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Effect of Plant Growth Promoting Rhizobacteria on Growth, Yield and Quality of Shankapushpi (*Clitorea ternatea* L.) Under Irrigated Situation

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ABSTRACT

A field experiment was conducted to assess the Effect of Plant Growth Promoting Rhizobacteria on growth, yield and quality of shankapushpi (Clitorea ternatea L.) under irrigated situation at College of Horticulture, UHS campus, GKVK Post, Bengaluru during 2016-2017. The experiments were comprised of ten treatments and they were replicated thrice in RCBD. The maximum plant height (16.25 cm, 32.15 cm, 65.50 cm and 84.07 cm at 30, 60, 90 and at harvest, respectively, number of branches (6.00, 9.00.12.50 and 16.50 at 30, 60, 90 and at harvest, respectively, early germination (5.73), germination percentage (89.07) and early flowering (41.33) were recorded with seed treatment of Bradyrhizobium japonicum + Pseudomonas fluorescence with application of full dose of RDF. The maximum number of pods per plant (60.48), length of pod (9.67 cm), width of pod (1.03 cm), weight of pod (0.74 g), seed weight (0.51 g) test weight (4.35 g), seed yield (16.50 Q ha^{-1}), biological yield (43.76 Q ha^{-1}), economical yield (34.93 Q ha^{-1}) and crude protein content (6.9%) were obtained with seed treatment of Bradyrhizobium japonicum + Pseudomonas fluorescence with application of full dose of RDF.

Key words: *Shankapushpi, Bradyrhizobium japonicum and Pseudomonas fluorescence.*

INTRODUCTION

India is the largest producer of medicinal herbs and known as the botanical garden of the world. Officially, over 3000 plants were recognized in India for their medicinal value and about 200 native plant species are in wide use for their curative properties. Shankapushpi also know as butterfly pea is one of the important medicinal plants used for boosting memory and improving intellect and also to cure mental illness.

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It is a perennial leguminous twiner, botanically know as *Clitoria ternatea* L. belonging to the family Fabaceae. The plant originated from tropical Asia and distributed widely in South and Central America. The genus *Clitoria* comprises of about 60 species distributed mostly within the tropical belt with a few species found in temperate areas. The most frequently reported species is *Clitoria ternatea* L, which is mainly used as a forage as it is highly palatable for live-stock apart from its various medicinal usage.

The plant is vigorous, strongly persistent and it is long-lived perennial herb with an erect growth habit. The stem is fine twining and sparsely pubescent at base, leaves are pinnate with 5-7 leaflets, petioles 1.5-3cm long, flowers are auxiliary, whitish blue to dark blue in colour resemble a conch shell. The pods are linear oblong, flattened 4-13 cm long the tap root which may grow to more than 2m deep, bears one to several purplish lateral roots. The plant is adaptable to a wide range of temperature, rainfall and altitude, but susceptible to frost and does not grow well during cold spells in winter. The rainfall requirements ranges from 400mm to 1500 mm per annum, sensitive to water logging and flooding and it is claimed to have some tolerance to salinity. The shankapushpi is considered as Madhya-Rasayana in *Ayurveda* and reported as nervine tonic and laxative. The leaves of shankapushpi conatains glycosides *viz*., kaempferol-3-glucoside, kaempferol-3-rutinoide and kaempferol-3-neohesperidoside. The root contains ternatins, alkaloids, flavonoids, saponins, tannins, carbohydrates, proteins, resins, starch, taraxerol and taraxerone. The seeds have nucleoprotein with its aminoacid sequence similar to insulin, delphinidin-3, 3, 5 - triglucoside, essential amino-acids, pentosan and water soluble mucilage[9]. The root powder of *clitorea* is used as one of the ingredients in the preparation of the drug "SULAK" and its ointment to treat leprosy. The flower had been used to dye rice cake in Malaysia and being eaten as vegetable in India and Philippines. The flower is also being used traditionally as diuretic, anthelmintic, purgative, demulcentand remedy for rheumatism, bronchitis, urinogenital disorderand cancer[7].

