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Combine Inoculation Effect of Pink Pigmented Facultative *Methylotrophs* and *B. Japonicum* on Plant Growth and Yield of Soybean

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ABSTRACT

A field experiment was conducted during kharif 2017-18 was carried out at Department of Plant Pathology and Agricultural Microbiology, PGI MPKV, Rahuri.(Maharashtra) to study the combine effect of inoculation of Methylobacterium and B.Japonicum and foliar spray of Methylobacterium isolates on plant growth and yield of soybean under field conditions. All growth and yield parameters of soybean as well as the population of Methylobacterium were influenced by the (S.T of Rhizobium +S.T of PPFMs +Foliar spray consortium of PPFMs + 100 % N) which was significantly superior over rest of all the treatments and it was at par with treatment (S.T of Rhizobium +S.T of PPFMs +Foliar spray consortium of PPFMs + 75 % N) during flowering and harvesting stage. This shows that the consortium of efficient strain of PPFMs could reduce N fertilizer application in field condition.

Keyword: *Methylobacterium, Pink pigmented facultative methylotrophs, Soybean.*

INTRODUCTION

In a recent years, the pink pigmented facultative methylotrophs (PPFMs) is one of such a bacteria receiving more attention as a plant growth promoting bacteria. They are known to play an important role in increasing crop yields and land fertility. These are physiologically an interesting group of bacteria able to grow on methanol, methylamine as well as on a variety of C_2, C_3 and C_4 compounds as sole sources of carbon and energy (Lidstrom, 1992), potentially dominating the *phyllosphere* population. They are commonly found in soil as well as on the surfaces of leaves, seeds and in the rhizosphere of a wide variety of plants (Holland & Polacco, 1994; Chanprame et al., 1996; Holland, 1997; & Shepelyakovskaya et al., 1999).

The association of PPFMs with plant possesses an associative symbiotic relationship in which PPFMs utilizes the methanol emitted from leaves of plants as sole carbon and energy source.

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In response, PPFMs produces plant growth promoting substance such as Indole acetic acid (IAA), Gibberlic acid (GA) etc which are known to stimulate plant growth (Ivanova et al., 2001 & Koenig et al., 2002); fix the atmospheric nitrogen (Sy et al., 2001); solubilise mineral phosphate (Jones et al. 2007); induce systemic resistance against plant pathogens (Madhaiyan et al., 2006) and chelation of inorganic compound such as iron. Due to these importance, the PPFMs have received a great deal of attention as bioinoculants for use in agriculture. Combined use of two or three beneficial microorganisms as inoculation have been found to perform better than single inoculations (Alagawadi & Gaur, 1988; Jisha & Alagawadi, 1996; & Prathibha et al., 1995). Therefore the present study was undertaken to study the effect of combined inoculation PPFMs (*Methylobacterium*) and *B Japonicum* on growth and yield of soybean.

MATERIALS AND METHODS

The PPFM consortium was prepared by using Sterilized Ammonium mineral salt (AMS) broth and base material such as Fe. EDTA, Arabinose, Glycerol, PVP and Trehalose and was inoculated with loopful of efficient isolates of *Methylobacterium* cultures and kept in temperature controlled shaker (150 rpm/min) at 30^0C for 5 days. After attaining the full growth (10^{-9} cfu/ml) it was diluted to1:100(1%) and sprayed @ 3.0 lit/ha after 45 days of sowing. The consortium of *B.Japonicum* was obtain from the Department of plant pathology and Agricultural microbiology, MPKV, Rahuri The treatments were as follows.

