

## Combine Inoculation Effect of Pink Pigmented Facultative methylotrophs and *B. japonicum* on Nutrient Status of Soybean

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Received: 29.08.2020 | Revised: 8.10.2020 | Accepted: 13.10.2020

### 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 nutrient status of soybean under field conditions. The available nitrogen in soil and the uptake of nutrients (N P K )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 clearly indicate that the PPFMs along with the *B Japonicum* plays an imp role in reducing N fertilizer application in field condition. There is also a positive correlation between PPFMs and available nitrogen as well as uptake of nutrients along with the yield of crop which clearly shows that the application of efficient strain of PPFMs also increases the available nitrogen as well as uptake of nutrients along with the yield of crop.

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

### INTRODUCTION

As a result of the green revolution, there have been notable increases in agricultural production with the use of technology that released crop cultivation from cultural, religious, and environmental constraints. Chemical fertilizers, an essential component of modern agriculture, supply nutrients required for optimal crop improvement that are not available in the soil and or from other

available organics sources. Excessive use of chemical fertilizer has an “environmental footprint”: It diminishes crop yield, destroys the soil nutrient balance, leads to poor soil quality in terms of physiochemical properties, and gives rise to a variety of plant diseases. The use of chemical fertilizers is not only costly but also hazardous for both the environment and humans.

**Cite this article:** Govekar, Y. R., & Navale, A. M. (2020). Combine Inoculation Effect of Pink Pigmented Facultative methylotrophs and *B. japonicum* on Nutrient Status of Soybean, *Ind. J. Pure App. Biosci.* 8(5), 429-435. doi: <http://dx.doi.org/10.18782/2582-2845.8368>

In addition, manufacturing of such fertilizers deplete nonrenewable natural resources. Thus, there should be a biological substitute for chemical fertilizers that will efficiently provide nitrogen and phosphorus to plants and prevent the depletion of soil fertility and soil quality. In a recent years biofertilizers has emerged as a commercial alternative to chemical fertilizers to improve soil fertility and crop production in a sustainable farming. It reduces the dose of chemical fertilizer. Beside this, it also reduces adverse environmental effects, diseases and promote plant growth. Bioinoculants are microbial preparations of a single or consortia of living microorganisms. So far considerable number of bacterial bioinoculants mostly associated with the plant *rhizosphere* and *Phyllosphere* have been found to be beneficial to plant growth, yield and crop quality These groups of bacteria are known as plant growth promoting rhizobacteria (PGPR) which include strains from genera such as PPFMs and *Rhizobium*.

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. 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 nutrients status of soybean.

## MATERIALS AND METHODS

The PPFMs 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°C for 5 days. After attaining the full growth ( $10^9$  cfu/ml) it was diluted to 1:100(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 chemical analysis of the soil available nutrients (NPK) and nutrient (N,P,K )uptake by plant is done by standard procedures at flowering and harvesting stage. The PPFMs population count was recorded by serial dilution technique. The field treatments were as follows.

Treatment No.	Treatment details
T <sub>1</sub>	Absolute control
T <sub>2</sub>	Seed Treatment of <i>B.Japonicum</i> .
T <sub>3</sub>	S.T.of <i>B.Japonicum</i> +S.T.of Reference strain + F.S. of Reference strain
T <sub>4</sub>	S.T.of <i>B.Japonicum</i> +S.T.of Reference strain + F.S. of Reference strain +50%N
T <sub>5</sub>	S.T.of <i>B.Japonicum</i> +S.T.of Reference strain + F. S. of Reference strain+75%N
T <sub>6</sub>	S.T.of <i>B.Japonicum</i> +S.T.of Reference strain of PPFM+ F.S. of Reference strain +100%N
T <sub>7</sub>	S.T.of <i>B.Japonicum</i> +S.T.of PPFM+ F.S. of PPFM
T <sub>8</sub>	S.T.of <i>B.Japonicum</i> +S.T.of PPFM + F.S. of PPFM +50%N
T <sub>9</sub>	S.T.of <i>B.Japonicum</i> +S.T.of PPFM+ F.S. of PPFM +75%N
T <sub>10</sub>	S.T.of <i>B.Japonicum</i> +S.T.of PPFM + F.S.of PPFM+100%N
T <sub>11</sub>	Only 100%N

## RESULT AND DISCUSSION

The field experiment was conducted 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).

