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International Journal of Current Research
Vol. 3, Issue, 7, pp.314-319, July, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

NITROGEN FIXING DIAZOTROPH *Azospirillum* AND PHOSPHOBACTERIA ON THE GROWTH AND DEVELOPMENT OF *CROSSANDRA* (*Crossandra infundibuliformis* L.)

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ARTICLE INFO

Article History:
Received 5th April, 2011
Received in revised form
8th May, 2011
Accepted 5th June, 2011
Published online 16th July 2011

Key words:
Biofertilizers:
Azospirillum,
Phosphobacteria,
NPK fertilizers,
Crossandra.

ABSTRACT

Crossandra plants bearing very attractive and colourful flowers for more than four months in a year by consuming minimum amount of chemical fertilizers. *Azospirillum* and phosphobacteria are biofertilizers which induces or enhances the growth, development and yield of crop plant through 'N' fixation, 'P' solubilization and also by synthesizing growth promoting substances like IAA, GA and cytokinines. In the present investigation about six isolates of *Azospirillum* were isolated from roots of *Crossandra* from different locations of Cuddalore District, Tamilnadu, India. Among the six isolates one of the best isolate AVCR* was selected and used in the field studies along with phosphobacteria from the Department of Microbiology, Annamalai University. The present results revealed that the *Crossandra* plants receiving 75 per cent recommended dose of NPK fertilizers along with *Azospirillum* and phosphobacteria (T_{10}) registered maximum growth and yield when compared to other treatments and also the treatments T_6 and T_{14} registered appreciable values in growth and yield of *Crossandra*, particularly the treatment T_6 – 100 per cent NPK + *Azospirillum* and phosphobacteria registered nearly on par values with T_{10} in many growth parameters. Hence there are lot of ways to reduce 25 per cent of N and P fertilizers by intending *Azospirillum* and phophobacteria to the flower crop *Crossandra*.

INTRODUCTION

India is one of the traditional country in the cultivation of different types of flower crops, most of the South Indian peoples from India cultivating or growing the flower crops in the backyard of the houses, in some cases they are growing the flower crops like jasmine, marigold, rose and *Crossandra* etc. as a ornamental plants in front of their houses or in their gardens and in borders of lawns, among the flower crops *Crossandra* has got much important as compare to other flower crops due to its perennial nature and flower yielding capacity for about 3-4 months in a year and also consumes minimum amount of fertilizers. Small farmers in India particularly in Tamilnadu are having and showing lot of interest in the cultivation of *Crossandra* because of assured and regular income and most of the South Indian peoples need different flowers for their usage in their daily life style. The area under cultivation of *Crossandra* has increasing day by day due to increasing demand in local market as well as in abroad. The estimated area under the cultivation of *Crossandra* in India is around 1700 hectares with the production of 430 tonnes. In Tamilnadu, it is being cultivated in an area of 980 hectares with an average production of 250 tonnes per annum (Subramaniyan *et al.,* 1997).

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All the flower crops are having specific recommendation by the Department of Horticulture and even though fertilizer recommendations are available for this crop, modern crop production with eco-friendly technology with integrated nutrient management which embraces judicial combination of organic and inorganic fertilizers. Due to raising cost of fertilizers and their pollution in environment it has become imperative to find an integrated nutrient management schedule for this crop for an optimum and economic use of plant nutrients to reduce the cost of production as well as environmental pollution. Bio-fertilizers are nothing but the preparations containing live or latent cells of efficient strain of microorganisms recommended to improve the availability of nutrients to the crop plants. *Azospirillum* is an important bio-fertilizer which not only aid in nitrogen fixation but also produces growth promoting substances like auxin, gibberellins and cytokinins and helps to reduce the use of nitrogenous fertilizer to an extent of 25 per cent. Phosphobacteria is another important bio-fertilizer used in different crops to enhance the availability of phosphorus by solubilizing different types of phosphorus sources in soil likes tricalcium, aluminium and iron phosphates as well as rock phosphate and making the phosphorus available to the crop plants that induces early root growth and leads to vigour in the growth and development of various flower crops.

