum* L. variety CIM-Saumya) was procured from Central Institute of Medicinal and Aromatic Plants, Bengaluru, India.

Inoculum Preparation

The pure cultures of PGPR *Bacillus sonorensis* and *Pantoea dispersa*, for inoculating chilli and basil respectively, were sub-cultured on Luria Bertani (LB) agar and Modified Nutrient Glucose (MNG) agar respectively. The plates were incubated at 37°C for 24 hours. A single colony was picked from each subcultured plate and inoculated into 300ml LB broth and 300ml MNG broth respectively and incubated at 37°C for 48 hours. Fully grown broth cultures were cold centrifuged and the bacterial pellets collected separately were mixed in dilute phosphate buffer and used as inoculum. The population of B. sonorensis and *P. dispersa* were 1.9×10^8 CFU/ ml and 2×10^8 CFU/ ml of inoculum respectively which was estimated by serial dilution and plating method [24,25]. The AMF *F. mosseae* and *F. monosporus*, for inoculating chilli and basil respectively, were multiplied using 'Pot Culture' technique in plastic pots under glasshouse conditions using vermiculite, perlite and soilrite in the ratio of 3:1:1 (v/v/v) as the substrate and Rhodes grass (*Chloris gayana* Kunth) as the host. After 75 days of growth, shoots of Rhodes grass were severed and the substrate containing spores, hyphae and root bits (cut into about 1 cm pieces) was air dried and used as the inoculum. The infective propagule (IP) numbers of the AMF cultures was estimated by the most probable number (MPN) method as described by Porter [24]. The IP numbers of *F. mosseae* and *F. monosporus* was 7000/ g and 9000/ g of substrate.

Experimental Setup

The experiment was performed in plastic pro trays containing 50 cells. Each cell of the pro tray was filled with 18.5g of the substrate mentioned above and 1.5g of vermicompost and mixed well. There were two treatments for each plant type. Hundred cells of two pro trays served as uninoculated control and 100 cells of two pro trays served as inoculated treatment for each plant under study. A planting hole was made in the substrate and 1g of *F. mosseae* inoculum and 2ml of *B. sonorensis* inoculum was added before sowing chilli. Similarly 1g of *F. monosporus* and 2 ml of *P. dispersa* was added before sowing basil. The pro trays were maintained in a polyhouse 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 (DAS) [26].

Parameters Evaluated

The various plant parameters such as shoot, root and seedling length, and stem diameter were determined just before harvesting the seedlings 60 DAS. Shoot length was

measured from the substrate surface to the tip of the plant. Stem diameter was measured 1cm above the substrate using Vernier calipers. The bio-volume index was calculated using the formula given by Hatchell [27]. The seedling vigour index was calculated using the standard formula [28]. The plant strength was calculated using the formula given by Maskina, et al. [29]. Root length and fresh weight of the plants were determined. 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 [30]. Phosphorus concentration was estimated by Vanadomolybdate phosphoric yellow colour method [30]. Potassium concentration was determined by Flame photometer method [31]. The micronutrient analysis of the samples was performed using atomic absorption spectrophotometer with a hallow cathode lamp set to standard wavelengths [32]. 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 [33]. The AM spore number in the substrate was determined by wet-sieving and decantation method [34]. The *B. sonorensis* and *P. dispersa* population in the substrate was enumerated by serial dilution and plating on LB agar and MNG agar plates respectively [35]. Dehydrogenase activity in the substrate was determined by the method outlined by Casida, et al. [36]. The data was subjected to T-test at a significance level of $P \leq 0.05$ to determine the effect of microbial consortia on chilli and basil seedlings.

Results and Discussion

It was observed that the shoot length, root length, stem diameter, fresh and dry weight (Table 1) of chilli and basil seedlings inoculated with the selected microbial consortia were significantly higher compared to the uninoculated seedlings. Inoculation of microbial consortia to the chilli and basil seedlings increased the bio volume index (Table 1) which indicates the total volume of the seedlings was more in inoculated than the uninoculated seedlings. Vigour index of seedlings inoculated with microbial consortia was significantly higher than uninoculated seedlings representing an elevated rate of germination of seeds. Increased plant strength was noticed in seedlings inoculated with microbial consortia in comparison with uninoculated seedlings, bringing out that the quality of inoculated seedlings is significantly superior.