MATERIAL AND METHODS

The field experiment was conducted at College of Horticulture, University of Horticultural Sciences Campus, Gandhi Krishi Vignana Kendra (Post), Bengaluru during June to November 2016-17. Shankapushpi seeds (Local type) were collected from Sanjeevini vatika, Division of Horticulture, University of Agricultural Science, Gandhi Krishi Vignana Kendra, Bengaluru

The native Rhizobium stain was collected from root nodules of shankapushpi and *Pseudomonas fluorescence* was collected from the Department of Agricultural Microbiology, University of Agricultural sciences, Gandhi krishi Vignana Kendra, Bengaluru and used for seed treatment of shankapushpi with three replication by using RCBD design and treatments *viz*. T_1 - Recommended dose of fertilizers (control) T_2 - Recommended dose of fertilizers + *Bradyrhizobium japonicum* T_3 -Recommended dose of fertilizers + *Pseudomonas fluorescens* T_4 -Recommended dose of fertilizers +*Bradyrhizobium japonicum*+ *Pseudomonas fluorescens* T_5 -75 % Recommended dose of fertilizers + *Bradyrhizobium japonicum* T_6 - 75% Recommended dose of fertilizers + *Pseudomonas fluorescens* T_7 -75% Recommended dose of fertilizers + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* T_8 - 50 % Recommended dose of fertilizers + *Bradyrhizobium japonicum* T_9 -50 % Recommended dose of fertilizers +*Pseudomonas fluorescens* T_{10} -50 % Recommended dose of fertilizers+ *Bradyrhizobium japonicum* +*Pseudomonas fluorescens.*

RESULT AND DISCUSION

The seed treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* and application of full dose of RDF has recorded early germination (5.73 days), 50 per cent germination (9.85 days) and maximum

germination (89.07%) these is due to the applied PGPR's might have attributed for creating favorable condition through secretions of vitamins and growth promoting substances thus helped in maximum germination of seeds. The results are in line with those obtained by Abdolshakoor *et al*.[1] in Isabgol. The seed treatment of *Bradyrhizobium japonicum*+ *Pseudomonas fluorescens* along with application of full dose of RDF has resulted in early flowering (41.33 days), days for 50 per cent flowering (50.17 days), early pod setting (16.83 days). The days from pod setting to maturity was observed early (30.67 days) And Seeds treated with *Bradyrhizobium japonicum*+ *Pseudomonas fluorescens* and application of full dose RDF has recorded early flowering and crop maturity under rainfed condition. The less water supply to the plants resulted in accumulation of secondary metabolites and reduced vegetative growth and attaining the early reproductive growth (Table1).

The seeds treated with combination of two plant growth promoting rhizobacteria along with the application full dose of NPK has recorded the maximum plant height of 16.25 cm, 32.15 cm, 65.50 cm and 84.07 cm at 30, 60. 90 days after sowing and at harvest, respectively under irrigated condition. It was followed by seed treatment of *Bradyrhizobium japonicum* and application of full dose RDF (15.54 cm, 31.06 cm, 63.41 cm and 82.12 cm at 30, 60, 90 days after sowing and harvest, respectively). While, the least plant height was recorded with seed treatment of *Pseudomonas fluorescens* + 50 per cent RDF at all the growth stages. The increased plant height may be due to inoculation of plant growth promoting rhizobacteria on the seed before sowing, so that, they are able to establish themselves on the roots and directly affect on plant growth and development through the fixation of atmospheric nitrogen, increases the availability and uptake by free living biological nitrogen fixer *Rhizobium* and also increased phosphorus solubilization, mobilization and uptake by *Pseudomonas fluorescens* and also might be due to indirect effect of these rhizobacteria through the production of plant hormones. These results are in confirmity with the works of Abdolshakoor *et al*.[1] in isabgol. The seeds treated with combination of two plant growth promoting rhizobacteria and application of full dose RDF has recorded the maximum number of branches (6.00, 9.00, 12.50 and 16.50 at 30, 60, 90 days after sowing and at harvest, respectively) under irrigated condition. It was *on par* with seed treatment of *Bradyrhizobium japonicum* and application of full dose of RDF at all the plant growth stages. While, the least number of branches was recorded with the application of seed treatment of *Pseudomonas fluorescens*and 50 per cent RDF at all the growth stages. Rhizobium and *Pseudomonas fluorescens* performed better when the soil is well supplied with nutrients particularly nitrogen through organics, which resulted in increased in cell division and cell elongation which in turn increased the plant height and number of branches. Increased number of branches could be attributed by application of nutrients and activation of bioactive substances in plants due the application of PGPRs, which would in turn have a increased availability of nutrients to the plant. These findings are in line with. Abdolshakoor *et al*.[1] in isabgol (Table 2).

