

## MM 2.3 : Bioinoculants for sustainable and cost effective production of high quality fibre

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### Targets and achievements

Activity	Target	Achievement
A1: Survey, Isolation and enumeration of <i>Azospirillum</i> , PSB, PGPR from cotton based cropping system	Isolation of efficient bio-inoculant isolates for further screening	In the centers Indore, Parbani, Rahuri, Hissar and TNAU, survey was conducted to isolate efficient strains of bio-inoculants. Collected isolates were screened for their efficiency to promote plant growth.
A2 : Screening of Isolates	Isolation of efficient bio-inoculant isolates suitable of particular region.	Efficient strains of <i>Azospirillum</i> , Phosphobacteria and PPFM were selected at regional level. Selected PPFM isolates were sequenced for 16s rDNA gene and these isolates were identified at species level.
Q2: On farm testing of microbial consortium consisting of <i>Azospirillum</i> + Phosphate solubilizing bacterium + PPFM (Azophosmet)	Popularizing the bioinoculants application among farmers.	A net saving of 25% cost of inorganic fertilizer application with increased plant growth and yield was demonstrated in farmers fields.
A2: Field trial Field trial II to study the individual and combined effect of the bioinoculants on cotton	To evaluate the performance of individual and combined effect of bio-inoculants on the yield and quality of cotton	The newly designed microbial inoculant formulation (Azophosmet) has recorded better performance in all the centers studied. In field trial studies, combined inoculation of <i>Azospirillum</i> + Phosphobacteria + PPFM with 75% application of inorganic fertilizers has recorded maximum yield. However, bio-inoculant treatment didn't exert any significant influence of cotton fibre quality and boll weight.
Field Trial II : Standardization of phyllosphere application of PPFM	To standardize phyllosphere application of PPFM inoculant to cotton crop.	Phyllosphere application of PPFM at 45, 60 and 90 DAS has recorded significant yield increase in all the centers studied.

**Summary of result of the experiments conducted in lead centre and co-operating centres**

<b>Laboratory studies</b>	
<b>A1: Survey, isolation and enumeration of bio inoculant from rhizosphere soils of cotton and cotton based cropping system.</b>	
<b>Target: To isolate different strains of <i>Azospirillum</i>, PSB and PPFM.</b>	
<b>Center</b>	<b>Result</b>
Indore	The soil samples were collected from various villages of district Khandwa and Indore to see the performance of native strain <i>Azospirillum</i> and PSB. The maximum <i>Azospirillum</i> population 1.42/g of soil was recorded in the samples collected at Sanawad (Khandwa) while the population of PSB was recorded higher 25/g of soil from the samples collected at village Patoli (Khandwa).
Parbani	Survey was carried out in Marathwada region and rhizosphere soil samples from fields of cotton and cotton based cropping system were collected and brought to the laboratory. After isolation of different bioagents from these samples their population was counted and it was found that in the soils of Marathwada region the <i>Azospirillum</i> was present in the range of 23 – 31 x 10 <sup>3</sup> CFU/g soil, PSB in the range of 12 – 23 x 10 <sup>2</sup> CFU/g soil and PGPR and PPFM in the range of 3 – 6 x 10 <sup>1</sup> CFU/g soil.
Rahuri	Survey was carried out in central Maharashtra region from 27 places and 27 soil samples were collected from cotton field. Isolations were made for <i>Azospirillum</i> and Phosphobacteria and their population was counted. It was observed that the <i>Azospirillum</i> was present in the range of 7.3 - 19.6 X 10 <sup>4</sup> cfu/g soil and PSB in the range of 52.6-98.6 X 10 <sup>4</sup> cfu /g soil.
Hissar	Microbial count was done from the soil of CCSHAU, Hisar and Sirsa for PSB <i>Azotobacter</i> (PGPR) and <i>Azospirillum</i> by plate count method using specific media. The survival count was observed 4.6x10 <sup>4</sup> – 6.2 x10 <sup>4</sup> in the both sample.
TNAU	The survey was conducted in Coimbatore, Aruppukottai and Kovilpatti to isolate more efficient strains of <i>Azospirillum</i> and phosphobacteria. Phosphobacteria was found as the dominant bacteria in the rhizosphere region. More than 60 isolates of PPFM were obtained from the phyllosphere region. PPFM isolates were screened through cytokinins synthesis and antagonistic potential.