Parameter	Chilli			Basil		
	Uninoculated control	Inoculated	T-test Value*	Uninoculated control	Inoculated	T-test Value*
Shoot Length (cm)	4.26	5.09	4.5	19.68	25.18	19.2
Root Length (cm)	3.31	4.68	2.4	9.06	12.14	4.7
Stem Diameter (mm)	0.93	1.47	10.2	1.68	2.51	15
Fresh Weight (g)	19.09	35.12	24.9	94.42	124.19	5.6
Dry Weight (g)	8.49	10	8.1	19.04	22.27	2.8
Bio-volume Index	4	7.61	9.5	33.25	63.29	20.3
Vigour Index	356.2	752	5.2	1791.2	2984	11.9
Plant Strength (dry matter/ unit area)	0.94	1.4	7.2	0.46	0.65	9.4
Mycorrhizal spore Count (number / 50g)	3	72	33.1	2	94.4	23.6
Mycorrhizal root olonization %	7.3	65.33	19.2	5.33	88	16.3
Dehydrogenase (µg of TPF released/ g of soil/ hr)	280	760	20.3	350	950	22.2
Bacillus sonorensis (CFU/ g) Pantoea dispersa (CFU/ g)	1.2×10^2	2.4×10^4	14.4	1×10^2	2.2×10^4	14.4

*T- test value significant at P value (<0.05)

Table 1: Influence of microbial consortia on growth of chilli and basil seedlings raised in pro trays.

PGPR like *B. sonorensis* and *P. dispersa* are known to possess traits such as IAA, siderophore and HCN production and P-solubilization, promoting plant growth [37,38]. AMF are known to improve plant growth by increased uptake

of diffusion limited nutrients like P, Zn, Cu, etc. and also through the production of phytohormones, synergistic interaction with other beneficial soil organisms and their role in alleviation of biotic and abiotic stresses [7]. Microbial consortia of *F. mosseae + B. sonorensis* added to the substrate in pro trays of chilli enhancing seedling height, stem diameter, biovolume index, plant strength, vigour index and dry weight has been reported earlier in seedlings of tomato and capsicum raised in pro trays [20]. Dual inoculation with the *F. monosporus + P. dispersa* improving growth on *Ocimum tenuiflorum* (holy basil) under pot culture and field conditions has been reported earlier [23,39]. The nutrient analysis showed that there is a significant difference in uptake of nitrogen, phosphorus, potassium and calcium in chilli and basil seedlings treated with the microbial consortia compared to uninoculated seedlings (Table 2). It was observed that there is a striking increase in the uptake of zinc, copper, manganese, molybdenum and iron in both the seedlings treated with microbial consortia compared with uninoculated seedlings (Table 2). This is mainly due to the activity of PGPR, which changes the morphology of roots by the phytohormones that are synthesized which result in an increased root surface area and in turn increased colonization and uptake of nutrients by AMF [35]. The dehydrogenase activity in the substrate of inoculated chilli and basil was 2.7 times more compared to uninoculated substrate. This suggests significant increase in the overall metabolic state and activity of microorganisms in the substrate of inoculated seedlings compared to uninoculated seedlings [40].

	Parameters	Chilli			Basil		
		Control	Inoculated	T-test value*	Control	Inoculated	T-test value*
Macronutrients (%)	Nitrogen	3.4	4.83	22	2.33	3.49	16
	Phosphorus	3.29	4.17	18	2.37	3.19	12
	Potassium	2.63	2.89	15	1.6	2.19	17
	Calcium	1.45	1.57	8.9	2	2.32	14
	Magnesium	1.04	1.92	17	0.88	0.91	1.7
Micronutrients (ppm)	Zinc	43.42	74.3	17	52.67	55.22	14
	Copper	110.9	146.5	25	129	138.3	9
	Manganese	18.27	100.8	23	15.56	20.54	7.4
	Boron	44.2	34.41	16	29.93	32.11	9.9
	Molybdenum	45.07	280.8	11	38.64	43.72	10.9
	Iron	1516	11450	26.8	1509	1645	12

*T- test value significant at P value (<0.05)

Table 2: Influence of microbial consortia on macro and micro nutrients uptake of chilli and basil seedlings raised in pro trays.