The seed treatment of *Bradyrhizobium japonicum*+ *Pseudomonas fluorescens* with full dose of RDF has recorded maximum pods per plant (60.48), maximum length of pod (9.67 cm), width of pod of (1.03 cm), pod weight (0.74 g), seeds per pod (8.42), seed weight per pod (0.51 g), seed weight per plant (22.27 g) and test weight (4.35 g) The maximum number of pods may be attributed to the role of *Rhizobium* which can helped in increased vegetative growth in terms of higher plant height and number of branches. The increased vegetative growth resulted in synthesis of greater amount of food materials, which in turn supported profused flowering and pod setting and resulted in maximum number of pods. The results are in conformity with the works of Yadav and Kumawat[8], Jat and Purbey and Sen[6] in fenugreek.

The increased pod length and width may be due to better vegetative growth exhibited by plants because of optimum dose of nutrients and role of *Rhizobium* which intern responsible for vigorous growth and of production of greater amount of photosynthates, which helped in production longer pods as compared to other treatments. The increased test weight could be due to higher nutrients levels leads to accumulation of functional photosynthate which in turn might have resulted in bolder seeds leading to maximum seed weight. These results are in concurrence with Yadav and Kumawat[8] in fenugreek (Table 3).

The seeds treated with the combination of two plant growth promoting rhizobacteria with full dose of RDF has recorded maximum fresh herb yield per plant (72.57 g) dry herb yield per plant (24.88 g) fresh weight of roots per plant (24.50 g) dry weight of roots per plant (11.92 g) dry herb yield (18.43 Q ha^{-1}), seed yield (16.50 Q ha^{-1}), maximum biological yield (43.76 Q ha^{-1} Economic yield (34.93 Q ha^{-1}) Increased fresh herb yield and dry herb yield may be due to duel inoculation of a bio-fertilizer significantly increased the plant biomass yield. However, such increased effects have been found to be further enhanced significantly due to dual or other compatible mixtures of inoculants resulting from their strong synergistic relationships among themselves. These findings are in line with Ordookhani *et al*.[4] in *Cymbopogon martini*. The seed yield is an output of sequential metamorphosis from source to sink. Hence, higher growth parameters, in-turn resulted in increased the seed yield. Partitioning of photosynthates in to vegetative and reproductive parts has gone simultaneously in the later growth phases, which resulted in higher seed yield A positive correlation was observed between nutrient level and seed yield indicating the fact that, seed yield increases with increases in nutrient dose. These results are in accordance with the findings of Parakhia *et al.*[5], Jat, Purbey and Sen[6] in fenugreek. The increase in herb and seed yield contributed for the enhancement of economical yield in plants where seeds were treated with PGPR and with the application of full dose of RDF. The findings of the present investigation are in agreement with those of the Meena[3] in fenugrrek. The seed treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* and application of full dose of RDF has recorded significantly maximum crude protein content and crude protein yield (6.90% and 112.18 kg ha^{-1}, respectively) these is might be due to the *Rhizobium* and *pseudomonas* helped in fixation of atmospheric nitrogen which resulted in translocation and accumulation of secondary metabolites. The results of the present study are in agreement with those obtained by Purbey and Sen[6] in fenugreek (Table 4).

Table 1: Influence of plant growth promoting rhizobacteria on germination and flowering of *Clitorea ternatea* L.