**A2 : Screening of Isolates****Target: To select efficient strains of *Azospirillum*, PSB and PPFM.**

Center	Result
Indore	The ten different samples were isolated to evaluate the performance of <i>Azospirillum</i> on germination root length, shoot length, and vigour index of cotton seeds. The germination range was recorded 70-90%. The minimum germination was 70% in isolate No.3 and 9, where as the maximum root length, shoot length and vigour index have been noted under isolate No.4 i.e. 6.0 cm 7, 58 cm, 1086.4 respectively. In case of PSB the germination was over all good in all isolate and root length was higher 5.66 cm in isolate No.8 where as shoot length and vigour index was maximum in isolate No.10 (8.25 cm and 1083.2).
Parbani	The results showed that the vigour index of <i>Azospirillum</i> isolates was in the range of 570.5 – 1215.0 (Table 2a). The vigour index of cotton seedlings as influenced by different isolates of PSB were in the range of 581.0 – 1229.4. The PGPR isolates exhibited the vigour index in the range of 684.0 – 1175.4. Similarly PPFM isolates exhibited vigour index in the range of 665.0 – 1285.2. In a pot culture experiment different isolates of <i>Azospirillum</i> , PSB PGPR and PPFM were evaluated for their efficiency in dry matter production The data clearly showed that the bio-inoculants produced highest dry matter, production i.e. 0.390 g plant-1 ( <i>Azospirillum</i> ), 0.290 g plant-1 (PSB), 0.310 g plant-1 (PGPR) and 0.280 g plant-1 (PPFM), respectively.
Rahuri	The seeds of var. LRA-5166 treated with 27 isolates <i>Azospirillum</i> and PSB individually and was sown in earthen pots to see the efficiency of the same. The biometric observations (plant height and dry matter weight) were recorded on 45 <sup>th</sup> DAS. The plant height and dry matter weight in different isolates was in the range of 41.4 to 47.4 cm and 4.1 to 5.8 g, respectively. The maximum plant height (47.4 cm) and dry matter weight (5.8 g) was recorded in soil sample from Manmad (Nashik). The data revealed that the vigour index of <i>Azospirillum</i> isolates was in the range of 967.2 - 1280 and the maximum vigour index (1280) was recorded in isolate from Pathardi (Ahmednagar) soil. The vigour index for PSB isolates was in the range of 940–1251.3 and the maximum vigour index (1251.3) was recorded in isolate from Shrirampur (Ahmednagar) soil.
Hissar	The strains PSB-602 was superior over PSB-601 and PSB-603 at 100%RDF, 75% RDF and 0% RDF, but PSB-601 and PSB-603 at par in 100% RDF and 0% RDF. <i>Azotobacter</i> 611 with 100% and 75% RDF was superior over <i>Azotobacter</i> 612 and <i>Azotobacter</i> 613, while in 0% RDF <i>Azotobacter</i> 613 was superior over <i>Azotobacter</i> 611 and <i>Azotobacter</i> 612. <i>Azospirillum</i> 632 with 100% RDF, 75% RDF and 0%RDF was superior over <i>Azospirillum</i> 631 and <i>Azospirillum</i> 633.
TNAU	Isolates were screened for plant growth promotion efficiency through vigor index studies. Among the best performing isolates, best suited isolate was selected through ARA assay. PPFM isolates were grouped based on RAPD profiles. One isolate from each group was (11 isolates in total) PCR amplified with 16s rDNA primers. PCR products were cloned in pTZ vector. Cloned products were sequenced by single pass analysis in forward and reverse direction. Sequence analysis indicated <i>Methylobacterium populi</i> as the dominant species found in cotton phyllosphere.