**Table 1: Effect of liquid consortium of PPFMs on soil available nutrients of soybean during flowering and harvesting stage**

Treatment		Soil available nutrient (Kgha <sup>-1</sup> )					
		Flowering stage			Harvesting stage		
		N	P	K	N	P	K
T <sub>1</sub>	Absolute control	167.03	13.03	325.96	139.10	10.02	322.49
T <sub>2</sub>	Seed treatment of <i>Rhizobium</i>	176.96	19.79	376.88	143.45	15.69	371.55
T <sub>3</sub>	S.T of <i>B.Japonicum</i> +S.T of R.S+ F.S. of R.S.	179.78	19.93	376.94	146.51	15.81	371.84
T <sub>4</sub>	S.T of <i>B.Japonicum</i> +S.T of R.S+ F.S. of R.S+50%N	184.78	20.13	377.69	149.23	16.13	372.19
T <sub>5</sub>	S.T of <i>B.Japonicum</i> +S.T of R.S+ F.S. of R.S+75%N	190.23	21.47	378.36	154.51	17.15	372.88
T <sub>6</sub>	S.T of <i>B.Japonicum</i> +S.T of R.S + F.S. of R.S +100%N	191.08	21.50	378.54	155.72	17.62	373.13
T <sub>7</sub>	S.T of <i>B.Japonicum</i> +S.T of PPFMs+ F.S. of PPFMs	183.36	20.01	377.37	148.58	15.49	371.88
T <sub>8</sub>	S.T of <i>B.Japonicum</i> +S.T of PPFMs+ F.S. of PPFMs +50%N	187.35	20.17	377.90	151.68	16.42	372.62
T <sub>9</sub>	S.T of <i>B.Japonicum</i> +S.T of PPFMs + F.S. of PPFMs+75%N	193.45	21.60	378.79	157.99	17.76	373.21
T <sub>10</sub>	S.T of <i>B.Japonicum</i> +S.T of PPFMs+ F.S. of PPFMs+100%N	194.03	21.85	378.86	158.24	17.83	373.78
T <sub>11</sub>	Only 100%N	188.23	20.40	378.04	152.40	16.87	372.53
S.Em ±		0.69	0.71	0.72	0.69	0.72	0.79
CD at 5%		2.02	2.10	2.12	2.03	2.14	2.34

F-Flowering stage, H-Harvesting stage, S.T:-Seed Treatment, F.S-Foliar spray, R.S. =Reference strain of PPFMs.

In present study, available nitrogen and total nutrient uptake (N,P,K) was influenced by the treatment T<sub>10</sub> (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. It was at par with treatment T<sub>9</sub> (S.T of *Rhizobium* +S.T of PPFMs +Foliar spray consortium of PPFMs + 75 % N) in respect of available nitrogen and uptake of nutrients (N,P,K) at flowering and harvesting stages. The treatment absolute control recorded the least nutrient status at flowering and harvesting stage.

The superiority of T<sub>10</sub> treatment is due the high dose of nitrogenous fertilizer and beneficial effect of both *Rhizobium* and PPFMs. The increase in higher available nitrogen is be may due to the graded dose nitrogenous fertilizer as reported by Krishnanjali 2017 and also due to

the nitrogen fixation in soil by PPFMs isolates without formation of nodulation as reported by Palanisamy Raja et al. (2006). The Krishnanjali, reported that combine effect of *Rhizobium* along with increase in graded doses of nitrogenous fertilizer results in increase in the available nitrogen in soil. While Palanisamy Raja et al. (2006) reported that the *Methylobacterium* able to fix atmospheric nitrogen in *phyllophere* without any formation of nodule.

Among the dual and single application, the treatment T<sub>7</sub> (S.T of *Rhizobium* +S.T of PPFMs+ Foliar spray consortium of PPFMs) was significantly higher available nitrogen and uptake of nutrients (N, P, K) as compared to T<sub>2</sub> (Seed treatment of *Rhizobium*) treatment at flowering and harvesting stage.