Treatments		Days to First Germination	Days to 50% Germination	Germination Percentage	Days to First Flowering	Days to 50% Flowering	Days from Flowering to Pod Setting	Days from Pod Setting to Maturity
T_1 -	RDF (control)	9.50	14.10	72.18	49.67	59.33	20.33	41.00
T_2 -	RDF + *Broadyrhizobium japonicum*	6.57	11.47	83.57	43.67	55.00	18.17	33.33
T_3 -	RDF + *Pseudomonas fluorescence*	7.17	12.47	79.07	44.00	56.00	19.00	35.50
T_4 -	RDF + *Broadyrhizobium japonicum*+ *Pseudomonas fluorescence*	5.73	9.85	89.07	41.33	50.17	16.83	30.67
T_5 -	75% RDF + *Broadyrhizobium japonicum*	8.67	14.94	71.47	47.00	57.00	19.33	37.61
T_6 -	75% RDF + *Pseudomonas fluorescence*	10.13	15.58	78.23	50.67	58.67	20.00	40.00
T_7 -	75% RDF + *Broadyrhizobium japonicum*+ *Pseudomonas fluorescence*	7.87	13.51	76.20	47.33	58.33	18.67	37.00
T_8 -	50% RDF + *Broadyrhizobium japonicum*	10.50	16.73	62.20	50.67	57.67	22.67	42.67
T_9 -	50% RDF + *Pseudomonas fluorescence*	12.50	17.83	60.73	59.00	61.67	26.00	44.00
T_{10} -	50% RDF + *Broadyrhizobium japonicum*+ *Pseudomonas fluorescence*	8.67	16.63	74.27	53.00	57.67	22.33	41.33
F test		*	*	*	*	*	*	*
S.Em±		0.19	0.93	0.41	0.97	1.34	0.64	1.73
CD at 5 %		0.56	2.76	1.21	2.88	3.98	1.91	4.56

DAS – Days After Sowing, RDF – Recommended Dose of Fertilizer

Table 2: Plant height and number of branches as influenced by plant growth promoting rhizobacteria in *Clitorea ternatea* L

Treatments	Plant height (cm)				Number of branches			
	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest
T_1 - RDF (control)	13.90	28.55	61.19	78.89	4.98	7.78	11.25	14.54
T_2 - RDF + *Bradyrhizobium japonicum*	15.54	31.06	63.41	82.12	5.75	8.75	12.22	15.93
T_3 - RDF + *Pseudomonas fluorescens*	15.35	30.63	62.92	81.08	5.72	8.57	11.97	15.71
T_4 - RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	16.25	32.15	65.50	84.07	6.00	9.00	12.50	16.50
T_5 - 75% RDF + *Bradyrhizobium japonicum*	14.57	29.03	61.92	80.78	5.20	8.25	11.68	14.81
T_6 - 75% RDF + *Pseudomonas fluorescens*	14.39	28.83	61.00	79.75	4.83	8.22	11.37	14.75
T_7 - 75% RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	14.89	29.67	60.61	81.65	5.33	8.50	11.86	15.50
T_8 - 50% RDF + *Bradyrhizobium japonicum*	13.23	27.55	60.32	77.92	4.00	7.40	10.78	14.07
T_9 - 50% RDF + *Pseudomonas fluorescens*	12.69	25.67	57.89	76.83	3.78	7.25	10.27	13.83
T_{10} - 50% RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	13.27	28.23	60.58	78.62	4.75	7.73	10.82	14.45
F test	*	*	*	*	*	*	*	*
S.Em±	0.21	0.15	0.16	0.15	0.11	0.21	0.25	0.31
CD at 5 %	0.62	0.44	0.48	0.45	0.32	0.63	0.74	0.92

DAS – Days After Sowing, RDF – Recommended Dose of Fertilizer

Table 3: Effect of plant growth promoting rhizobacteria on pod and seed characteristics in *Clitorea ternatea* L