**On farm testing of microbial consortium consisting of *Azospirillum* + Phosphate solubilizing bacterium + PPFM (Azophosmet)**  
**This trial was carried out at five different locations**  
**Target: Field level demonstration of bio-inoculant application to cotton**

Center	Impact
Indore	Field level demonstrations were conducted.
Khandwa	Five field level demonstrations were conducted.
Parbani	Five field level demonstrations were conducted.
Rahuri	Five farmers field at different locations of Village- M. Wadgaon Tal. Shirampur were conducted.
Hissar	The five experiments were conducted at the farmer's field in different villages i.e. Arya Nagar, Dandure , Birbabran, Talwandi Rana, Panihari of Hisar district. It was observed that maximum seed cotton yield in the treatments Azophosmet + 75% RDF (814 kg /acre) followed by 100% RDF (803 kg / acre) and 75% RDF (768 kg/ acre) .The spray of Phyllosphere @ 400ml PPFM ( $10^{10}$ cell /ml) in 400 litre water /acre at 90 <sup>th</sup> DAS .There was saving of 25% of fertilizer dose.
Nandyal	Farmers field demonstrations have recorded favorable results. The application of 75% of RD of N & P along with the application of Azophosmet (Azospirillum+ PSB + PPFM) PPFM recorded seed cotton yield of 1018 kg ha <sup>-1</sup> which was 8.8% increase over check I i.e., 75% RDof N & P alone and very slightly lower than 100% RD of N & P. Hence, by using the bio-fertilizers the farmers can save about 25% of expenditure incurred on inorganic fertilizers in addition to quality produce with the use of bio-inoculants.
TNAU	Three farmer's field level demonstrations were conducted in Aruppukottain and two field level demonstrations were conducted in Kovilpatti. Results of field level demonstrations were encouraging. Application of 75% RDF along with azophosmet has recorded on par yield with 100% RDF alone applied treatments. Results of the demonstrations indicated by applying Azophosmet along with 75% RDF can save 25% of inorganic fertilizer cost.
CICR, Coimbatore	The On farm testing of bio inoculants on farmers field during 2006-07 using RCH 2 Bt as test cultivar revealed that application of bio inoculants + 75 % recommended N and P recorded the highest (2612 kg/ha) seed cotton yield which was 15.8 % higher yield than 75 % recommended N and P alone without bio inoculants.