Treatment No.	**Treatment details**
T_1	Absolute control
T_2	Seed Treatment of *B.Japonicum*.
T_3	S.T.of *B.Japonicum* +S.T.of Reference strain + F.S. of Reference strain
T_4	S.T.of *B.Japonicum* +S.T.of Reference strain + F.S. of Reference strain +50%N
T_5	S.T.of *B.Japonicum* +S.T.of Reference strain + F. S. of Reference strain+75%N
T_6	S.T.of *B.Japonicum* +S.T.of Reference strain of PPFM+ F.S. of Reference strain +100%N
T_7	S.T.of *B.Japonicum* +S.T.of PPFM+ F.S. of PPFM
T_8	S.T.of *B.Japonicum* +S.T.of PPFM + F.S. of PPFM +50%N
T_9	S.T.of *B.Japonicum* +S.T.of PPFM+ F.S. of PPFM +75%N
T_{10}	S.T.of *B.Japonicum* +S.T.of PPFM + F.S.of PPFM+100%N
T_{11}	Only 100%N

RESULT AND DISCUSSION

The field experiment was conducted on at the research farm of Department of Plant Pathology and Agricultural Microbiology, MPKV, Rahuri, on soil with PH 8.04.The available nitrogen, available phosphorus and available potassium were 170.8, 16.12 and 370.20.There were 11 treatment combinations with three replications for each treatment following the method of Randomized block design (RBD) The observations on plant growth and PPFMs population were taken during flowering and harvesting stage. The population of PPFMs in *phyllosphere* and *rhizosphere* was enumerated by serial dilution plate count method.

Table 1: Effect of liquid consortium of PPFMs on plant height and shoot and root length and root length and height of soybean during flowering and harvesting stage

Treatment		Plant height (cm)		Shoot length (cm)		Root length (cm)	
		(F)	(H)	(F)	(H)	(F)	(H)
T_1	Absolute control	29.63	33.84	9.02	17.66	36.85	42.04
T_2	Seed treatment of *B.Japonicum*	30.91	36.70	10.64	18.80	38.48	44.11
T_3	S.T of *B.Japonicum* +S.T of R.S+ F.S. of R.S.(PPFMs)	32.73	38.26	11.86	20.17	40.03	46.23
T_4	S.T of *B.Japonicum* +S.T of R.S+ F.S. of R.S+50%N	37.18	41.25	14.05	21.72	42.65	48.88
T_5	S.T of *B.Japonicum* +S.T of R.S + F.S. of R.S +75%N	42.51	47.01	17.97	24.39	48.03	54.29
T_6	S.T of *B.Japonicum* +S.T of R.S + F.S. of R.S +100%N	43.59	48.66	18.02	25.05	49.31	56.56
T_7	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs	36.16	40.49	13.01	21.26	41.94	48.31
T_8	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs +50%N	38.77	43.24	15.66	22.83	45.56	50.92
T_9	S.T of *B.Japonicum* +S.T of PPFMs + F.S. of PPFMs+75%N	44.98	51.88	19.57	26.96	52.17	59.77
T_{10}	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs+100%N	45.71	52.37	20.54	27.94	53.12	61.61
T_{11}	Only 100%N	39.04	44.45	16.70	23.27	46.46	52.04
S.Em ±		0.52	0.40	0.58	0.38	0.36	0.68
CD at 5%		1.54	1.18	1.70	1.13	1.05	2.01

F-Flowering stage, H-Harvesting stage, S.T:-Seed Treatment, F.S-Foliar spray

In present study, significant differences were observed between various treatments by applications of PPFMs and *Rhizobium* consortium under graded levels of nitrogenous fertilizers on all growth attributing parameters viz, plant height, root and shoot length, fresh and dry weight of shoot and root, number of effective and non- effective nodules, of soybean during both flowering and harvesting stage. The *phyllosphere* as well as *rhizosphere* PPFMs population along with no. of pods per plant, stover yield and grain yield also shows similar trend. All the above growth parameters along with PPFMs population and the no. of pods per plant, stover and grain yield of soybean influenced by the treatment T_{10} (S.T of *Rhizobium* +S.T of PPFMs +Foliar spray consortium of PPFMs + 100 % N) which was significantly superior over rest of all the treatments and it was at par with treatment T_9 (S.T of *Rhizobium* +S.T of PPFMs +Foliar spray consortium of PPFMs + 75 % N) in respect of their influence on all growth attributing character at flowering and harvesting stage.

It is also found that the treatment T_7 (S.T of *Rhizobium* +S.T of PPFMS+ Foliar spray consortium of PPFMs) recorded all the above parameters which was significantly higher over T_2 (Seed treatment of *Rhizobium*) (86.75) treatment.

