



RESEARCH ARTICLE

# Seed treatment with microbial consortia of biofertilizers enhanced growth and yield of hybrid rice ADTRH1

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## Abstract

Improved seeds and inorganic sources of fertilizer application are the core techniques of green revolution to reach the potential yield. Application of bio-fertilizer would improve the physical, chemical and biological condition of the soil, The recent concept of integrated nutrient management with the use of biofertilizer acquired greater importance not only for the increased yield, but also for farmers profit maximization and contribution to environment preservation. In this context the experiments were conducted to study the response of hybrid rice ADTRH1 with biofertilizers viz., *Azospirillum*, phosphobacteria and *Methylobacterium*, compared with GA<sub>3</sub> spraying at different level of chemical fertilizer application. Results revealed that microbial consortia at 100 % recommended levels of NPK fertilizer application with pink pigmented facultative methylotrophic bacteria (PPFM) foliar spray recorded maximum growth, germination percentage (95.81 %) and yield of hybrid rice (7627 kg/ha) over other treatments and uninoculated control.

**Keywords:** *Azospirillum*; hybrid rice; *Methylobacterium*; phosphobacteria

## Introduction

Rice is the most important cereal crop and staple food of approximately one half of the world's population. In Asia, one of the constraints to high yields is the limited availability and high prices of nitrogen and phosphorus fertilizers. The idea of utilizing biological nitrogen fixation (BNF) as an alternative or supplementary source of nitrogen for rice is not new (1, 2). Nitrogen fixing green manures have been used for centuries in some rice growing areas and research on biofertilizers, including algal and bacterial inoculants, began in the early 1930s. However, the productivity of these sustainable systems is low, with yields less than four tonnes per hectare. Modern agriculture has increased rice yields to five to eight tonnes per hectare but requires the input of 60 to 100 kg ha<sup>-1</sup> of nitrogenous fertilizer to supply additional nitrogen requirement. Decreasing the amount of industrially produced nitrogenous fertilizer needed in agricultural systems is an important goal for agricultural scientists in general (3). The fact that the total world BNF is three times that of industrially produced nitrogen demonstrates the significance of BNF in agriculture and natural nitrogen cycles (4).

*Methylobacterium* are a group of non-pathogenic phyllosphere microbes ubiquitously distributed on plants. Successful co-existence of facultative methylotrophic bacteria (FM) with plants is largely due to their ability to

assimilate methanol which is a plant exometabolite. They provide plant with increased nitrogenase activity and growth promoting substances, vitamins and enhance their resistance to various stresses. Although they are not well known, these bacteria are coevolved, interacting partners in plant metabolism. They do not merely colonize the plants but are symbiotically related to them (5, 6).

Improved seeds and inorganic sources of fertilizer application are the core techniques of green revolution to reach the potential yield to reach a sustainable food production system. The recent concept of integrated nutrient management with the use of bio-fertilizer is not only economical but also environmentally sustainable to the crop production system. Application of bio-fertilizer would improve the physical, chemical and biological properties of the soil, The beneficial effect of soil bacteria as a biofertilizer in enhancing crop productivity is being exploited due to their ability to produce various components including ammonia excretion, production of phytohormones, organic acids, siderophores, fixation of atmospheric nitrogen, phosphorous solubilisation, production of antimetabolites that suppress the deleterious rhizobacteria and control various plant diseases or due to some other unidentified mechanism. This strategy acquired greater importance recently not only for the increased yield, but also for farm profit maximization and contribution to environment sustainability.

So far, most of the work on facultative methylotrophic bacteria has been carried out on temperate crop plants and a lot of evidence was accumulating on the beneficial role of facultative methylotrophic bacteria on various temperate crop plants (5, 6). As such, very few research works were carried out on tropical plants (7, 8). Studies carried out on the natural occurrence of facultative methylotrophs in the phyllosphere region of tropical plants revealed that almost all the crops tested were found to harbour PPFM in their phyllosphere. The population of PPFM on the phyllosphere region of tropical crops varied widely ranging from 2 to 70 cfu cm<sup>-2</sup> of leaf. The varied agro-climatic conditions in India make it possible to grow a wide variety of crops and vegetables (7, 9).