Mycorrhizal root colonization in the inoculated chilli and basil seedlings were 65% and 88% compared to uninoculated seedlings with 7% and 5% respectively. Similarly mycorrhizal spore numbers in the substrate of inoculated chilli and basil were 72 and 94 per 50g in contrast to 3 and 2 per 50g in uninoculated seedlings respectively. The increased mycorrhizal root colonization and spore numbers in the inoculated plants indicate the compatibility and host preference of the respective AMF for the 2 crop plants used in the present study. This is in conformity with the earlier reports made in flowering ornamentals raised in pro trays [41]. The CFU of *B. sonorensis* in the substrate of chilli and that of *P. dispersa* in the substrate of basil were 200 and 220 times more respectively in inoculated seedlings compared to uninoculated seedlings. This finding supports the earlier report observed in tomato and capsicum [20]. The results of the present study brings out that inoculation of the substrate with the microbial consortia viz., *F. mosseae + B. sonorensis* for chilli and *F. monosporus + P. dispersa* for basil significantly enhanced the growth, nutrition, development and quality of seedlings raised in pro trays. This simple inoculation technology can easily be followed by nurserymen to produce vigorously growing chilli and basil seedlings which will fetch them higher income. As far as we are aware this is the first report of raising any medicinal plant seedlings in pro trays with the selected microbial consortia.

Author Contribution

LS – Data collection and processing; DJB – Materials, concept, design and critical review; NSN –Literature search and analysis; AR – Writing manuscript.

References

1. FAO (2020) World Food and Agriculture - Statistical Yearbook 2020.

2. Kanchana D, Jayanthi M, Usharani G, Saranraj P, Sujitha D (2014) Interaction effect of combined inoculation of PGPR on growth and yield parameters of chilli var k1 (*Capsicum annuum* L.). International Journal of Microbiology Research 5(3): 144-151.

3. Geetha R, Selvarani K (2017) A study of chilli production and export from India. IJARIIE 3(2): 205-210.

4. Gowtham HG, Murali M, Singh SB, Lakshmeesha TR, Murthy KN, et al. (2018) Plant growth promoting rhizobacteria- *Bacillus amyloliquefaciens* improves plant growth and induces resistance in chilli against anthracnose disease. Biological Control 126: 209-217.

5. Gupta SK, Prakash J, Srivastava S (2002) Validation of traditional claim of Tulsi, Ocimum sanctum Linn. as a medicinal plant. Indian J Exp Biol 40(7): 765-773.

6. Manaharan T, Thirugnanasampandan R, Jayakumar R, Kanthimathi MS, Ramya G, et al. (2016) Purified essential oil from *Ocimum sanctum* Linn. Triggers the apoptotic mechanism in human breast cancer cells. Pharmacogn Mag 12(Suppl 3): S327-S331.

7. Bagyaraj DJ (2020) Microbial Biotechnology for Sustainable Agriculture, Horticulture & Forestry. New India Publishing Agency, India.

8. Altieri MA (2018) Agroecology: The Science of Sustainable Agriculture. CRC Press, USA.

9. Vandenberghe LPDS, Garcia LMB, Rodrigues C, Camara MC, Pereira GVDM, et al. (2017) Potential applications of plant probiotic microorganisms in agriculture and forestry. AIMS Microbiology 3(3): 629-648.

10. Raklami A, Bechtaoui N, Tahiri AI, Anli M, Meddich A, et al. (2019) Use of rhizobacteria and mycorrhizae consortium in the open field as a strategy for improving crop nutrition, productivity and soil fertility. Frontiers in Microbiology 10: 1106.

11. Vessey JK (2016) Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255: 571-586.

12. Havlin JL, Tisdale SL, Nelson WL, Beaton JD (2016) Soil Fertility and Fertilizers, 8th(Edn.), Pearson Education, India.

13. Bagyaraj DJ, Jamaluddin (2017) Microbes for Restoration of Degraded Ecosystems. New India Publishing Agency, India.

14. Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. World J Microbiol Biotechnol 28(4): 1327-1350.

15. Kehri HK, Akhtar O, Zoomi I, Pandey D (2018) Arbuscular mycorrhizal fungi: taxonomy and its systematics. International Journal of Life Sciences Research 6(4): 58-71.

16. Nanjundappa A, Bagyaraj DJ, Saxena AK, Kumar M, Chakdar H (2019) Interaction between arbuscular mycorrhizal fungi and *Bacillus* spp. in soil enhancing growth of crop plants. Fungal Biology and Biotechnology 6: 1-10.

17. Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica (Cairo) 2012: 963401.

18. Bisen K, Keswani C, Mishra S, Saxena A, Rakshit A, et al. (2015) Unrealized potential of seed biopriming for versatile agriculture. In: Rakshit A, (Eds.), Nutrient Use Efficiency: from Basics to Advances, Springer, New Delhi, pp: 193-206.