Table 2: Effect of liquid consortium of PPFMs on shoot and root fresh weight of soybean during flowering and harvesting stage

Treatment		Shoot fresh weight		Root fresh weight	
		(F)	(H)	(F)	(H)
T_1	Absolute control	19.18	6.76	4.32	2.01
T_2	Seed treatment of *B.Japonicum*	20.98	8.20	5.49	2.90
T_3	S.T of *B.Japonicum* +S.T of R.S+ F.S. of R.S.(PPFMs)	22.55	9.58	6.56	3.81
T_4	S.T of *B.Japonicum* +S.T of R.S+ F.S. of R.S+50%N	25.87	11.09	8.01	5.06
T_5	S.T of *B.Japonicum* +S.T of R.S + F.S. of R.S +75%N	31.59	15.55	11.35	6.94
T_6	S.T of *B.Japonicum* +S.T of R.S + F.S. of R.S +100%N	32.50	16.38	11.60	7.01
T_7	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs	24.65	10.94	7.72	4.73
T_8	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs +50%N	28.51	12.49	9.39	5.97
T_9	S.T of *B.Japonicum* +S.T of PPFMs + F.S. of of PPFMs+75%N	34.05	18.77	12.93	8.01
T_{10}	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs+100%N	34.60	19.89	13.92	8.89
T_{11}	Only 100%N	29.80	13.75	10.31	6.02
S.Em ±		0.51	0.45	0.34	0.29
CD at 5%		1.50	1.34	1.02	0.87

F-Flowering stage, H-Harvesting stage, S.T:-Seed Treatment, F.S-Foliar spray

The superiority of T_{10} treatment is due the high dose of nitrogenous fertilizer and combine beneficial effect of both *Rhizobium* and PPFMs. The increase in growth parameters is due to the as per increase in graded dose nitrogenous fertilizer as reported by Ntambo et al. (2017). The Krushnanjali (2017) reported that combine effect of *Rhizobium* along with increase in graded doses of nitrogenous fertilizer results in increase in the growth parameters of the plant.

The significant improvement found in the growth parameter such as height of the plant, development of root and shoot, and nodulation and yield parameters of the crop in the treatment inoculated with *Rhizobium* and PPFMs. This is due to combine beneficial effect of these two organisms. Rao and Dhir, (1993) reported that the presence of *Rhizobia* in the legume *rhizosphere* influencing the legume roots to release plant growth and promoting substances which, in turn might have enhanced the growth of *Methylobacterium in situ* and synergistic effect might have occurred in treatments.

Table 3: Effect of liquid consortium of PPFMs on shoot and root dry weight of soybean during flowering and harvesting stage

Treatment		Shoot dry weight		Root dry weight	
		(F)	(H)	(F)	(H)
T_1	Absolute control	1.11	0.36	0.15	0.13
T_2	Seed treatment of *B.Japonicum*	1.37	0.46	0.23	0.19
T_3	S.T of *B.Japonicum* +S.T of R.S+ F.S. of R.S.(PPFMs)	1.57	0.55	0.30	0.25
T_4	S.T of *B.Japonicum* +S.T of R.S+ F.S. of R.S+50%N	2.16	0.67	0.38	0.32
T_5	S.T of *B.Japonicum* +S.T of R.S + F.S. of R.S +75%N	2.67	1.12	0.54	0.45
T_6	S.T of *B.Japonicum* +S.T of R.S + F.S. of R.S +100%N	2.70	1.15	0.55	0.46
T_7	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs	2.10	0.64	0.37	0.31
T_8	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs +50%N	2.40	0.76	0.45	0.38
T_9	S.T of *B.Japonicum* +S.T of PPFMs + F.S. of of PPFMs+75%N	2.84	1.28	0.62	0.52
T_{10}	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs+100%N	2.95	1.31	0.66	0.53
T_{11}	Only 100%N	2.47	0.80	0.47	0.39
S.Em ±		0.04	0.03	0.02	0.02
CD at 5%		0.13	0.08	0.06	0.05

F-Flowering stage, H-Harvesting stage, S.T:-Seed Treatment, F.S-Foliar spray

The development of root and shoot is due to the action of plant growth promoting substances such as IAA and GA produced by the PPFMs. This has been supported by Suresh Reddy, 2002 who reported that these growth promoters allows a balanced growth of shoot and root system. When roots become more extensive due to the action of IAA, then the cytokinins of the plant signals the shoot system to form more branches.

