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Effect of plant growth promoting rhizobacteria on soil microbial load and nodulation at different growth stages in *Clitorea ternatea* L. under irrigation condition

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Abstract
A field experiment was conducted to assess the Effect of Plant Growth Promoting Rhizobacteria on soil microbial load and nodulation at different growth stages in *Clitorea ternatea* L. under irrigation condition 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 *Bradyrhizobium japonicum* (13.75 CFU) and *Pseudomonas fluorescence* population (14.18 CFU) was recorded with seed treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescence* with application of full dose of RDF and number of nodules (3.51, 19.14 and 26.15), fresh weight of nodules (136.69 mg, 860.67 mg and 1196.40 mg) and dry weight of nodules (50.21 mg, 284.40 mg and 563.06 mg) are found maximum at 30, 60 and 90 days after sowing respectively, with seed treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescence* with application of full dose of RDF.

Keywords: 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. 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 know 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 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.

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) [6]. 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 (Subramanian and Prathyusha, 2011) [4]. 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) [5].

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 (13.75 CFU) and was followed by seed treatment of *Bradyrhizobium japonicum* and application of full dose of RDF (12.55 CFU). While, the lowest population of *Bradyrhizobium japonicum* was observed with control (4.18 CFU) and significant maximum *Pseudomonas fluorescens population* was recorded with seeds treatment of *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* and application full dose of RDF (14.18 CFU). While, the minimum *Pseudomonas fluorescens* population was observed with control (4.63 CFU) 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 was a positive and significant improvement in nodulation due to treatment effects. The seeds treated with combination of two plant growth promoting rhizobacteria along with the application full dose of NPK has recorded the significantly maximum number of nodules (3.51, 19.14 and 26.15 at 30, 60 and 90 days after sowing, respectively). It was followed by seed treatment of *Bradyrhizobium japonicum* and application of full dose RDF (3.34, 17.84 and 24.29 at 30, 60 and 90 days after sowing, respectively). While, the lesser number of nodules (1.84, 6.12 and 10.12 at 30, 60 and 90 days after sowing, respectively) was recorded with seed treatment of *Pseudomonas fluorescens* and application of 50 per cent RDF. Similarly, the seeds treated with *Bradyrhizobium japonicum* + *Pseudomonas fluorescens* and application of full dose of RDF has recorded significantly maximum fresh weight of nodules (136.69 mg, 860.67 mg and 1196.40 mg at 30, 60 and 90 days after sowing, respectively). It was followed by seed treatment of *Bradyrhizobium japonicum* and application of full dose RDF (117.95 mg, 796.57 mg and 1192.92 mg at 30, 60 and 90 days after sowing, respectively). While, the lesser fresh weight of nodules (51.21 mg, 242.08 mg and 419.33 mg at 30, 60 and 90 days after sowing, respectively) was recorded with seed treatment of *Pseudomonas fluorescens* and application of 50 per cent RDF under irrigated condition. Whereas, maximum dry weight of nodules (50.21 mg, 284.40 mg and 563.06 mg at 30, 60 and 90 days after sowing, respectively) was obtained with seeds treated with combination of two plant growth promoting rhizobacteria along with the application full dose of RDF and It was followed by seed treatment of *Bradyrhizobium japonicum* and application of full dose RDF (49.56 mg, 272.80 mg and 398.30 mg at 30, 60 and 90 days after sowing, respectively). While, the lesser dry weight of nodules (30.76 mg, 83.05 mg and 143.44 mg at 30, 60 and 90 days after sowing, respectively) was recorded with seed treatment of *Pseudomonas fluorescens* and application of 50 per cent RDF under irrigated condition The increased number of nodules may be attributed that, the *Bradyrhizobium japonicum* helped in nitrogen fixation and colonize the rhizosphere of the plant. Further, Pseudomonas play a vital role in cell division, photosynthesis and root growth. Harish (2010) [3] have reported similar results in garden cress (*Lepidium sativum* L.) Table 2.

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

Treatments	***Bradyrhizobium japonicum*** **(No.×10^6 CFU of soil)**	***Pseudomonas fluorescence*** **(No.×10^6 CFU of soil)**
T_1 - RDF (control)	3.45	3.32
T_2 - RDF + *Bradyrhizobium japonicum*	12.55	11.83
T_3 - RDF + *Pseudomonas fluorescens*	11.40	13.08
T_4 - RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	13.75	14.18
T_5 - 75% RDF + *Bradyrhizobium japonicum*	8.90	9.08
T_6 - 75% RDF + *Pseudomonas fluorescens*	7.08	7.00

T_7 - 75% RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	10.33	10.25
T_8 - 50% RDF + *Bradyrhizobium japonicum*	5.22	5.15
T_9 - 50% RDF + *Pseudomonas fluorescens*	4.18	4.63
T_{10} - 50% RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	6.07	6.63
F test	*	*
S.Em±	0.13	0.07
CD at 5%	0.38	0.20

Table 2: Influence of plant growth promoting rhizobacteria on nodulation at different growth stages in *Clitorea ternatea* L. under irrigation condition

Treatments	Number of nodules			Fresh weight of nodules (mg)			Dry weight of nodules (mg)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1 - RDF (control)	2.31	13.2	17.07	63.73	538.77	688.11	33.49	194.43	234.35
T_2 - RDF + *Bradyrhizobium japonicum*	3.34	17.84	24.29	117.95	796.57	1192.92	49.56	272.80	398.3
T_3 - RDF + *Pseudomonas fluorescens*	3.07	16.8	23.17	117.88	735.44	1059.87	45.86	231.46	376.55
T_4 - RDF+ *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	3.51	19.14	26.15	136.69	860.67	1196.40	50.21	284.40	563.06
T_5 - 75% RDF + *Bradyrhizobium japonicum*	2.58	14.83	19.32	112.82	623.8	860.17	34.58	210.78	285.35
T_6 - 75% RDF + *Pseudomonas fluorescens*	2.47	13.77	18.27	106.67	561.67	852.27	33.75	205.84	251.57
T_7 - 75% RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	2.81	16.13	21.93	110.47	685.27	787.89	40.84	223.96	366.07
T_8 - 50% RDF + *Bradyrhizobium japonicum*	2.14	13.13	16.23	104.34	536.64	655.8	32.38	191.32	226.94
T_9 - 50% RDF + *Pseudomonas fluorescens*	1.84	6.12	10.12	51.21	242.08	419.33	30.76	83.05	143.44
T_{10} - 50% RDF + *Bradyrhizobium japonicum* + *Pseudomonas fluorescens*	2.2	12.75	16.27	74.8	542.9	643.84	38.11	198.17	216.36
F test	*	*	*	*	*	*	*	*	*
S.Em±	0.06	8.99	2.43	0.07	2.85	2.34	0.07	8.57	5.98
CD at 5%	0.17	26.70	7.22	0.21	8.47	6.94	0.20	25.47	17.76

Note: DAS- Days After Sowing

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 obtained maximum *microbial* load of the soil and root nodules at different growth stages in shankapushpi under irrigated condition.

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