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Enhancement of seedling quality in small cardamom (*Elettaria cardamomum* Maton.) through inoculation of VAM fungi and liquid microbial inoculants as PGPR

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ABSTRACT

An experiment was carried out to evaluate the effect of liquid microbial inoculants on quality seedlings production in small cardamom at ZAHRS, Mudigere, Karnataka during 2020-21with nine treatments and three replications in CRD. The results of study revealed that, among the different treatments studied, combined inoculation of liquid PGPR (*Azotobacter chroococcum, Bacillus megaterium, Bacillus mucilaginosus*) and VAM recorded significantly maximum pseudostem height, pseudostem girth, number of leaves, leaf area, leaf area index, total chlorophyll content, total dry matter production, number of primary and secondary roots, root length, root thickness, root volume, total dry matter production, tillering percentage, Dickson quality index and volume index at 150 days after inoculation in the secondary nursery. Similarly, significantly higher microbial population in terms total bacteria, fungi and actinomycetes were observed in the nursery media with conjuctive use of liquid microbial inoculants and VAM.

Key words *: Azotobacter chroococcum, Bacillus megaterium, Bacillus mucilaginosus, VAM, PGPR, Liquid microbial inoculants.*

Introduction

Small cardamom (*Elettaria cardamomum* Maton) belongs to the family Zingiberaceae. It is originated from Western Ghats of South India and is a rich spice obtained from the seeds. Cardamom is used in three forms *viz*., whole, decorticated seeds and ground as a spice and flavouring agent in various food, confectionary, beverages and liquors. Cardamom is a perennial spice crop, propagated sexually through seeds and vegetatively by suckers and tissue culture. But, the seed propagation is commercially preferred over asexual methods to overcome viral infections as they easily transmit through suckers.

Area under small cardamom is increasing due to better market price for dried capsules. Productive period for cardamom is only 12 to 15 years. Later it needs to be replanted with fresh seedlings after its economic life span. Hence, there is a greater demand for quality planting materials. The application of liquid PGPR at nursery stage in single or combination to boost the seedling growth and vigour. When applied to seed, plant surfaces or soil which colonizes

the rhizosphere or the interior of the plant and promotes growth by increasing the availability of primary nutrients to the host plant and also by secreting plant hormones.

Liquid PGPR formulation is the promising and updated technology than the carrier based biofertilizers. Liquid PGPR has many advantages over carrier based inoculants. The shelf life is the first and foremost problem of the carrier based biofertilizers which is up to three months and it does not retain throughout the crop cycle, liquid PGPR on the other hand facilitates the long survival of the organisms by providing the suitable medium which is sufficient for the entire crop cycle. Carrier based bio fertilizers (CBBF) are not so tolerant to the temperature which is mostly unpredictable and uncertain in the crop fields while temperature tolerance is the other advantage of the liquid PGPR. Moisture retaining capacity of the CBBF is very low which does not allow the organism viable for longer period and the liquid PGPR facilitates the enhanced viability of the organism. Hence, the present investigation was undertaken to study the effect of liquid microbial inoculants on growth of cardamom seedlings in the secondary nursery.

Materials and Methods

Seed collection and primary nursery preparations

Fully matured bold capsules from high yielding and disease-free mother clumps were collected at second harvest during the month of September. The seeds were extracted by gently pressing the capsules and then washed 3- 4 times in cold water to remove the mucilage adhering to the seeds. After that, the acid scarification with 25 per cent nitric acid for 10 minutes was carried out to ensure the higher germination percentage. After the acid treatment, the seeds were washed repeatedly in cold water to remove traces of acid. The washed seeds were shade dried for 24 hours and broadcasted on the beds of 6 × 1 m under low cost polyhouse. Germination commenced in about 20-25 days and was allowed to grow for a month in primary nursery

Source of planting material and microbial inoculants

Seedlings collected from ZAHRS, nursery (Variety: Mudigere-2) and liquid microbial inoculants were procured from the Department of Agricultural Microbiology, KSNUAHS, Shivamogga was used for the study.