<b>Field trial I : To study the individual effect of the bio inoculants on cotton</b>	
<b>Target: To find out individual and combined effect of <i>Azospirillum</i>, PSB and PPFM at 75% N&amp;P of recommended dose.</b>	
<b>Center</b>	<b>Impact</b>
Indore	The significant differences in yield and yield attribute values due to various treatments were prevailed. Application of 75% RDF along with seed treatment as well as application of <i>Azospirillum</i> + PSB + PPFM + SSB (T <sub>7</sub> ) was yielded higher 1307 kg /ha over all other treatments, though the application of 75% RDF+ <i>Azospirillum</i> + PSB + PPFM as seed treatments + soil application was noted at par while comparing to 100 % RDF alone (1109 kg and 1172 kg/ha ). Similar trend was also noted in respect of yield attributing characters and B: C ratio respectively. The application of various bioinoculant did not have any impacts in ginning outturn.
Khandwa	Fertilizer levels as well as bio inoculants significantly influenced seed cotton yield per hectare during 2006-07. Maximum seed cotton yield of 1150,46 Kg.ha <sup>-1</sup> was obtained from <i>Azospirillum</i> + PSB TNAU + PPFM followed by 1143,52 Kg ha <sup>-1</sup> from 100% RDF while, it was minimum (709,10 Kg ha <sup>-1</sup> ) under control. Similar results were noted for number of bolls and seed cotton yield per plant. Boll weight remained unchanged.
Parbani	The data clearly indicated that fertilizer and bio-inoculants application increased the seed cotton yield, single plant yield, boll number per plant and boll weight. Maximum seed cotton yield, single plant yield, boll number per plant and boll weight was found in the treatment of <i>Azospirillum</i> + PSB TNAU <sub>1</sub> + PPFM + SSB.
Rahuri	The data indicated that there were significant differences in seed cotton yield/ha due to the effect of various treatments. The maximum seed cotton yield (884 Kg/ha) was recorded in treatment of 100 % RDF and was at par with the treatment 75% RDF + seed treatment with <i>Azospirillum</i> + PSB + PPFM + SSB (879 Kg/ha) and also with the treatment 75% RDF + seed treatment with <i>Azospirillum</i> + PSB + PPFM (842 Kg/ha). The minimum seed cotton yield (672 Kg/ha) was recorded in treatment of 75% RDF only. Also, the treatment differences in seed cotton yield per plant were non significant.
Hissar	The application of bioinoculant <i>Azospirillum</i> surat + PSB TNAU <sub>1</sub> + PPFM (2054 kg/ha) gave significantly higher seed cotton yield over other treatments. The application of <i>Azospirillum</i> surat+ PSB TNAU <sub>1</sub> + PPFM+SSB (1885 kg/ha), PPFM (1878kg/ha), <i>Azospirillum</i> surat(1857 kg/ha) and 100%. RDF (1812 kg/ha) were at par and significantly higher over PSB TNAU <sub>1</sub> (1712kg/ha), SSB (1712kg/ha) and without bioinoculant(1647kg/ha).
Nandyal	Application of 75% RDF in addition to <i>Azospirillum</i> + PSB + PPFM + SSB (T <sub>7</sub> ), significantly increased the number of bolls (25.9) per plant and boll weight (3.2g) However, it was on par with application of 100% RDF (T <sub>8</sub> ) and <i>Azospirillum</i> + PSB + PPFM (T <sub>6</sub> ). Similarly, significantly higher seed cotton yield per plant (72.9g) and seed cotton yield (1040 kg ha <sup>-1</sup> ) were registered with application of 75% RDF in combination with <i>Azospirillum</i> + PSB + PPFM + SSB (T <sub>7</sub> ) than control but it was comparable with other treatments (which received bio-inoculants either alone or combined) in addition to 75% RDF.
TNAU	The experiment has indicated a net saving of 25% in the cost of inorganic fertilizer application when Azophosmet and 75% inorganic fertilizers are combined together.
CICR, Coimbatore	Application of 75 % recommended N and P with <i>Azospirillum</i> + PSB + PPFM as seed and soil treatment with two foliar spraying of PPFM at flowering to boll development recorded the highest (2106 kg/ha) seed cotton yield and it was on par with all other bio inoculated treatments with 75 % recommended N and P or 100 % recommended N and P without bio inoculants. Application of 75 % recommended N and P without bio inoculants recorded significant yield reduction to the tune of 438 kg/ha as compared to Application of 75 % recommended N and P with <i>Azospirillum</i> + PSB + PPFM.