The results also supported by some workers such as Senthilkumar, 2003; Madhaiyan et al., 2009. They reported that the plant growth promoters such as IAA and GA also enhanced root growth enabling the plants to absorb more nutrient in soybean ultimately resulting in better growth, dry matter production.

Table 4: Effect of liquid consortium of PPFMs on number of effective and non-effective nodule of soybean during flowering and harvesting stage

		Number of effective nodule (Plant $^{-1}$)		Number of non-effective nodule (Plant $^{-1}$)	
		(F)	(H)	(F)	(H)
T_1	Absolute control	14.67	4.33	18.00	24.00
T_2	Seed treatment of *B.Japonicum*	16.33	5.67	16.67	22.33
T_3	S.T of *B.Japonicum* +S.T of R.S+ F.S. of R.S.(PPFMs)	17.67	7.00	15.33	20.67
T_4	S.T of *B.Japonicum* +S.T of R.S+ F.S. of R.S+50%N	19.67	8.67	12.33	18.67
T_5	S.T of *B.Japonicum* +S.T of R.S + F.S. of R.S +75%N	23.67	12.33	9.00	13.00
T_6	S.T of *B.Japonicum* +S.T of R.S + F.S. of R.S +100%N	24.67	13.33	8.00	12.67
T_7	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs	19.33	8.33	12.67	19.00
T_8	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs +50%N	21.67	10.00	10.67	16.33
T_9	S.T of *B.Japonicum* +S.T of PPFMs + F.S. of of PPFMs+75%N	26.00	16.67	6.33	10.67
T_{10}	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs+100%N	27.00	17.00	5.33	9.67
T_{11}	Only 100%N	22.33	10.33	10.33	15.67
S.Em ±		0.38	0.43	0.37	0.44
CD at 5%		1.13	1.27	1.09	1.29

F-Flowering stage, H-Harvesting stage, S.T:-Seed Treatment, F.S-Foliar spray

The maximum nodule number may be attributed to the presence of flavonoid compounds taken up by the roots of soybean plants. This has been supported by Subba Rao who reported that the flavonoid compounds secreted by the roots of soybean plants helps to release naringenin, genistein and diadzein (iso flavones).So these flavonoid molecules by legumes can either induce or block the transcription of *nod* genes in *rhizobium* and *Bradyrhizobium* resulting in increased nodulation as well as nitrogen fixation .

Similar results were shown by Radha (2007) and Meenakshi (2009) Radha et al. (2009) reported that the combined inoculation of *Methylobacterium sp* with *Rhizobium sp* .significantly increased plant growth parameters such as root and shoot development, number of leaves, nodulation, compared with individual inoculation of *Rhizobium* sp or uninoculated control. While, Meenakshi (2008) reported significantly higher number of leaves, shoot dry weight and root dry weight and nodulation was recorded in the treatments that received *Bradyrhizobium* and PPFM spray than *Bradyrhizobium* alone and uninoculated control.

Table 5: Effect of liquid consortium of PPFMs on PPFM count in phyllosphere and rhizosphere of soybean during flowering and harvesting stage

