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Effect of beneficial microorganisms on the nursery plant production in crossandra (C*rossandra infundibuliformis* (L.) Nees) cv. Bangalore local

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Abstract
An experiment was conducted at Tamil Nadu Agricultural University, Coimbatore to find out the effect of beneficial microorganisms on nursery plant production of crossandra (*Crossandra infundibuliformis* (L.) Nees) cv. Bangalore local. On analyzing total N, P, K and phytochemical content of the leaves in crossandra seedlings, it was observed that the total nitrogen (3.450 %) and total potassium (3.416 %) were observed highest in *Methylobacterium* sp. (PPFM) @ 2 % volume. *Methylobacterium* sp. (PPFM) @ 1 % volume (3.420 %) was on par with each other in total nitrogen content. Total phosphorous in leaf was observed highest (0.550 %) in *Bacillus amyloliquefaciens* @ 2 % volume. Total chlorophyll content was recorded highest in *Methylobacterium* sp. (PPFM) @ 2 % volume (8.836 mg g^{-1}). The highest quantity of leaf soluble protein (120.740 mg g^{-1}) and total sugar (26.500 mg g^{-1}) was recorded in *Bacillus amyloliquefaciens* @ 1 % volume. The highest quantity of total phenol (5.750 mg g^{-1}) and proline (9.08 mg g^{-1}) was recorded in *Bacillus amyloliquefaciens* @ 2 % volume. The results of the experiment indicates that *Methylobacterium* sp. followed by *Bacillus amyloliquefaciens* were suitable microbes for utilizing in the nursery plants production of crossandra.

Keywords: *Methylobacterium, Bacillus, Pseudomonas, Crossandra*, biochemical parameters, beneficial microorganisms.

Introduction
Floriculture is one of the potential sectors in Global Horticulture. The area under floriculture production in India was around 248.51 thousand hectares (Anon., 2015 (1)) [1]. The biological approaches for improving crop production have become a prevailing idea among agronomists and environmentalists (Ahemad and Kibret, 2014) [2] due to various recent environmental degradable facts of utilizing synthetic plant protection chemicals and fertilizers. Thus the use of beneficial microbes which possessing novel traits like salinity tolerance, biological control of phytopathogens and insects (Hynes *et al.,* 2008 [14] and Russo *et al.*,2007 [27]) along with the normal plant growth promoting properties for the development of more efficient crop production programme is necessitated (Chaudhary, 2010) [7]. In this regard, developing a microbial community for nursery plant production of certain flower and ornamental crops, to enhance the quality of planting materials by way of increased nutrient uptake and protection from soil borne pathogens is a low cost input for initial crop production. Nowadays, many microorganisms such as *Bacillus* sp., *Methylobacterium* sp., *Pseudomonas* sp. and Arbuscular Mycorrhizae (AM) are gaining commercial significance in crop production due to their efficiency in making association with plants system and improving growth of plants. With the above futuristic background, the present investigation was carried out with the objectives of identifying and standardizing the quantity and mode of application of crop specific beneficial microorganism's role on nursery plants production in Crossandra (C*rossandra infundibuliformis* (L.) Nees) cv. Bangalore local.

Materials and methods
The investigation was carried out at Department of Floriculture and Landscaping, Horticultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Coimbatore during 2016-2017. The study was conducted with three replications of ten treatments namely T_1 - *Methylobacterium* sp. (PPFM) @ 1 % volume, T_2 - *Methylobacterium* sp. (PPFM) @ 2 % volume, T_3 - *Bacillus amyloliquefaciens* @ 1 % volume, T_4 - *Bacillus amyloliquefaciens* @ 2 % volume, T_5 - *Bacillus subtilis* @ 1 % volume, T_6 - *Bacillus subtilis* @ 2 % volume, T_7 - *Pseudomonas fluorescens* @ 1 % volume, T_8 -*Pseudomonas fluorescens* @ 2 % volume, T_9 – Water soaking and T_{10} – Control in Completely Randomized Block Design (CRBD). For each replication 50 seeds were sown in 50 cell protrays. Crossandra seeds were sown in protrays, after water soaking for 4

hours and shade drying followed by implying treatments by treating seeds with desired microbes for 15 minutes. The protray media comprised of Soil + FYM + Cocopeat + Vermicompost in ratio of 6:4:1:1 + Arbuscular Mycorrhizae @ 10 g Kg^{-1} of media respectively. Nutrient and biochemical traits of the crossandra seedlings at transplanting stage was observed by analysing Total nitrogen, Total phosphorus, Total potassium (Piper, 1966) [26], Total chlorophyll (Yoshida *et al*., 1976) [28], Soluble protein (Lowry *et al*., 1951) [18], Total sugar(Hedge and Hofreiter, 1962) [13], Total phenols (Malik and Singh, 1980) [21] and Proline (Bates *et al.*,1973) [6] on leaf samples of the seedlings. The statistical analysis was done by adopting the standard procedures of Panse and Sukhatme (1985) [25].

