International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(3): 4602-4605

Received: 04-03-2019
Accepted: 06-04-2019

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Effect of plant growth promoting rhizobacteria on soil microbial load, chemical properties of soil and economics in *Clitorea ternatea* L. under rain fed condition

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Abstract
A field experiment was conducted to assess the Effect of plant growth promoting rhizobacteria on microbial load, chemical properties of soil and economics in *Clitorea ternatea* L. under rain fed condition at College of Horticulture, UHS campus, GKVK, Bengaluru, during 2016-2017. The experiments were comprised of ten treatments and they were replicated thrice in RCBD. The maximum *Bradyrhizobium japonicum* (13.17 CFU) and *Pseudomonas fluorescens* population (11.58 CFU) was recorded with seed treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* with application of full dose of RDF and higher nitrogen and phosphorous content of the soil (242.16, 51.46 kg ha^{-1} respectively) was obtained with seed treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* with application of full dose of RDF. Whereas, the potassium in soil was found higher (170.82 kg ha^{-1}) with control. The higher B: C Ratio was found with seed treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* with application of full dose of RDF.

Keywords: Shankapushpi, *Bradyrhizobium japonicum* and *Pseudomonas fluorescense*

Introduction
India with a rich biodiversity supports many systems of medicines by producing and exporting various medicinal plants approximately, one third of pharmaceutical are plant origin and are used for reliving and curing ailments. Plants are the important source of medicine ever since the dawn of human civilization and in spite of tremendous developments in the field of allopathy during the 20th century, plants still remain as one of the major source of drugs in modern as well as traditional systems of medicine across the world (Ghosh., 1998) [2]. Approximately, one third of pharmaceuticals are of plant origin and are used for relieving and curing ailments (Dubey *et al*., 2004) [1].

Shankapushpi also known as butterfly pea is one of the important medicinal plants used for boosting memory and improving intellect and also to cure mental illness. It is a perennial leguminous twiner, botanically known 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.

Butterfly pea 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 axillary, 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 400 mm 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 (Zingare *et al.*, 2013) [7].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, demulcent and remedy for rheumatism, bronchitis, urino genital disorder and cancer (Subramanian and Prathyusha, 2011) [5]. The application of PGPR strains can provides an effective, economical and practical way of plant protection via disease suppression, P-solubilization, phytohormone production *etc.* The PGPR strain mixtures often show synergistic action in plant protection and growth promotion involving many mechanisms (Zahir and Arshad, 2004) [6].

Materials 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 discussion

Significantly maximum *Bradyrhizobium japonicum* population was recorded with seed treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* and application full dose of RDF (13.17CFU) and was followed by seed treatment of *Bradyrhizobium japonicum* and application of full dose of RDF (11.25CFU). While, the lowest population of *Bradyrhizobium japonicum* was observed with control (2.17CFU) and significant maximum *Pseudomonas fluorescens population* was recorded with seeds treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* and application full dose of RDF (11.58CFU). While, the minimum *Pseudomonas fluorescens* population was observed with control (2.37CFU). This might be due to synergistic effect among these microbial populations and congenial microclimate for their reproduction. Harish (2010) [3] have reported similar results in garden cress (*Lepidium sativum* L.). Table 1.

There is no significant difference among the treatments with respect to pH, electrical conductivity and organic carbon content of the soil under rain fed condition. The nitrogen in soil was maximum (242.16kg ha^{-1}) with seed treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* and application of full dose RDF and was on par with application of full dose of RDF, seed treatment of *Bradyrhizobium japonicum* + full dose of RDF, and seed treatment of *Pseudomonas fluorescens*+ full dose of RDF (226.05, 229.37 and 221.20kgha^{-1}, respectively). While, the lowest Nitrogen (188.13kgha^{-1}) was found in soil when seeds were treated with *Pseudomonas fluorescens* and application of 50 per cent RDF under rain fed condition. The Phosphorous in soil was maximum (51.46) with seed treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* and application of full dose RDF and was *on par* with application of full dose of RDF, seed treatment of *Bradyrhizobium japonicum* + full dose of RDF, seed treatment of *Pseudomonas fluorescens*+ *Bradyrhizobium japonicum* and application of 75 per cent RDF and seed treatment of *Pseudomonas fluorescens* and application of 75 per cent RDF (48.45, 48.25, 46.17 and 47.25 kg ha^{-1}, respectively). While, the lowest Phosphorous (40.40 kg ha^{-1}) was found in soil when seeds were treated with *Bradyrhizobium japonicum* and application of 50 per cent RDF under rainfed condition. The potassium in soil was maximum (170.82kgha^{-1}) application of full dose RDF and was on par with with the seed treatment of *Bradyrhizobium japonicum*+ full dose of RDF, seed treatment of *Pseudomonas fluorescens*+ full dose of RDF and seed treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* and application of full dose RDF (170.62, 170.80 and 170.15 kg ha^{-1}, respectively).While, the lowest potassium (130.00 kg ha^{-1}) was found in soil when seeds were treated with *Pseudomonas fluorescens* and application of 50 per cent RDF under rain fed condition. The higher available nitrogen, phosphorus and potassium content may be due to biological nitrogen fixation, phosphorus solubilization and increasing availability of potassium. These findings are in support with the work carried out by Harish (2010) [3] in garden cress (*Lepidium sativum* L.) Table 2.