<b>Field Trial II : Standardization of phyllosphere application of PPFM</b>	
<b>Target : To standardize phyllosphere application of PPFM to cotton</b>	
<b>Center</b>	<b>Impact</b>
Indore	A significant difference in seed cotton yield was recorded due to phyllosphere spray with PPFM at various stages of crop growth. The phyllosphere spray with PPFM at 45 DAS, 60 DAS and 90 DAS recorded significantly higher seed cotton yield 1189 kg/ha as compared to all rest treatments. Although the spray with PPFM at various stages was also found significant except for the treatment 45 day spray as compared to control. Similar trend was also noted in respect of yield attributes, B:C ratio and ginning outturn.
Khandwa	Phyllosphere spray of PPFM at different stages and 100% N&P application significantly enhanced the seed cotton yield over unsprayed control except the spray of PPFM at 45 <sup>th</sup> day(T2), 60 <sup>th</sup> day (T3) and 45 <sup>th</sup> + 60 <sup>th</sup> (T5) where the increase was not up to mark. The per plant yield and boll number also markedly increased due to the phyllosphere spray at 45 <sup>th</sup> and 90 <sup>th</sup> day(T6), 60 <sup>th</sup> & 90 <sup>th</sup> day(T7) and 45 <sup>th</sup> + 60 <sup>th</sup> + 90 <sup>th</sup> day(T8) and 100% N&P application over un sprayed control. Boll weight could not exhibit any response to the different treatments.
Parbani	The data clearly indicated that fertilizer and bio-inoculants application increased the seed cotton yield, single plant yield, boll number per plant and boll weight. Maximum seed cotton yield, single plant yield, boll number per plant and boll weight was found in the treatment of phyllosphere spray of PPFM at 45 <sup>th</sup> , 60 <sup>th</sup> and 90 <sup>th</sup> day.
Rahuri	There were significant differences in seed cotton yield/ha due to the effect of various treatments. The highest seed cotton yield (892 Kg/ha) was recorded in treatment of 75% RDF + seed treatment with Azophosmet + three sprays of PPFM at 45, 60 & 90 DAS and was at par with all the treatment except treatments T <sub>1</sub> & T <sub>3</sub> .
Hissar	The application of phyllosphere spray of PPFM at 45, 60 and 90 DAS + Azophosmet bioinoculants(1589kg/ha) gave significantly higher seed cotton yield over other treatments except the application of phyllosphere spray of PPFM at 45 and 60 DAS + Azophosmet bioinoculants(1565kg/ha), phyllosphere spray of PPFM at 45 and 90 DAS + Azophosmet bioinoculants(1534kg/ha) and phyllosphere spray of PPFM at 60 and 90 DAS + Azophosmet bioinoculants(1526kg/ha) were at par.
Nandyal	application of 75% RDF + two phyllosphere sprays of PPFM at any two stages of 45, 60 and 90 DAS resulted into higher number of bolls, boll weight and yield per plant compared to single spray at any stage. Application of 100% RDF alone produced higher number of bolls, boll weight and also yield per plant (80.5 g). Application of 75% RDF along with phyllosphere spray at 45 and 60 DAS produced significantly higher yield (1060 kg ha <sup>-1</sup> ) than control (859 kg ha <sup>-1</sup> ). However, it was comparable with all other treatments including application of 100% of RDF alone without any spray 1038 (kg ha <sup>-1</sup> ).
TNAU	Application of PPFM as foliar spray has brought considerable increase plant growth and yield. Among the different treatments tested, foliar spray of PPFM at 45 <sup>th</sup> day, 60 <sup>th</sup> day and 90 <sup>th</sup> day has recorded the maximum plant growth and yield (19.28% increase over control).
CICR, Coimbatore	Among the treatments evaluated, application of 75 % recommended N and P ( k 100 %) with Azophosmet as soil and seed treatment combined with two foliar application of PPFM 60 and 90 DAS or at 45 and 60 DAS recorded numerically higher number of productive bolls/plant which was on par with all other treatments. The boll weight was not influenced by the treatments. Application of 75 % RDF with Azophosmet as seed and soil treatment was on par with 100 % RDF without bio inoculants.

- ◆ Significant effect in application of bio-inoculants (Azophosmet) was seen in increase in the number of bolls. Application of Azophosmet has increased the number of bolls per plant in all the centres studied. On average, an increase of 43% over control in number of bolls was evident. The results were followed by 100% RDF applied treatment (31% over control) and PPFM alone applied treatment (24% over control). Increase in number of bolls has become the cause of increased yield in Azophosmet applied treatments.
- ◆ Application of bio-inoculants had little influence over boll weight when compared to influence of bio-inoculants application over number of bolls per plant. Application of PPFM as seed treatment and foliar spray has recorded maximum boll weight in all the centres except Nandyal. PPFM alone applied treatment has recorded more boll weight (7% over control) followed by Azophosmet treatment (4% over control).
- ◆ Effect of bio-inoculants application was clearly seen in increase of seed cotton yield. Except in Rahuri and Indore, Azophosmet application has recorded maximum seed cotton yield followed by 100% RDF alone

applied treatment. In Rahuri and Indore, Azophosmet applied treatment has recorded on par yield with 100% RDF applied treatment. On average, Azophosmet has recorded an increase of 28% over control followed by 100% RDF applied treatment (24.5% over control) applied treatment and PPFM alone applied treatment (15% over control).