Results

On analyzing total N, P, K and phytochemical content of the leaves in crossandra, it was observed that the total nitrogen (3.450 %) and total potassium (3.416 %) were observed highest in *Methylobacterium* sp. (PPFM) @ 2 % volume (T_2). *Methylobacterium* sp. (PPFM) @ 1 % volume (T_1) (3.420 %) was on par with highest value in total nitrogen content. Likewise, on analyzing total potassium content, *Bacillus subtilis* @ 1 % volume (T_5) (3.126 %), T_4 (*Bacillus amyloliquefaciens* @ 2 % volume) (3.125 %) and T_3 (*Bacillus amyloliquefaciens* @ 1 % volume) (3.124 %) which are on par with each other next to *Methylobacterium* sp. (PPFM) @ 2 % volume (T_2). Total phosphorous in leaf was observed highest (0.550 %) in (T_4) *Bacillus amyloliquefaciens* @ 2 % volume followed by *Methylobacterium* sp. (PPFM) @ 2 % volume (T2) (0.450 %). (Table 1).

Total chlorophyll content was recorded highest in *Methylobacterium* sp. (PPFM) @ 2 % volume (T_2) (8.836 mgg^{-1}). It was followed by the treatments *Bacillus amyloliquefaciens* @ 1 % volume (T_3) (8.390 mg g^{-1}), *Methylobacterium* sp. (PPFM) @ 1 % volume (T_1) (8.362 mg g^{-1}) and *Pseudomonas fluorescens* @ 2 % volume (T_8) (8.317 mg g^{-1}) which were on par with each other. The highest quantity of leaf soluble protein (120.740 mg g^{-1}) and total sugar (26.500 mgg^{-1}) was recorded in *Bacillus amyloliquefaciens* @ 1 % volume (T_3). *Methylobacterium* sp. (PPFM) @ 1 % volume (T_1) were on par with highest value on both leaf soluble protein (113.415 mg g^{-1}) and total sugar (22.00 mg g^{-1}) respectively. The highest quantity of total phenol (5.750 mg g^{-1}) and proline (9.08 mg g^{-1}) was recorded in *Bacillus amyloliquefaciens* @ 2 % volume (T_4) (Table 2). The highest value of total phenol was followed by *Bacillus subtilis* @ 2 % volume (T_6) (5.250 mg g^{-1}). Likewise, the highest value of proline was followed by *Methylobacterium* sp. (PPFM) @ 1 % volume (T_1) (8.38 mg g^{-1}).

Discussion

Effect on leaf nutrient content

In crossandra, it was observed that the increased total leaf nitrogen and total potassium content were recorded in PPFM @ 2 % volume and (PPFM) @ 1 % volume (T_1) (3.420 %) was on par with PPFM @ 2 % volume in total nitrogen content. The total phosphorous content was highest in *Bacillus amyloliquefaciens* @ 2 % volume. This result similar to the report given by Madhaiyan *et al.*, (2009) [20] that the PPFM strain CMSA322 formed more number of nodules and increased shoot nitrogen and dry weight in *M. atropurpureum*. Regarding *Bacillus* strains, the experimental statement given by Farzana and Radizah (2005) [11] suits with the present experimental outcome that *Bacillus* helps to stimulate plant growth and increased the uptake of N, P, K, Ca and Mg in sweet potato cultivar. Etesami *et al.*, (2009) [10] who found that production of IAA in plants helped to increase root dry weight and thereby increase the plants ability to take up N, P, K from soil compared to non-inoculated control. This is how *B. amyloliquefaciens* improves the N, P, K uptake in plants. The report on *B. amyloliquefaciens* possessing multiple plant growth-promoting traits which included production of indole-3-acetic acid (IAA), solubilization of zinc, production of ACC deaminase, solubilization of phosphate, production of phytases, HCN and cellulases by Ajilogba (2013) [3] again confirmed the role of this microorganism on increasing nutrient content, particularly total phosphorous of the plant.

Effect on biochemical parameters

Total chlorophyll content was observed highest in *Methylobacterium* sp. (PPFM) @ 2 % volume (T_2). Report on PPFM application with 0.5 % to 1.0 % accelerating vegetative growth, leaf area index, chlorophyll content, earliness in flowering, fruit set and maturation support this study very well (Anon., 2015(2)) [4].

Action of PPFM on total nitrogen and total chlorophyll content of the seedlings assured that it will promote vegetative growth of the seedlings by inducing the plants for the production of more carbon compounds through biosynthesis of amino acids and single cell proteins and bioconversion of some substrates unusable by other organisms (such as methanol emitted by the stomata of plants) (Marshall *et al.*, 1995) [22]. The effective action of PPFM on vegetative growth of the seedlings are due to the colonization of the bacteria on both rhizosphere as well as the phyllosphere (Madhaiyan *et al.*, 2004) [19].

Past report on the increased seedling weight and shoot length of *Nicotiana tabacum, Lycopersicon esculentum, Sinapis alba* and *Fragaria vesca* in the presence of the pink-pigmented facultative methylotroph (PPFM) strain *Methylobacterium extorquens* ME4 (Abanda, 2006) [1] is on support with the current experiment. The seedling growth promotion of PPFM was already reported in other crops by Corpe and Basile (1982) [8], Madhaiyan *et al.* (2004) [19] and Lee *et al.*, (2006) [15].