Like PGPR plant growth promoting rhizobacteria, plant growth phyllosphere bacteria are also reported to coexist on the leaf surface by leading saprophytic/symbiotic life in the phyllosphere. Leaf diffusates and leaf exudates encourage growth and multiplication of these microorganisms. The pink pigmented facultative methylotrophic bacteria (PPFM) belonging to the genus *Methylobacterium* are a group of non-pathogenic phyllosphere microbes ubiquitously distributed on plants, successful co-existence of FM with plants is largely due to their ability to assimilate methanol which is a plant exometabolite. They provide plant with increased nitrogenase activity, plant growth promoting substances (5, 6) vitamins and enhance their resistance to various stresses like dehydration, freezing on hygroscopic carriers, ultraviolet and ionizing radiations and elevated temperatures, able to resist certain degree of desiccation and to scavenge traces of nitrogen and carbon, which make them well suited for survival in stressful environment. As such more than 70 isolates of PPFM were isolated from the phyllosphere of tropical plants such as cereals, pulses, oil seeds, vegetables, fruit crops, spices, commercial crops, flower crops, trees and weeds (5, 6). The effect of PPFM on crop growth and yield was studied by several workers under *in-vivo* and *in-vitro* condition in cotton and rice (7, 8); groundnut, tomato (9), soybean, black gram, sugarcane, sunhemp and in tissue culture studies; cytokinin biosynthesis in association with plant tissue in cowpea, callus induction and regeneration of cowpea and rice plants. The non-pigmented facultative *Methylobacterium* (NPFM) isolates from the nodules of various legumes had significantly influenced the growth of legume crop (7). However, NPFM bacteria isolates are host specific, produces plant growth promoting substances (8, 10), a strain *Methylobacterium nodulans* ORS 2060 forms nodules only in *Crotalaria juncea* increases growth and yield of crop plants (11).

In this context the present study was undertaken with the aim of exploiting facultative methylotrophic bacteria with conventional biofertilizer *Azospirillum* and phosphobacteria for increasing the productivity of rice crops. The PPFM and NPFM were isolated from different parts of rice crops. The best performing isolates were screened based on the growth hormone production and *in-vitro* germination tests along with the reference strain and utilized in the experiment. GA<sub>3</sub> application was very effective in increasing seed set rate and grain yield in rice production especially in hybrid rice (12, 13) but its application on rice crop for improvement of growth and yield is meagre and costly, also there is no field study to

trace the beneficial role of facultative methylotrophic bacteria with other bioinoculants on the tropical crops especially in rice crop. Hence the studies were undertaken using hybrid rice ADTRH1. This variety was released by Tamil Nadu Rice Research Institute Aduthurai Tamil Nadu for irrigated regions of Tamil Nadu, Semi tall, non-lodging, low shattering, high milling (75 %) and moderately resistant to stem borer and leaf folder.

## Materials and Methods

### Preparation of standard inoculum

The standard strains of nitrogen fixing bacteria *Azospirillum lipoferum* Az204 and phosphate solubilising bacterium, *Bacillus megatherium* var *phosphaticum* PSB-1 and PPFM bacterial isolates viz., *Methylobacterium* sp. PPFM-Os-07 were obtained from the Department of Agricultural Microbiology, TNAU, Coimbatore, Tamil Nadu, India. NPFM bacterial isolates viz., *Methylobacterium* sp. NPFM-Os-04 were isolated from rice crop (*Oryza sativa* L.), characterized and identified based on the presence of *mxoF* gene by PCR amplification (555 bp) in all the isolates. In this study the NPFM isolates were screened based on growth hormone production and *in-vitro* germination tests. Based on screening the efficient NPFM isolate *Methylobacterium* sp. NPFM-Os-04 were used in the experimental study. Laboratory studies confirm that *Azospirillum*, phosphate solubilising bacterium (PSB) and *Methylobacterium* sp. were found to be compatible with each other. The standard inoculum of bioinoculants was prepared by inoculating 72 h old log phase cultures of bioinoculants viz., *Azospirillum lipoferum* Az-204 in N-free malic acid medium, phosphate solubilizing bacterium, *Bacillus megatherium* var *phosphaticum* PSB1 in nutrient broth and *Methylobacterium* sp. in AMS broth respectively (14). The flasks were kept over a gyratory shaker (100 strokes per min) at 28 ± 2 °C for five days till it reached the O.D. value of 1.0 at 620 nm for *Azospirillum lipoferum* Az-204 *Methylobacterium* sp. at 660 nm phosphate solubilizing bacterium to get population of 10<sup>9</sup> cells/mL of liquid culture. For individual inoculum treatment the individual inoculum was diluted in 1:100 ratio with water so that the population remains 10<sup>7</sup> cells/mL and for microbial consortia the individual inoculum were mixed in equal cell ratio of 10<sup>9</sup> cells/mL and diluted with water in 1:100 ratio to get a final population of 10<sup>7</sup> cells/mL (15).