The maximum net profit of Rs. 74171 per hectare was obtained from seed treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* with full dose of RDF under rain fed situation. While, the least profit of Rs.38935 per hectare was obtained with seed treatment of *Pseudomonas fluorescens* and application of 50 per cent RDF under rain fed condition and the maximum benefit per rupee invested 1.21 was obtained from shankapushpi seeds treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* and application of full dose of RDF under rain fed situation as compared to seed treatment of *Pseudomonas fluorescens* and application of 50 per cent RDF (0.69) rain fed condition. These results are similar to the results reported by Jat *et al*. (2004) in fenugreek, Patel *et al*. (2011) [4] in fenugreek and Harish (2010) [3] in garden cress (Table 3).

Table 1: Influence of plant growth promoting rhizobacteria on load of *Bradyrhizobium japonicum* and *Pseudomonas fluorescens* in the soil after harvest of *Clitorea ternatea* L.

Treatments	*Bradyrhizobium japonicum* (No.×10^6 CFU of soil)	*Pseudomonas fluorescens* (No.×10^6 CFU of soil)
T_1 -RDF (control)	2.17	2.37
T_2 RDF + *Bradyrhizobium japonicum*	11.25	9.50
T_3 -RDF + *Pseudomonas fluorescens*	10.08	10.17
T_4 -RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	13.17	11.58
T_5 -75% RDF + *Bradyrhizobium japonicum*	7.25	6.67
T_6 -75% RDF + *Pseudomonas fluorescens*	6.83	5.17
T_7 -75% RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	8.17	7.67
T_8 -50% RDF + *Bradyrhizobium japonicum*	4.67	3.75
T_9 -50% RDF + *Pseudomonas fluorescens*	3.17	3.20
T_{10} -50% RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	5.50	4.67
F test	*	*
S.Em±	0.11	0.08
CD at 5%	1.29	0.25

Table 2: Effect of plant growth promoting rhizobacteria on chemical properties of soil after harvest of *Clitorea ternatea* L. under rain fed condition

Treatments	pH	EC (dSm^{-1})	OC (%)	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
T_1 -RDF (control)	6.62	0.15	0.55	226.05	48.45	170.82
T_2 -RDF + *Bradyrhizobium japonicum*	6.79	0.12	0.60	229.37	48.25	170.62
T_3 -RDF + *Pseudomonas fluorescens*	6.76	0.15	0.58	221.20	50.42	170.80
T_4 -RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	6.80	0.15	0.61	242.16	51.46	170.15
T_5 -75% RDF + *Bradyrhizobium japonicum*	6.69	0.14	0.57	210.24	42.47	143.13
T_6 -75% RDF + *Pseudomonas fluorescens*	6.64	0.14	0.56	212.34	47.25	143.04
T_7 -75% RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	6.67	0.15	0.59	214.34	46.17	144.62
T_8 -50% RDF + *Bradyrhizobium japonicum*	6.57	0.18	0.53	191.87	40.40	133.09
T_9 -50% RDF + *Pseudomonas fluorescens*	6.54	0.17	0.51	188.13	43.34	130.00
T_{10} -50% RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	6.54	0.19	0.54	190.13	42.44	132.48
F test	-	-	-	*	*	*
S.EM±	0.00	0.00	0.00	7.45	2.43	4.21
CD at 5%	NS	NS	NS	22.34	6.23	13.43

Table 3: Influence of plant growth promoting rhizobacteria on cost of cultivation, gross, net returns and B: C ratio in *Clitorea ternatea* L. under rain fed condition

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
Seeds	**2000**	**2000**	**2000**	**2000**	**2000**	**2000**	**2000**	**2000**	**2000**	**2000**
Manures, fertilizers and PGPR	34969	35049	35049	35129	32557	32557	32637	30065	30065	30145
Tractor hire charges (8H)	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000
Total labour required	20000	20000	20000	20000	20000	20000	20000	20000	20000	20000
Cost of cultivation (Rs ha^{-1})	60969	61049	61049	61129	58557	58557	58637	56065	56065	56145
Yield (Q Kg ha^{-1})	11.20	12.83	12.68	13.53	11.80	11.50	12.00	9.65	9.50	10.02
Gross return (Rs ha^{-1})	112000	128300	126800	135300	118000	115000	120000	96500	95000	100200
Net return (Rs ha^{-1})	51031	67251	65751	74171	59443	56443	61363	40435	38935	44055
B:C Ratio	0.84	1.10	1.08	1.21	1.02	0.96	1.05	0.72	0.69	0.78

Conclusion

The present investigation reveals that, the seed treatment with *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* and application of full dose of RDF has resulted in significantly found maximum *microbial* load of the soil, chemical properties of soil, B:C Ratio and Net return in shankapushpi under rain fed condition.

Acknowledgement

The author is thankful to Dr. A.P. Mallikarjuna Gowda, Seiner scientist and Head, KVK Doddabalapura, GKVK, Bengaluru for his constant inspiration and immense support.

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