19. Mahmood A, Turgay OC, Farooq M, Hayat R (2016) Seed biopriming with plant growth promoting rhizobacteria: a review. FEMS Microbiology Ecology 92(8).

20. Desai S, Bagyaraj DJ, Ashwin R (2020) Inoculation with microbial consortium promotes growth of tomato and capsicum seedlings raised in pro trays. Proceedings of the National Academy *of* Sciences, India, Section B: Biological Sciences 90: 21-28.

21. Fernandez M, Nachu NS, Revanna A, Bagyaraj DJ (2021) Influence of microbial consortium in the production of China aster and gaillardia seedlings. Journal of Horticultural Research 28(2): 21-28.

22. Thilagar G, Bagyaraj DJ, Rao MS (2016) Selected microbial consortia developed for chilly reduces application of chemical fertilizers by 50% under field conditions. Scientia Horticulturae 198: 27-35.

23. Jyothi E, Bagyaraj DJ, Rao EVSP (2018) Microbial consortia developed for *Ocimum tenuiflorum* reduces application of chemical fertilizers by 50% under field conditions. Medicinal Plants - International Journal of Phytomedicines and Related Industries 10(2): 138-144.

24. David AB, Davidson CE (2014) Estimation method for serial dilution experiments. J Microbiol Methods 107: 214-221.

25. Porter WM (1979) The 'Most Probable Number' method for enumerating infective propagules of vesicular arbuscular mycorrhizal fungi in soil. Soil Research 17(3): 515-519.

26. Smith GS, Johnston CM, Cornforth IS (1983) Comparison of nutrient solutions for growth of plants in sand culture. New Phytolo 94: 537-48.

27. Hatchell GE (1985) Production of bare root seedlings. Proceedings of the 3rd Biology South S.I. Research Conference, pp: 395-402.

28. Baki AAA, Anderson JD (1973) Vigor determination in soybean seed by multiple criteria. Crop Science 13(6): 630-633.

29. Maskina MS, Meelu OP, Roberts DL (1984) Effect of organic and inorganic manuring on rice nurseries. International rice research newsletter 9: 265-75.

30. Jackson ML (1973) Soil Chemical Analysis, Prentice-Hall of India Private Limited M-97. New Delhi, India.

31. Barnes RB, Richardson D, Berry JW, Hood RL (1945) Flame photometry a rapid analytical procedure. Ind Eng Chem Anal Ed 17(10): 605-11.

32. Manjeet A (2018) Experiment-32 Determination of Copper, Zinc, Lead and Cadmium in Food Products by Atomic Absorption Spectroscopy. IGNOU, India.

33. Philips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55(1): 158-161.

34. Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society 46(2): 235-244.

35. Johnson LF, Curl EA (1972) Methods for Research on the Ecology of Soil-borne Plant Pathogens. Burgess Publishing Co, Minneapolis, pp: 247.

36. Casida LE, Klein DA, Santoro T (1964) Soil dehydrogenase activity. Soil Sci 98(6): 371-376.

37. Kulkarni GB, Nayak AS, Sajjan SS, Oblesha A, Karegoudar TB (2013) Indole-3-acetic acid biosynthetic pathway and aromatic amino acid aminotransferase activities in *Pantoea dispersa* strain GPK. Lett Appl Microbiol 56(5): 340-347.

38. Thilagar G, Bagyaraj DJ, Podile AR, Vaikuntapu PR (2018) *Bacillus sonorensis*, a novel plant growth promoting rhizobacterium in improving growth, nutrition and yield of chilly (*Capsicum annuum* L.). Proceedings of the National Academy *of* Sciences, India, Section B, 88: 813-818.

39. Jyothi E, Bagyaraj DJ (2017) Inoculation with microbial consortia enhances the growth, nutrition and oil concentration of *Ocimum sanctum*. Medicinal Plants - International Journal of Phytomedicines and Related Industries 9(4): 237-241.

40. Meena KK, Sorty AM, Bitla UM, Choudhary K, Gupta P, et al. (2017) Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. Front Plant Sci 8: 172.

41. Sukeerthi D, Sai NN, Ashwin R, Bagyaraj DJ (2020) Microbial consortium promotes growth of zinnia and balsam seedlings raised in pro trays. Journal of Floriculture and Landscaping 6: 4-8.