- ◆ In all the centres, application of PPFM as seed inoculant followed by foliar spray of the inoculant along with 75% of RDF has increased the number of bolls per plant. Among all the treatments, application of PPFM as seed treatment followed by PPFM foliar spray has increased the number of bolls significantly. On average, this treatment has recorded an increase of 35% over control followed by 100% RDF alone applied treatment and PPFM foliar spray at 60<sup>th</sup> day and 90<sup>th</sup> day.
- ◆ Application of PPFM has recorded considerable influence on cotton boll weight. Foliar spray of PPFM at 45<sup>th</sup> day and 90<sup>th</sup> day (T6), at 60<sup>th</sup> day and 90<sup>th</sup> day (T7), at 45<sup>th</sup> day, 60<sup>th</sup> day and 90<sup>th</sup> day has recorded on increase in boll weight when compared to 100% RDF alone applied treatment. Foliar spray of PPFM at above timings records a minimum increase of 12% over control which is on par with 100% RDF treatment.
- ◆ Application of PPFM as seed treatment followed by foliar spray along with 75% RDF has definite influence on seed cotton yield. This can be correlated to increase in number of bolls per plant and increase in boll weight. On average, with a single spray of PPFM, a minimum increase of 13% in seed cotton yield over control was recorded. PPFM spray at 45<sup>th</sup> day, 60<sup>th</sup> day and 90<sup>th</sup> day has recorded the maximum seed cotton yield (24% over control) followed by PPFM spray at 60<sup>th</sup> day and 90<sup>th</sup> day (21% over control).

Comparison of Field Trial II in all centres: Effect of phyllosphere application of PPFM on Seed cotton yield (Kg/ha)										
Sl No.	Treatments	TNAU	CICR, Cbe.	Parbhani	Rahuri	Hisar	Nandyal	Indore	Khandwa	Average
1.	Control	830	1978	792.17	767	1313	859	26	37.11	825
2.	Spray at 45 <sup>th</sup> day	893	2259	907.41	839	1510	950	36	38.67	929
3.	Spray at 60 <sup>th</sup> day	880	2254	971.19	830	1502	988	37	45.11	938
4.	Spray at 90 <sup>th</sup> day	876	2138	1076.1	843	1494	1031	38	47.56	943
5.	Spray at 45 <sup>th</sup> and 60 <sup>th</sup> day	930	2257	1113.2	847	1565	1060	40	51.00	983
6.	Spray at 45 <sup>th</sup> and 90 <sup>th</sup> day	905	2157	1220.2	866	1534	1004	41	56.78	973
7.	Spray at 60 <sup>th</sup> and 90 <sup>th</sup> day	910	2296	1299.4	861	1526	971	42	57.89	995
8.	Spray at 45 <sup>th</sup> , 60 <sup>th</sup> and 90 <sup>th</sup> day	990	2226	1384.8	892	1589	1018	52	57.00	1026
9.	100% RDF alone	902	2021	1331.3	873	1439	1038	44	61.44	964

On farm testing of Azophosmet Effect of Azophosmet on seed cotton yield in different centres						
Treatments	TNAU	Nandyal	Rahuri	Hisar	CICR, Coimbatore	Average
T1 - Azophosmet + 75 % NP ( K 100 % )	944	1018	488	1717	2612	1339
- 75 % N and P ( K 100 % )	828	935	416	1670	2256	1238
T3 – 100 % NPK	904	1025	507	1750	2595	1356

Field level demonstrations conducted in all the centres have recorded the beneficial effects of bio-inoculants application. Azophosmet application as seed treatment followed by field application of bio-inoculant has recorded on par yield with 100% NPK alone applied treatment.