**Table 2: Effect of liquid consortium of PPFMs on N, P and K uptake by soybean during flowering and harvesting stage**

Treatment		Total nutrient uptake(Kgha <sup>-1</sup> )					
		Flowering stage			Harvesting stage		
		N	P	K	N	P	K
T <sub>1</sub>	Absolute control	50.13	4.01	12.01	66.48	20.01	53.86
T <sub>2</sub>	Seed treatment of <i>Rhizobium</i>	52.93	5.07	14.53	78.26	20.76	55.50
T <sub>3</sub>	S.T of <i>B.Japonicum</i> +S.T of R.S+ F.S. of R.S.	58.50	6.14	15.76	83.04	21.50	57.08
T <sub>4</sub>	S.T of <i>B.Japonicum</i> +S.T of R.S+ F.S. of R.S+50%N	65.05	7.82	17.61	95.96	23.04	59.66
T <sub>5</sub>	S.T of <i>B.Japonicum</i> +S.T of R.S+ F.S. of R.S+75%N	78.68	10.72	21.65	104.90	24.87	65.03
T <sub>6</sub>	S.T of <i>B.Japonicum</i> +S.T of R.S+ F.S. of R.S+100%N	79.76	11.68	22.94	104.92	25.79	66.32
T <sub>7</sub>	S.T of <i>B.Japonicum</i> +S.T of PPFMs+ F.S. of PPFMs.	63.85	7.25	16.98	91.82	22.78	58.95
T <sub>8</sub>	S.T of <i>B.Japonicum</i> +S.T of PPFMs+ F.S. of PPFMs +50%N	73.42	8.91	18.80	98.84	23.95	62.58
T <sub>9</sub>	S.T of <i>B.Japonicum</i> +S.T of PPFMs + F.S. of PPFMs+75%N	84.91	12.92	24.22	109.73	26.98	69.22
T <sub>10</sub>	S.T of <i>B.Japonicum</i> +S.T of PPFMs+ F.S. of PPFMs+100%N	92.73	13.96	25.19	117.09	27.16	70.17
T <sub>11</sub>	Only 100%N	74.63	9.21	19.92	99.09	24.07	63.51
S.Em ±		0.43	0.35	0.38	0.59	0.25	0.52
CD at 5%		1.26	1.03	1.13	1.73	0.72	1.54

F-Flowering stage, H-Harvesting stage, S.T:-Seed Treatment, F.S-Foliar spray, R.S. =Reference strain of PPFMs

The increase in N, P, K uptake is due to the production of GA by *Rhizobium* and PPFMs. This has been supported by Anurajan (2003) who reported that GA which are known to modify the plant morphology by extending the plant tissue particularly the stem, which induces the uptake of minerals like K, Ca and thereby influence the chlorophyll content, soluble sugars and protein content of the plants. Similar results in case of uptake of nitrogen has been documented by worker Devananda, 2000. who reported uptake of nitrogen in Pigeon pea inoculated

with rhizobacteria. Hoflich et al. (1995); Biswas et al. (2000), Senthilkumar (2003) and Holland (1997) have well documented the increase in nutrient use efficiency when inoculated with *Rhizobium leguminosarum* by *trifoli* in wheat, corn, radish, mustard, rice and by PPFMs in soybean respectively.

The results are in conformity with Meenakshi (2008) reported that combine application of PPFMs and *Bradyrhizobium* influenced the uptake of nutrient(N,P,K) of soybean as compared to the application of *Rhizobium* alone.

**Table 3: Effect of liquid consortium of on PPFM population count of soybean during flowering and harvesting stage**

Treatment		PPFM Count (Phyllosphere) (x10 <sup>6</sup> cfu/g)		PPFM Count (Rhizosphere) (x10 <sup>3</sup> cfu/g)	
		(F)	(H)	(F)	(H)
T <sub>1</sub>	Absolute control	85.06	12.01	10.33	3.10
T <sub>2</sub>	Seed treatment of <i>Rhizobium</i>	87.77	14.02	11.96	4.17
T <sub>3</sub>	S.T of <i>B.Japonicum</i> +S.T of R.S+ F.S. of R.S.	89.82	15.07	13.56	5.11
T <sub>4</sub>	S.T of <i>B.Japonicum</i> +S.T of R.S+ F.S. of R.S +50%N	94.46	17.08	15.77	6.11
T <sub>5</sub>	S.T of <i>B.Japonicum</i> +S.T of R.S+ F.S. of R.S +75%N	99.82	20.19	19.37	7.98
T <sub>6</sub>	S.T of <i>B.Japonicum</i> +S.T of R.S+ F.S. of R.S +100%N	100.78	21.01	20.05	8.48
T <sub>7</sub>	S.T of <i>B.Japonicum</i> +S.T of PPFMs+ F.S. of PPFMs.	92.58	16.46	14.79	6.02
T <sub>8</sub>	S.T of <i>B.Japonicum</i> +S.T of PPFMs+ F.S. of PPFMs. +50%N	96.80	18.23	17.62	7.03
T <sub>9</sub>	S.T of <i>B.Japonicum</i> +S.T of PPFMs+ F.S. of PPFMs. +75%N	103.04	22.57	21.86	9.74
T <sub>10</sub>	S.T of <i>B.Japonicum</i> +S.T of PPFMs+ F.S. of PPFMs. +100%N	105.01	23.52	22.95	10.06
T <sub>11</sub>	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

The application of PPFM cultures significantly influenced the population of *Methylobacterium* in the *phyllosphere* and *Rhizosphere* of soybean. In both *Phyllosphere* and *Rhizosphere*, the PPFMs count was influenced by the treatment T<sub>10</sub> (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<sub>9</sub> (S.T of *Rhizobium* +S.T of PPFMs +Foliar spray consortium of PPFMs + 75 % N) in respect of their influence on *phyllosphere* and *rhizosphere* population at flowering and harvesting stage.