The rapid loss of nitrogenous fertilizers due to leaching and precipitation of phosphatic fertilizers leads to poor availability of N and P nutrients in soil, so it is imperative to go for some low cost technology, preferably the use of biofertilizers, to improve the availability of N and P nutrients. The use of *Azospirillum* and phosphobacteria has resulted in an increased yield and reduction in the recommended dose of nitrogenous and phosphatic fertilizers in many flower crops (Wange *et al.,* 1995a; Balasubramanian, 1989). Based on the above trait, a research work has formulated to reduce the use of inorganic fertilizers to reduce the pollution and increase the usage of bio-fertilizers to improve the growth and yield of *Crossandra* cv Dindigul Local and also to improve the soil health.

MATERIALS AND METHODS

The root samples of *Crossandra* were collected from Annamalainagar, Killai, Orathur, Sivapuri, Vallampadugai and Vilagam, Cuddalore District, Tamilnadu, were surface sterilized with 1% cholormine-T and macereate the root bits with the help of foreceps and place the root bits into a serum vials containing 5.0 ml of Nfb semisolid medium. Incubate the vials for 48 hours at 32-35°C and observe for a thin, disc like pellicle in the inoculated vials. The subsurface growth in the form of pellicle shows a positive indication for the successful isolation of *Azospirillum*. Subsequently, the cultures were streaked on Nfb plates for purification and finally mass multiplied in Nfb broth for the further studies.The present investigation was conducted in farmer's field at Vilagam, a village located 12 km away from Annamalinagar, during the year 2009-2010. *Crossandra* cv. Dindigal Local which had proven to perform well in a comparative evaluation trial (Reddy, 2000) was selected and utilized for the field experiment.

The inoculants of *Azospirillum* was selected based on the efficiency in nitrogen fixation, the best isolate was mass multiplied in Nfb broth and used in field studies and phosphobacteria used in the field studies were obtained from the Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University. The treatments details are as follows: Absolute control (T_o), *Azospirillum* only (T_1), Phosphobacteria only (T_2), 100% NPK alone (T_3), 100% NPK + *Azospirillum* (T_4), 100% NPK + phosphobacteria (T_5), 100% NPK + *Azospirillum* + phosphobacteria (T_6), 75% NPK alone (T_7), 75% NPK + *Azospirillum* (T_8), 75% NPK + phosphobacteria (T_9), 75% NPK + *Azospirillum* + phosphobacteria (T_{10}), 50% NPK alone (T_{11}), 50% NPK + *Azospirillum* (T_{12}), 50% NPK + phosphobacteria (T_{13}), 50% NPK + *Azospirillum* + phosphobacteria (T_{14}). The soil of the experimental field having available N (178kg/ha), available P_2O_5 (15kg/ha), available K_2O (126 kg/ha), Soil reaction (pH) (6.7), soil type (Clay loam).

Crop Management

Seeds of the selected cultivar were sown on raised beds. Soil was drenched with Bavistin and Furadon granules before sowing. Seedlings were ready for transplanting after 50DAS and were planted at a spacing of 60 × 30 cm. FYM @ 5 kilograms per plot was incorporated initially. The major nutrients viz., N, P_2O_5 and K_2O were applied as per the treatment schedule (75:50:125 kg per hectare). The recommended dose of *Azospirillum* and phosphobacteria (each @ 2 kg per hectare) were inoculated through seedlings root dip for half an hour, as per the treatment schedule. Observations were recorded in five plants tagged at random leaving boarder rows in each replication (fifteen plants in each treatment) at different stages on the following characters. Height of the plants from ground level to the tip of the main stem was measured twice at initial and peak flowering period and was expressed in centimeters. Number of branches was also counted at the time of initial and peak flowering stage and expressed as number per plant. Number of days taken from the date of transplanting to first flowering was recorded and expressed in days. Numbers of leaves were counted at the time of initial and peak flowering stages was expressed as number per plant. Numbers of spikes were counted at the post flowering period and was expressed in number. Spikes on which the emergence of the flower had completed were considered and the length from the tip of the last pair of flowers to the base of the spike was recorded in centimeters. Total number of flowers that were produced in each spike was counted. Flowers picked at each harvest, per plant were weighed separately and added for flower yield per plant and was expressed in grams. The plant samples were dried in hot air over on 80 ± 5ºC for 48 hours. The oven dry weight of samples was recorded as dry matter production was expressed in grams per plant. Soil sample were collected from the experimental plot twice, once before the incorporation of organic and inorganic fertilizers and another after harvest. The samples were dried at shade and gently powdered using a wooden mallet and sieved through 2 mm sieve and are used for detailed analysis. Available nitrogen was estimated by alkaline permanganate method as suggested by Subbiah and Asija (1956) and expressed as $kgha^{-1}$. Available phosphorus was estimated by calorimeter method as suggested by Olsen *et al.,* (1954) and expressed in kg ha^{-1}. The experimental data were statistically analysed using the method suggested by Panse and Sukhatme (1978).