		PPFM Count (*Phyllosphere*) (x10^6cfu/g)		PPFM Count (*Rhizosphere*) (x10^3cfu/g)	
		(F)	(H)	(F)	(H)
T_1	Absolute control	85.06	12.01	10.33	3.10
T_2	Seed treatment of *B.Japonicum*	87.77	14.02	11.96	4.17
T_3	S.T of *B.Japonicum* +S.T of R.S+ F.S. of R.S.(PPFMs)	89.82	15.07	13.56	5.11
T_4	S.T of *B.Japonicum* +S.T of R.S+ F.S. of R.S+50%N	94.46	17.08	15.77	6.11
T_5	S.T of *B.Japonicum* +S.T of R.S + F.S. of R.S +75%N	99.82	20.19	19.37	7.98
T_6	S.T of *B.Japonicum* +S.T of R.S + F.S. of R.S +100%N	100.78	21.01	20.05	8.48
T_7	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs	92.58	16.46	14.79	6.02
T_8	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs +50%N	96.80	18.23	17.62	7.03
T_9	S.T of *B.Japonicum* +S.T of PPFMs + F.S. of of PPFMs+75%N	103.04	22.57	21.86	9.74
T_{10}	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs+100%N	105.01	23.52	22.95	10.06
T_{11}	Only 100%N	97.65	19.04	18.14	7.07
S.Em ±		0.68	0.35	0.38	0.30
CD at 5%		2.01	1.03	1.13	0.89

In case of *phyllosphere* and *rhizosphere* PPFM population. The results are mainly due to the growth hormone production by *Methylobacterium* sp. especially high cytokinin production in apical plant tissues and *rhizosphere* soil. The another reason behind that the foliar spraying PPFMs which significantly influenced the PPFMs population in the foliar region such as *phyllosphere*. of rice crop as reported by Holland, (1997b).

However, the *phyllosphere* region of the soybean recorded the highest PPFMs load compared to soybean *rhizosphere*. The increased population on the leaves is due to the fact that they utilize the gaseous methanol. This has been supported by Nemecek-marshal et al. 1995 and Daniel et al. (2006). They reported that the PPFMs population utilize gaseous methanol emitted by the stomata of the leaves of the plants during leaf expansion by pectin demethylation as carbon and energy source and promote the growth of their host through the release of metabolites.

Table 6: Effect of liquid consortium of PPFMs on pods, grain and Stover yield of soybean during flowering and harvesting stage

Treatment		No of Pods plant^{-1}	Grain yield (qha^{-1})	Stover yield (qha^{-1})
T_1	Absolute control	39.95	13.01	16.27
T_2	Seed treatment of *B.Japonicum*	45.71	15.39	18.60
T_3	S.T of *B.Japonicum* +S.T of R.S+ F.S. of R.S.(PPFMs)	48.55	16.42	21.07
T_4	S.T of *B.Japonicum* +S.T of R.S+ F.S. of R.S+50%N	52.41	18.36	23.04
T_5	S.T of *B.Japonicum* +S.T of R.S + F.S. of R.S +75%N	60.85	21.57	26.29
T_6	S.T of *B.Japonicum* +S.T of R.S + F.S. of R.S +100%N	61.18	22.01	27.07
T_7	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs	51.24	16.78	22.45
T_8	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs +50%N	55.01	19.08	24.35
T_9	S.T of *B.Japonicum* +S.T of PPFMs + F.S. of of PPFMs+75%N	63.98	23.78	28.12
T_{10}	S.T of *B.Japonicum* +S.T of PPFMs+ F.S. of PPFMs+100%N	65.06	24.13	29.22
T_{11}	Only 100%N	57.50	20.50	25.19
S.Em ±		0.87	0.35	0.35
CD at 5%		2.58	1.03	1.04

The increase in yield parameters in the treatment containing 100% N is due to as per graded dose nitrogenous fertilizer reported by Krushnajali (2017).

The Krushnajali (2017) and Ntambo et al 2017 both worker reported that increase in graded doses of nitrogenous fertilizer results in increase in the yield parameters of the plant. The superior performance of the soybean inoculated with PPFMs and *Rhizobium* isolates may be due to the cumulative effect of increased plant growth substances, enhanced nutrient uptake, nitrogen fixation and control of plant pathogen by sidrophore production resulting in higher yield of soybean crop.

This shows that the combination of *Rhizobium* and PPFMs along with 75%N enhances the all growth parameters, as well as yield of soybean crop concluding that there is a possibility of saving fertilizer nitrogen to an extent of 25% to soybean crop. It is also found that the PPFMs consortium in combination with *Rhizobium* without any nitrogen dose shows significant improvement in growth attributing characters and yield of soybean as compared to the *Rhizobium* alone indicating the importance of dose of PPFMs consortium along with *Rhizobium* as compared to *Rhizobium* alone.

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