Total soluble protein, total sugar, total phenol and proline levels had its highest level in different concentrations of *B. amyloliquefaciens* treatments. From the result it is clearly observed that the highest level of biochemical parameters were recorded in the seedlings treated with these microbes compare to control. *B. amyloliquefaciens* promotes plant growth by its one of the mode of improving stress tolerance capacity of the plant. This statement was supported by Miller *et al.* (2009) [24], that proline accumulation was important for the tolerance of certain adverse environmental conditions. Likewise, plants protect themselves from oxidative effects under stressful conditions by enhanced synthesis of secondary metabolites including phenolic compounds (Mazid, 2011) [23].

Other microbes such as *Pseudomonas fluorescens* and *Bacillus subtilis* promotes optimum growth of crossandra seedlings comparatively than control. *P. fluorescens* had been observed for promotion of plant growth and at 2 % volume concentration it promotes plant growth comparatively higher than 1 % volume concentration. Many reports were on *P. fluorescens* for plant growth promotion along with antagonistic effect on plant associated pathogens. Dubeikovsky *et al.* (1993) [9] documented that *P. fluorescens*

produced plant growth regulators like gibbrellin, cytokinin and indole acetic acid (IAA) which involved in indirect disease resistance by enhancing plant growth. It is interested to understand that use of *Pseudomonas fluorescens* even at increased concentration (2 % volume) which gives better effect than 1 % volume concentration. *Bacillus subtilis* performs very well than control and water treatment. There are many reports that *B. subtilis* have antagonistic effect against pathogens and nematodes [Liu *et al.,* (2007) [17], Lim and Kim (2009) [16], Grover *et al.,* (2009) [12]]. But other microbes used in the experiment such as *Methylobacterium* sp., *B. amyloliquefaciens*, had overruled in plant growth promotion along with antimicrobial and stress tolerant activity than *P. fluorescens* and *B. subtilis* in this experiment.

Conclusion

The results of the experiment on growth promotion in seedlings of *Crossandra infundibuliformis* (L.) Nees., by the beneficial microorganisms indicates that *Bacillus amyloliquefaciens* and *Methylobacterium* sp. were suitable microbes for utilizing in the nursery plants production. Further experiments may be promoted on effect of beneficial microorganism on nursery plants growth promotion which will gives good outcome for commercial utilization as stable technology.

Table 1: Effect of beneficial microorganisms on leaf total nitrogen, total phosphorous and total potassium of crossandra (*Crossandra infundibuliformis* (L.) Nees) cv. Bangalore local seedlings

Treatments	Total Nitrogen (%)	Total Phosphrous (%)	Total Potassium (%)
T1	3.420 (10.751)	0.325 (3.270)	3.042 (10.045)
T2	3.450 (10.783)	0.450 (3.845)	3.416 (10.651)
T3	3.100 (10.304)	0.410 (3.671)	3.124 (10.263)
T4	3.150 (10.385)	0.550 (4.252)	3.125 (10.181)
T5	2.690 (9.788)	0.280 (3.033)	3.126 (10.522)
T6	2.750 (9.890)	0.380 (3.534)	3.109 (10.154)
T7	3.100 (10.141)	0.285 (3.060)	2.972 (9.926)
T8	3.120 (10.385)	0.255 (2.896)	2.939 (9.870)
T9	2.180 (9.140)	0.265 (2.951)	2.930 (9.855)
T10	2.010 (8.151)	0.180 (2.434)	2.846 (9.712)
Mean	10.084	3.295	10.118
SE.d	0.102	0.027	0.137
CD (0.05)	0.213**	0.056**	0.286**

** - Significant, NS - Not significant

*Percentage values are converted to arc sine values for analysis of variance

Table 2: Effect of beneficial microorganisms on total chlorophyll, soluble protein, total sugar, total phenol and proline content of crossandra (*Crossandra infundibuliformis* (L.) Nees) cv. Bangalore local seedlings

Treatments	Total Chlorophyll (mg g^{-1})	Soluble protein (mg g^{-1})	Total sugar (mg g^{-1})	Total phenol (mg g^{-1})	Proline (mg g^{-1})
T1	8.362	113.415	22.000	4.873	8.38
T2	8.836	102.136	14.000	4.625	7.85
T3	8.390	120.740	26.500	4.500	7.00
T4	8.059	101.123	13.000	5.750	9.08
T5	7.207	102.125	19.500	4.938	4.10
T6	7.742	102.059	12.200	5.250	6.03
T7	7.827	101.500	16.500	4.625	5.45
T8	8.317	103.625	11.690	4.875	4.13
T9	7.270	95.003	8.667	4.623	3.31
T10	6.955	95.233	8.500	3.625	3.85
Mean	7.911	106.696	14.589	0.933	5.916
SE.d	0.197	5.251	3.011	0.019	0.125
CD (0.05)	0.412**	10.955**	6.281**	0.040**	0.262**

** - Significant, NS - Not significant

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