These 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 *Methylobacterium* 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-marshall 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.

The results are in conformity with Senthilkumar et al. (2002) and Madhaiyan (2002). Similar results was also noticed by Radha (2007) and Meenakshi (2008). They

reported the higher *phyllosphere* population as compared to the *rhizosphere* population in soybean.

**Table 4: Effect of liquid consortium of PPFMs on grain yield of soybean**

Treatment		Grain yield (qha <sup>-1</sup> )
T <sub>1</sub>	Absolute control	13.01
T <sub>2</sub>	Seed treatment of <i>Rhizobium</i>	15.39
T <sub>3</sub>	S.T of <i>Rhizobium</i> +S.T of Reference strain+ F.S. of Reference strain(PPFMs)	16.42
T <sub>4</sub>	S.T of <i>Rhizobium</i> +S.T of Reference strain+ F.S. of Reference strain+50%N	18.36
T <sub>5</sub>	S.T of <i>Rhizobium</i> +S.T of Reference strain+ F.S. of Reference strain+75%N	21.57
T <sub>6</sub>	S.T of <i>Rhizobium</i> +S.T of Reference strain+ F.S. of Reference strain+100%N	22.01
T <sub>7</sub>	S.T of <i>Rhizobium</i> +S.T of consortium+ F.S. of consortium of PPFMs	16.78
T <sub>8</sub>	S.T of <i>Rhizobium</i> +S.T of consortium+ F.S. of consortium of PPFMs +50%N	19.08
T <sub>9</sub>	S.T of <i>Rhizobium</i> +S.T of consortium + F.S. of consortium of PPFMs+75%N	23.78
T <sub>10</sub>	S.T of <i>Rhizobium</i> +S.T of consortium+ F.S. of consortium+100%N	24.13
T <sub>11</sub>	Only 100%N	20.50
S.Em ±		0.35
CD at 5%		1.03

It is interesting to note that the grain yield of soybean obtained by T<sub>10</sub> (S.T of *Rhizobium* +S.T of PPFMs +Foliar spray consortium of PPFMs + 100 % N) 24.13 q ha<sup>-1</sup> was satisfactorily at par with yield recorded in T<sub>9</sub> (S.T of *Rhizobium* +S.T of PPFMs +Foliar spray consortium of PPFMs + 75 % N) 23.78 q ha<sup>-1</sup>. These results clearly shown that there is possibility of saving nitrogenous fertilizers to an extent of 25% i.e 27.17k urea/ha to soybean crop.

The superiority of T<sub>10</sub> treatment is due the graded dose nitrogenous fertilizer and combine beneficial effects of both *Rhizobium* and PPFMs. 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 soyabean 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.

The development of the plant from germination to root, shoot and leaf due to production of plant growth promoting substances such as IAA, GA, and cytokinin by PPFMs and *Rhizobium*. The GA is also involved in more up take of K and Ca. This has been supported by Anurajan (2003) who reported that GA which are known to modify the plant morphology by extending the plant tissue particularly the stem, which induces more uptake of minerals like K, Ca and thereby influence the chlorophyll content, soluble sugars and protein content and finally the yield of the plants.

The combined effect of IAA and cytokinins, allows a better growth of shoot and root system. The formation of more branches results in more uptake of nutrients. This is supported by Suresh Reddy, (2002) who reported that 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.

However the more up take of nutrients (N, P, K) by plant due to inoculation of *Methylobacterium* and *Rhizobium* which was given by Senthilkumar et al. (2002). He reported that the uptake of nutrient is higher due to their compatible nature, combined influence and foliar spray by *rhizobium* and PPFMs.

**Table 5: Correlation between PPFMs population, available nitrogen and uptake of nutrient (N, P, K) and yield of soybean**

Parameter	PPFMs population	Available nitrogen	Uptake of nitrogen	Uptake of phosphorous	Uptake of potassium	Yield
PPFMs population	1					
Available nitrogen	0.978823**	1				
Uptake of nitrogen	0.995604**	0.981994**	1			
Uptake of phosphorous	0.996054**	0.96427**	0.986227**	1		
Uptake of potassium	0.997262**	0.968865**	0.986687**	0.997613**	1	
Yield	0.993309**	0.977126**	0.983524**	0.98751**	0.993885**	1

There is a positive correlation between PPFMs and available nitrogen as well as uptake of nutrients (N P K) and yield of crop. This clearly shows that the increased population of efficient strain of PPFMs and *B. Japonicum* increases the available nitrogen as well as uptake of nutrients along with the yield of crop.

The available nitrogen in soil and the uptake of nutrients (N P K) 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 clearly indicate that the PPFMs along with the *B Japonicum* plays an imp role in reducing N fertilizer application in field condition.

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