Treatments	Number of pods per plant	Length of pod (cm)	Width of pod (cm)	Pod weight (g)	Number of Seeds per Pod	Seed Weight per Pod(g)	Seed weight per plant(g)	Test Weight (g)
T_1 - RDF (control)	50.17	8.81	0.83	0.63	7.42	0.41	18.19	3.63
T_2 - RDF + *Broadyrhizobium japonicum*	58.33	9.54	0.96	0.73	8.08	0.50	20.95	4.34
T_3 - RDF + *Pseudomonas fluorescence*	57.25	9.48	0.95	0.71	7.89	0.48	20.16	3.98
T_4 - RDF + *Broadyrhizobium japonicum*+ *Pseudomonas fluorescence*	60.48	9.67	1.03	0.74	8.42	0.51	22.27	4.35
T_5 - 75% RDF + *Broadyrhizobium japonicum*	53.58	9.15	0.90	0.67	7.62	0.44	19.21	3.77
T_6 - 75% RDF + *Pseudomonas fluorescence*	52.60	9.03	0.87	0.65	7.55	0.42	19.06	3.68
T_7 - 75% RDF + *Broadyrhizobium japonicum*+ *Pseudomonas fluorescence*	54.58	9.22	0.93	0.69	7.75	0.46	19.30	3.86
T_8 - 50% RDF + *Broadyrhizobium japonicum*	46.40	7.54	0.75	0.59	7.17	0.36	16.10	3.55
T_9 - 50% RDF + *Pseudomonas fluorescence*	43.73	6.97	0.67	0.52	6.80	0.33	15.93	3.26
T_{10} - 50% RDF + *Broadyrhizobium japonicum*+ *Pseudomonas fluorescence*	47.20	8.77	0.80	0.62	7.31	0.38	16.47	3.59
F test	*	*	*	*	*	*	*	*
S.Em±	0.93	0.18	0.02	0.02	0.16	0.01	0.52	0.08
CD at 5 %	2.77	0.54	0.05	0.05	0.47	0.03	1.55	0.25

DAS – Days After Sowing, RDF – Recommended Dose of Fertilizer

Table 3: Effect of plant growth promoting rhizobacteria on yield and quality of *Clitorea ternatea* L

Treatments	Fresh herb yield per plant (g)	Dry herb yield per plant (g)	Fresh weight of root per plant (g)	Dry weight of root per plant (g)	Dry root yield (Q ha^{-1})	Dry herb yield (Q ha^{-1})	Seed yield (Q ha^{-1})	Biological yield (Q ha^{-1})	Economic yield (Q ha^{-1})	Crude protein content (%)	Crude protein yield (Kg ha^{-1})
T_1 - RDF (control)	64.20	22.32	22.17	10.47	7.76	16.53	13.48	37.77	30.01	5.50	71.12
T_2 - RDF + *Broadyrhizobium japonicum*	71.20	24.58	23.90	11.58	8.58	18.21	15.52	42.31	33.73	6.72	104.26
T_3 - RDF + *Pseudomonas fluorescence*	70.57	23.30	23.75	11.48	8.51	17.26	14.94	40.71	32.20	6.62	97.62
T_4 - RDF + *Broadyrhizobium japonicum*+ *Pseudomonas fluorescence*	72.57	24.88	24.50	11.92	8.83	18.43	16.50	43.76	34.93	6.90	112.18
T_5 - 75% RDF + *Broadyrhizobium japonicum*	67.23	22.57	22.60	11.12	8.24	16.72	14.23	39.19	30.95	6.22	88.75
T_6 - 75% RDF + *Pseudomonas fluorescence*	66.27	22.40	22.24	10.67	7.90	16.60	14.12	38.62	30.72	6.00	84.73
T_7 - 75% RDF + *Broadyrhizobium japonicum*+ *Pseudomonas fluorescence*	68.33	22.90	23.54	11.32	8.37	16.96	14.30	39.63	31.26	6.43	93.04
T_8 - 50% RDF + *Broadyrhizobiumjaponicum*	60.33	21.57	20.77	10.23	7.58	15.98	11.93	35.49	27.91	4.87	56.65
T_9 - 50% RDF + *Pseudomonas fluorescence*	59.13	20.80	20.27	10.00	7.41	15.41	11.80	34.62	27.21	4.57	57.31
T_{10} - 50% RDF + *Broadyrhizobium japonicum*+ *Pseudomonas fluorescence*	62.67	22.47	22.30	10.37	7.68	16.64	12.20	36.52	28.84	5.33	72.19
F test	*	*	*	*	*	*	*	*	*	*	*
S.Em±	1.31	0.32	0.39	0.19	0.14	0.33	0.39	0.92	0.62	0.24	4.13
CD at 5 %	3.89	0.95	1.17	0.58	0.43	0.99	1.15	2.72	1.83	0.72	12.26

DAS – Days After Sowing, RDF – Recommended Dose of Fertilizer

CONCLUSION

The present investigation reveals that, the seed treatment with *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* and application of full dose of RDF has resulted in better growth, maximum yield and good quality in shankapushpi under irrigated situation.

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