RESULTS

Growth and development of *Crossandra*

The present research was conducted with an objective to find out the influence of biofertilizers viz., *Azospirillum*, best isolate from screening; phosphobacteria and graded levels of inorganic fertilizers on the growth and yield of *Crossandra* cv. Dindigul Local. The results of the mean performance of various parameters recorded and statistically analysed and are presented below in tables 1 to 4. Gradual increase in plant height was observed in all the treatments combination at both the stages of observation. The highest plant height of 46.39 m at initial flowering and 60.28 cm at peak flowering stage was recorded in T_{10}. The next best value were observed in T_6 46.38 cm at initial flowering and 60.26 cm at peak flowering stage) which exhibited statistically insignificant difference among themselves whereas both the treatments recorded much significant difference compared with control. In both the stages of observation, highest number of branches was recorded in T_{10} (7.56 at initial flowering and 12.49 at peak flowering stages). The least number of branches was recorded in T_0 (2.60 at initial flowering and 5.16 at peak flowering stages). The inoculation of *Azospirillum* and phosphobacteria altered the flowering nature of *Crossandra*. The earliness in flowering was noticed in T_{10} (56.28 days) followed by T_6

(56.40 days). A significant difference was noticed between control and other treatments receiving *Azospirillum* and phosphobacteria. Significant differences were recorded in the number of leaves, among the treatments, the highest number of leaves was observed in T_{10} (67.80 at initial flowering and 86.28 at peak flowering stage). This was followed by treatments T_6 which also exhibited statistically significant differences compared with control. The least number of leaves was recorded in T_0 control.

the least was recorded by T_0 (14.25) and also on par values were observed in the treatments T_6 and T_{14}. When compared to that of control, all the treatments exhibited significant difference in spike length. The highest spike length was recorded in T_{10} (10.00 cm). The treatments T_9 and T_{12} recorded statistically insignificant differences among themselves.

Table 1. Isolation and nitrogen fixing efficiency of *Azospirillum*

Source	Name of the isolates	Gram reaction	Nitrogenase activity ($nmoles C_2H_4/hr/mg$ of cell protein)
Annamalainagar	AACR	Gram negative	288.65
Killai	AKCR	Gram negative	298.50
Orathur	AOCR	Gram negative	374.80
Sivapuri	ASCR	Gram negative	369.83
Vallampadugai	AVCR	Gram negative	372.56
Vilagam	AVCR*	Gram negative	376.96

*Isolation and nitrogen fixing studies only for selecting a best isolate to conduct field studies.

Table 2. Effect of *Azospirillum* and phosphobacteria with graded levels of NPK fertilizers on the growth and development of Crossandra

Treatments	Plant height (cm)		Number of branches		Number of leaves per plant	
	Initial flowering	Peak flowering	Initial flowering	Peak flowering	Initial flowering	Peak flowering
T_0	24.32	25.63	2.60	5.16	28.43	42.43
T_1	29.51	30.10	3.81	7.18	34.92	56.00
T_2	26.42	28.23	3.54	6.71	32.28	52.23
T_3	37.21	40.21	4.80	9.26	58.23	69.13
T_4	41.40	51.40	5.80	10.47	62.37	79.23
T_5	39.53	47.63	5.41	10.04	60.00	75.84
T_6	46.38	60.26	7.52	12.47	64.40	80.56
T_7	36.82	41.70	5.05	9.47	55.12	71.67
T_8	32.80	34.26	4.25	8.24	46.60	65.80
T_9	34.74	36.42	4.28	8.49	48.88	68.03
T_{10}	46.39	60.28	7.56	12.49	66.80	84.28
T_{11}	32.90	34.89	4.29	8.29	43.00	60.20
T_{12}	32.98	34.94	4.06	821	44.33	62.83
T_{13}	30.30	31.21	3.93	7.63	39.79	57.23
T_{14}	46.20	60.24	7.38	12.44	62.30	79.00
SED	0.7807	0.9847	0.1646	0.2896	0.6163	0.9850
CD (p=0.05)	1.5615	1.8792	0.3293	0.5793	1.2327	1.8798