Transplanting of seedlings in secondary nursery

The seedlings at three to four leaf stage in the primary nursery were transplanted in polybags (6 x 6 inches) containing 0.75 kg of potting mixture consisting of forest soil: sand: Farm Yard Mannure (FYM) @ 3:1:1. The media used for study was analyzed to record the initial chemical properties and which is presented in Table 1.

Treatment details

T_1: Control (Forest soil: Sand: FYM @ 3:1:1); **T_2:** VAM; T_3: *Azotobacter chroococcum* + VAM; **T_4:** *Bacillus megaterium* + VAM; **T_5:** *Bacillus mucilaginosus* + VAM; T_6 *Azotobacter chroococcum* + *Bacillus megaterium* + VAM; **T7:** *Azotobacter chroococcum* + *Bacillus mucilaginosus* + VAM; **T8:** *Bacillus megaterium* + *Bacillus mucilaginosus* + VAM **T9:** *Azotobacter chroococcum* + *Bacillus megaterium* + *Bacillus mucilaginosus* + VAM.

Microbial inoculation

The liquid microbial inoculants diluted @1:10 with clean tap water. The seedlings were inoculated with diluted liquid microbial inoculants at the rate of 50 ml per seedling as per the treatments while, Vesicular Arbuscular Mycorrhizae (VAM) applied at the rate of 5 g per seedling. The treated seedlings were maintained under 50 per cent shade net up to 150 days after inoculation

Observations recorded

The observations were recorded on seedling growth, root and quality parameters, chemical properties and nutrient status of the nursery media and microbial population at 150 days after inoculation. The mean value of the data recorded was taken to represent a particular treatment with respect to a character.

Quality parameters

Tillering percentage

The side shoots arising from the basal portion of seedling was counted and their mean values were computed as below

$$\text{Tillering percentage} = \frac{\text{Number of plants initiated tillering}}{\text{Total number of plants used for treatments}} \times 100$$

Dickson Quality index (DQI)

The quality index of seedling was calculated empirically by adopting the formula derived by Ritchie *et al.* (1984) as follows

$$\text{Quality index} = \frac{\text{Total dry weight (g)}}{\frac{\text{Pseudostem height (cm)}}{\text{Collar diameter (mm)}} + \frac{\text{Shoot dry weight (g)}}{\text{Root dry weight (g)}}}$$

Volume index

Volume index was computed by considering collar diameter of the seedlings and plant height with the formula as given below.

Volume index = Collar diameter (mm) × Height (cm)

Statistical analysis

The data recorded on various seedling growth, root and quality parameters during the period of investigation was tabulated and subjected to statistical analysis using Complete Randomized Design (CRD) as suggested by Panse and Sukhatme (1985)

Results and Discussion

Effect of liquid microbial inoculants on growth parameters

The seedlings inoculated with combined liquid microbial inoculants T_9 in Tables 1, the significantly highest growth parameters like pseudostem height (63.93 cm), pseudostem girth (5.74 cm), number of leaves (10.33), leaf area (1174.41 cm^2) per plant, leaf area index (9.81) and chlorophyll-a, b and total chlorophyll (1.47, 0.82 and 2.30 mg/g, respectively) as compared to uninoculated T_1 (49.18 cm, 4.01 cm, 7.20, 578.52 cm^2, 4.86, 1.14, 0.52 and 1.66 mg/g respectively) at 150 days after treatment. The increased growth parameters could be attributed to enhanced nutrient availability in the media as well as uptake of nutrients by the seedlings as nitrogen fixation by *Azotobacter,* solubilization of unavailable form to phosphorus and potassium to available form by *Bacillus* species and mobilization of phosphorus by VAM in the media and also production of plant growth promoting hormones like auxins (IAA) and gibberellins (GA) by liquid microbial inoculants which might have triggered the growth of meristematic tissue due to increased rate of cell multiplication and cell enlargement in the seedlings which in turn stimulated the better vegetative growth and highest accumulation of chlorophyll content in the leaves might be due to the microbial inoculants which enhanced the availability of major nutrients such as nitrogen, phosphorus and potassium and also other essential elements to the seedlings required for accumulation of higher chlorophyll content. The findings of Paulraj and Raj (2018) and Panchami *et al*. (2020) in cardamom seedlings are in consonance with the present findings.