Table 3. Influence of *Azospirillum* and phosphobacteria with graded levels of NPK fertilizers on flowering and yield of crossandra

Treatments	Days taken to first flowering	Number of spikes per plant	Spike length (cm)	Number of flowers per spike	Flower yield per plant (g)	Flower yield qha^{-1}
T_0	86.18	14.25	5.00	17.0	19.30	10.60
T_1	81.91	18.49	6.43	21.30	27.10	14.98
T_2	82.36	17.80	6.38	20.10	25.69	14.14
T_3	66.18	26.54	7.60	25.46	37.13	20.44
T_4	60.31	32.43	8.73	27.24	39.46	21.75
T_5	63.54	29.46	7.91	25.74	38.00	20.94
T_6	56.40	36.90	9.50	30.28	41.73	22.91
T_7	67.81	26.83	7.66	24.38	36.80	20.30
T_8	70.26	24.85	7.00	22.00	33.50	17.80
T_9	76.25	25.68	7.28	23.52	34.40	18.92
T_{10}	56.28	37.20	9.80	30.28	41.73	22.90
T_{11}	77.50	21.80	6.60	21.50	31.00	16.65
T_{12}	74.21	22.45	7.19	22.96	32.86	18.60
T_{13}	76.42	19.98	6.82	21.64	30.45	16.70
T_{14}	56.33	36.90	9.50	26.60	39.69	19.96
SED	0.8838	0.489	0.11	0.489	0.990	0.221
CD (p=0.05)	1.7676	0.979	0.229	0.979	1.991	0.461

Yield of *Crossandra*

In the present study all the treatments were recorded significant values compared with control. The treatment T_{10} recorded the highest value (37.20) for number of spikes while

The lowest spike length was recorded in T_0 (5.00 cm), on par values were also observed in the treatments T_6 and T_{14}. All the treatments exhibited significant differences when compared with control. The highest number of flowers per spike was

Close up view of Crossandra flower

Peak flowering in Crossandra plant

recorded in T_{10} (30.28) and the treatment T_{10} on par with T_6. The next best value was recorded by the treatment T_9 (23.52). The least number of flowers per spike was recorded in T_0 (17.0). When compared with control all the treatments recorded significant differences in flower yield. The highest yield per plant and per hectare was recorded in T_{10} 41.73 plant^{-1} and 22.91 ha^{-1}. Statistically insignificant different exhibited between T_9 (39.80 g per plant and 20.10 qha^{-1}) and T_{12} (39.69 plant and 19.96 qha^{-1}). The lowest yield per plant in T_0 (19.30 per plant and 10.60 qha^{-1}) were recorded.

followed by T_6 recording 23.30 gplant^{-1}. The lowest dry matter production was recorded in T_0 (14.03 g). The soil residual status for available nitrogen ranged from 165.50 kg to 185.50 kgha^{-1}. The treatments T_{10}, T_4 and T_6 registered maximum soil nitrogen and the values were on par with one another. The left over available phosphorus content in the soil ranged from 16.00 kg (T_0) to 24.75kgha^{-1} (T_{10}) on par values in phosphorus content was noticed in T_5, T_6, T_{10} and T_{14} treatments. The residual potassium content of the soil ranged from 305.50 kgha^{-1} (T_0) to 315.50 kgha^{-1} (T_{10}). The highest K content was noticed in the treatment T_{10} (315.50) and the treatments T_3, T_4, T_5 and T_6 recorded on par values with the best treatment.

DISCUSSION

The use of biofertilizers besides inorganic fertilizers in flower crops production has gained momentum in recent years due to their beneficial effects. However, the use of organic and inorganic fertilizers in flower crops has not been much explored and the research works based on integrated nutrient management in flower crops are scanty. Hence the present investigation was made with an aim to exploit the combined use of *Azospirillum* native isolate and phosphobacteria along with graded levels of NPK fertilizers to minimize the cost of productivity in *Crossandra* cv. Dindigul Local.

Influence on the growth components

The vegetative growth in *Crossandra* is measured in terms of plant height, number of branches per plant, days taken for first flowering, number of leaves per plant and dry matter production. Application of both *Azospirillum* and phosphobacteria in combination with 75 per cent recommended dose of NPK fertilizers had significant effect in improving the plant height. The possible reason for the increase in plant height may be due to the inoculation of *Azospirillum* and phosphobacteria which enhanced the uptake of nitrogen and phosphorus, since N is the chief constituent of protein, essential for the formation of protoplasm, which leads to cell division and cell enlargement resulting in increased

Table 4. Influence of *Azospirillum* and phosphobacteria with graded levels of NPK fertilizers on dry matter production and NPK content in crossandra soil

Treatments	Dry matter production per plant (g)	Available nitrogen (kgha^{-1})	Available phosphorus (kgha^{-1})	Available potassium (kgha^{-1})
T_0	14.03	169.50	16.00	305.50
T_1	16.82	174.00	16.25	306.50
T_2	15.24	171.50	17.75	306.25
T_3	21.49	183.00	20.50	315.00
T_4	23.73	185.50	20.75	315.25
T_5	22.52	183.20	24.50	315.00
T_6	23.00	185.50	24.75	316.00
T_7	20.24	182.00	20.75	312.00
T_8	18.65	178.50	19.60	310.00
T_9	19.65	180.00	21.50	311.50
T_{10}	25.91	185.50	24.60	315.50
T_{11}	16.95	176.80	17.50	308.25
T_{12}	18.82	179.00	18.50	309.25
T_{13}	17.66	177.50	19.50	309.25
T_{14}	22.56	185.50	24.59	315.00
SED	0.5261	0.8179	0.2672	1.1187
CD (p=0.05)	1.0523	1.6358	0.5344	2.2374

Significant differences were noticed between the treatments compared with control in dry matter production. The highest value in dry matter production was recorded in T_{10} (25.91)

plant growth and phosphorus induces early root growth and leads to develops vigour in the growth and development of *Crossandra*. Application of biofertilizers in the absence of

inorganic nutrients also significantly improved the plant height. This may be because of the proven fact involved in the production of phytohormones like IAA, GA and cytokinin like substances which would have enhanced to plant growth. Similarly, application of phosphobacteria also increased the plant height through the solubilization of phosphates. Increased availability of phosphorus in the very early stage would have helped in early vigorous root growth. This would have helped in the absorption of all the major and micronutrients required for the plants to put forth early vigorous vegetative growth, in another mechanism by which phosphobacteira augment plant growth by biosynthesis of growth promoting substances like auxin IAA (Barea *et al.,* 1976). Similar views were also expressed by Manonmani (1992) in Jasmine. Besides, the improvement bought out an interesting effect on number of branches. The application of 75 per cent recommended dose of NPK fertilizers along with *Azospirillum* and phosphobacteria markedly enhanced the number of branches in the present investigation. Flowerings are expected outcome in the cultivation of flower crops and early flowering was noticed in the treatment receiving 75 per cent recommended doses of fertilizer along with *Azospirillum* and phosphobacteria. It may be due to better nutritional status of the plants as influenced by the various treatments. Due to more photosynthetic activities, flowering was induced in the form of early initiation of flower bud formation. This finding was inaccordance with Ravichandran (1991) in *Crossandra* and Balasubramanian (1989) in French marigold.

Number of leaves per plant was found significantly higher in the treatment that received 75 per cent recommended dose of fertilizer along with *Azospirillum* and phosphobacteria. This increased number of leaves could be due to increased number of branches in the above said treatment. Higher leaf production with optimum level of fertilizer application was earlier reported by Ravichandran (1991) in *Crossandra*, Karunakaran (1997) in gladiolus. Productivity of the crop plants are desired by the dry matter production and in the present research. The dry matter production of the plants significantly increased when 75 per cent recommended dose of fertilizer along with *Azospirillum* and phosphobacteria was applied. This may be due to the application of optimum P which had greater influence on root system leading to enhanced uptake and translocation of N and other nutrients from soil and also N fixation by *Azospirillum* resulting in increased plant height, number of branches as well as more number of leaves and ultimately enhancing the dry matter production. These findings are inaccordance with Ravichandran (1991) in *Crossandra*, Manonmani (1992) in Jasmine and Karunakaran (1997) in gladiolus.