Effect of liquid microbial inoculants on root parameters, plant biomass and quality parameters

The root parameters (Table 2) like number of primary roots (11.30), number of secondary roots (78.81), length of longest primary root (45.39 cm), root thickness (1.71 mm), root volume (13.56 cc) total dry matter production (5.44 g per plant) and quality parameters are tillering percentage (37.17),

Table 1. Effect of liquid microbial inoculants on growth parameters in cardamom seedlings at 150 days after inoculation in the secondary nursery conditions

Treatment	*Pseudostem* height (cm)	Pseudostem girth (cm)	Number of leaves	Leaf area (cm^2) perplant	Leaf area index	Total Chlorophyll (mg/g)
T1	49.18	4.01	7.20	578.52	4.86	1.66
T2	52.24	4.07	8.13	839.59	8.03	2.13
T3	62.82	5.23	7.26	781.10	7.41	1.95
T4	52.73	5.08	8.73	755.23	6.29	1.96
T5	49.76	4.38	8.26	730.84	5.31	1.99
T6	60.80	5.33	9.33	1025.83	7.45	2.05
T7	59.25	4.55	8.33	885.31	7.38	1.94
T8	57.74	4.80	8.66	957.54	7.98	2.05
T9	63.93	5.74	10.33	1174.41	9.81	2.30
S.Em±	3.310	0.25	0.51	19.44	0.63	0.11
CD @ 5%	9.911	0.76	1.54	57.77	1.87	0.33

Dickson quality index (1.26) and volume index (557.17) was recorded maximum in the seedlings inoculated with T_9 as compared to uninoculated T_1 (7.89, 60.03, 33.78 cm, 0.98 mm and 8.33 cc, respectively). Application of phosphorus solubilizing bacteria enhanced the phosphorus solubilization in the potting mixture which increased the availability of phosphorus in available form and ultimately resulted in the production of maximum number of primary and secondary roots. The nitrogen fixing bacteria (*Azotobacter chroococcum*) could produce plant hormones like IAA, GA and cytokinin that might have promoted the root branching and root hair development. The higher root thickness could be attributed to availability of phosphorus, zinc, iron and other essential nutrients in the potting mixture in turn contributed for thicker roots. Increased root volume might be due to the production of large number of primary and secondary roots and longer roots. Ilngamudali and Senarathnel (2016) opined that the root volume could be correlated to improvement in seedling growth. VAM fungi secretes some of phosphatases and organic acids (oxalic acid) in the rhizosphere which catalyzing the hydrolysis of the phosphorous esters thus placing phosphorus at the disposal of the plants in coconut seedlings. The higher total dry matter production could be attributed to higher dry weight of the shoot and roots. Seedling quality might be due to the fact that, as they promote seedling vigour through cell division

Table 2. Effect of liquid microbial inoculants on root parameters, plant biomass and quality parameters at 150 days after inoculation in small cardamom seedlings in the secondary nursery