Influence on the yield

The flower yield was found to be higher in treatments that recorded greater values for number of branches and spikes per plant. The present study were in accordance to the earlier findings on correlation analysis carried out by Reddy (2000); there existed positive and significant association among these characters in *Crossandra*. The highest number of spikes per plant was noticed in the treatment that received 75 per cent recommended dose of fertilizer along with *Azospirillum* and phosphobacteria. Similar results were reported by Parthiban *et al.,* (1992) in tuberose. Combination of inorganic and biofertilizers exhibited the characteristic effect in spike length and number of flower per plant. Maximum spike length and number of flowers per spike were noticed in the treatment that received 75 per cent recommended dose of fertilizer along with biofertilizers. Similar results were reported earlier by Bavanisankar and Vanagamudi (2000a) in *Crossandra*, Karunakaran (1997) in gladiolus and Swaminathan *et al.* (2000) in tuberose.

The enhanced flower yield in terms of weight was observed to be maximum in the plants treated with 75 per cent recommended dose of fertilizer along with *Azospirillum* and phosphobacteria. The observations also revealed that the treatment receiving 100 per cent recommended dose along with *Azospirillum* and phosphobacteria were comparable with treatment receiving 75 per cent recommended dose of NPK, *Azospirillum* and phosphobacteria. This result seems to concede the reports of Bhavanisankar and Vanagamudi (2000a) in *Crossandra*, Balasubramanian (1989) in French marigold, Preethi (1990) in Edward rose, Manonmani (1992) and Bavanisankar and Vanagamudi (2000a) in Jasmine and Wange *et al.* (1995a) in tuberose.

Available nutrients in the biofertilizers inoculated soil

The residual availability of nutrients will be much helpful for the successful performance of the crop grown. In the present study, application of N fixers with recommended dose of fertilizer resulted in marked increase in the available N content in the soil. Among the treatment combinations, inoculation of *Azospirillum* and phosphobacteria along with 75 per cent recommended dose of NPK fertilizer recorded maximum available NPK content. This might be attributed to the nitrogen fixing capacity of *Azospirillum*. The phosphobacteria inoculation also aided in dissolving the insoluble phosphorus and making it available to the plants which is attributed to the P solubilizing capacity of organisms (Banik and Datta, 1988). The availability of nutrients in the soil increased the uptake of nutrients, in turn influencing the growth and yield. This is in accordance with the findings of Manonmani (1992) in *Jasminum sambac* and Mahendran *et al.* (1995b) in moringa beans and butter beans.

SUMMARY AND CONCLUSION

The present investigation on the influence of organic (biofertilizers) and inorganic fertilizers on growth and yield of *Crossandra* cv. Dindigul Local was carried out in a farmer's field at Vilagam, a village located just 12 kms away from Annamalainagar. *Crossandra* cv. Dindigul Local grown with the supply of 75 per cent NPK along with *Azospirillum* and phosphobacteria exhibited improved growth in respect of plant height, number of branches, days taken for first flowering, number of leaves per plant and dry matter production. The treatment that received 100 and 50 per cent NPK along with *Azospirillum* + phosphobacteria were registered as the next best treatments. Highest flower yield per plant was obtained in the plants grown under 75 per cent NPK along with *Azospirillum* and phosphobacteria with 100 and 50 per cent NPK + *Azospirillum* and phosphobacteria were comparable with the best treatment. Maximum number of spikes per plant was recorded in plants grown under 75 per cent NPK along

with *Azospirillum* and phophobacteira. This was followed by the treatment with the supplementation of 100 and 50 per cent inorganic fertilizers along with *Azospirillum* + phosphobacteria. Spike length and number of flowers per spike were highest in plants receiving 75 per cent NPK along with *Azospirillum* and phosphobacteria. The available soil nitrogen, phosphorus and potassium were more in the treatment that received 75 per cent NPK along with *Azospirillum* and phosphobacteria. In the present investigation, the plants receiving 75 per cent recommended dose of NPK fertilizers, *Azospirillum* and phosphobacteria recorded enhanced growth values and flower yield, dry matter production and NPK content in soil. In contrast, the plants receiving 100 and 50 per cent NPK fertilizers, *Azospirillum* and phosphobacteria also recorded same type of growth values and flower yield. Hence, by intending *Azospirillum* and phosphobacteria to the flower crop *Crossandra*, based on the findings of present research paves way for reducing the use of NPK fertilizer to the extent of 25 per cent.

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