Treatments	Root parameters					Plant biomass	Quality parameters		
	Number of primary roots	Number of secondary roots	Length of longest primary root (cm)	Root thickness (mm)	Root volume (cc)	Total dry matter production (g)	Tillering percentage	Dickson quality index	Volume index
T1	7.89	60.03	33.78	0.98	8.33	2.73	9.73	0.64	198.68
T2	10.41	65.37	36.89	1.03	9.07	2.91	13.67	0.67	225.80
T3	9.70	64.81	34.72	1.06	9.85	3.16	11.70	0.79	443.32
T4	8.16	73.37	44.11	1.11	9.44	3.22	15.63	0.90	355.60
T5	9.94	61.92	40.22	1.29	8.89	2.99	13.67	0.81	249.20
T6	10.78	73.85	43.18	1.62	11.56	4.07	33.27	0.97	430.66
T7	9.78	70.44	39.44	1.35	10.11	3.63	19.57	0.78	311.05
T8	10.31	75.26	40.97	1.34	10.44	4.39	29.40	1.07	327.69
T9	11.30	78.81	45.39	1.71	13.56	5.44	37.17	1.26	557.17
S. Em ±	0.41	3.57	2.38	0.15	0.81	0.04	1.73	0.09	36.76
CD @ 5%	1.22	10.61	7.08	0.47	2.40	0.23	5.14	0.27	109.23

Table 3. Effect of liquid microbial inoculants on microbial population at 150 days after inoculation

Treatments	Total bacteria (*cfu*/g of soil)		Total fungi(*cfu*/g of soil)		Total actinomycetes(*cfu*/g of soil)	
	10-5	10-6	10-3	10-4	10-2	10-3
T1	43.00	34.00	15.00	9.00	11.00	3.00
T2	53.00	37.00	23.00	12.00	15.00	6.00
T3	67.00	40.00	26.00	16.00	13.00	5.00
T4	71.00	48.00	19.00	8.00	17.00	7.00
T5	63.00	39.00	29.00	19.00	14.00	9.00
T6	73.00	38.00	31.00	19.00	20.00	9.00
T7	82.00	41.00	36.00	23.00	19.00	11.00
T8	89.00	46.00	33.00	21.00	21.00	13.00
T9	94.000	53.00	46.00	29.00	23.00	15.00
S. Em ±	1.598	0.905	0.680	0.424	0.386	0.212
CD @ 5%	4.785	2.711	2.036	1.268	1.157	0.634

Note: Initial microbial population of the potting mixture, total bacteria (35.08 cfu/g of soil at 10^{-5}), total fungi (6.02 cfu/g of soil at 10^{-3}), total actinomycetes (8.99 cfu/g of soil at 10-2)

and shoot differentiation. The results similar to present study were reported by Latha (2017) in arecanut seedlings and Panchami *et al*. (2020) in cardamom.

Effect of liquid microbial inoculants on microbial population

The higher bacterial (94.00 *cfu*/g of soil at 10^{-5}), fungal (46.00 *cfu*/g of soil at 10^{-3}) and actinomycetes (23.00 *cfu* /g of soil at 10^{-2}) population was observed in the nursey media inoculated with *Azotobacter chroococcum* + *Bacillus megaterium* + *Bacillus mucilaginosus* + VAM over untreated control (43.00, 15.00 and 11.00, *cfu*/g of soil at 10^{-5}, 10^{-3} and 10^{-2} respectively) and is presented in Table 3. The increased bacterial population might be due to the multiplication of inoculated bacteria and also their synergistic interaction with other soil bacterias. The increase in fungal population might be due to the fact that the multiplication and survival of soil fungi as availability of organic matter, root exudates and other nutrients. The increase in actinomycetes population might be due to increased microbial activity and multiplication as it was inoculated with microbial consortium and organic manure. Similar results were reported by Thankamani *et al*. (2011) for black pepper.

Conclusion

The results of present investigation revealed that the conjuctive use of liquid PGPR (*Azotobacter chroococcum, Bacillus megaterium* and *Bacillus mucilaginosus*) and VAM resulted in quality seedlings production which exhibited in terms of better seedlings growth, root parameters and microbial population as compared to single inoculation and uninoculated control. Use of liquid microbial inoculants in the cardamom nursery is less expensive, ecofriendly and sustainable way of producing quality planting material. Hence, combined inoculation of liquid microbial inoculants (*Azotobacter chroococcum, Bacillus megaterium, Bacillus mucilaginosus*) and VAM proved to be the best combination than either single inoculation or combination of two and uninoculated for raising the quality seedlings in small cardamom.

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