### Field location

A field trial experiment was conducted in the field number M8 in wet land of Tamil Nadu Agricultural University, Coimbatore to study the effect of different combination of bioinoculants on growth and yield of hybrid rice (ADTRH1). The wet land is situated in the North Western climatic zone of Tamil Nadu at 11 °C North latitude and 77 °C East longitude at an altitude of 426.72 m above main sea level. There is no rainfall during the experimental period. Soil of the experimental field is clay loam. Initial soil samples were collected below 20 cm depth for testing physical and chemical characteristics of soil (16) and are given in Table 1. Soil pH and electrical conductivity (EC) were determined in a 1:2.5 (soil: water) solution (w/v). Organic carbon (OC) was estimated through the chromic acid wet digestion method, available nitrogen (N) through the alkaline permanganate method and available phosphorus (P)

**Table 1.** Initial characteristics of experimental field soil

S. No.	Particulars	Values
I	Physical properties	
1	Clay (%)	37.50
2	Silt (%)	16.00
3	Fine sand (%)	34.20
4	Coarse sand (%)	12.10
5	Texture	Clay loam
II	Chemical properties	
1	Soil pH	7.30
2	EC	0.50
3	Available nitrogen (kg/ha)	190.10 (Low)
4	Available phosphorus (kg/ha)	24.90 (Medium)
5	Available potassium (kg/ha)	750.00 (High)

was assessed through the Bray method (17-19). Available potassium (K) was calculated by ammonium acetate extract method with a flame photometer (20). All analyses were conducted in the Department of Soil Science and Agricultural Chemistry laboratory, Tamil Nadu Agricultural University, Coimbatore. The pH of the soil sample was 7.30 and electrical conductivity (EC) was 0.50 dS/m. The available NPK contents were 190.10, 24.90 and 750 kg/ha, respectively. The soil was low in nitrogen content, medium in phosphorus and high in potassium contents.

### Nursery preparation

The experimental soil in which the nursery was raised was sandy loam in texture with pH of 7.3. The soil was low in available nitrogen, medium in available phosphorous and high in available potassium. A plot size of 0.9 × 0.9 m was adopted for nursery. For seed bed preparation three weeks before sowing three dry ploughing were given to the seed bed, followed by puddling and levelling with planks. After draining the water, the field was divided into 24 plots of 0.9 × 0.9 m each. Bunds and channel were formed at 90 cm around each plot and individual plots were levelled thereafter. A seed rate of 20 kg ha<sup>-1</sup> was adopted and the sowing was done as per the treatment details. The details of method of application of biofertilizers were as discussed under previous section. Seedlings were raised by wet nursery technique/wet bed method. The paddy seeds were broadcasted uniformly on the nursery. DAT @ 2 kg/cent applied in nursery and calculated quantity of urea and super phosphate were applied as per the recommended dose and broadcasted after levelling.

### Field experiment details

The field experiments were conducted to study the influence of *Methylobacterium* isolates along with *Azospirillum* and phosphate solubilizing bacterium on hybrid rice (ADTRH1). The field experiment details are given in Table 2. The experiment consisted of 24 treatments, three replications with the field layout made using a split plot design. Treatment details were given in Table 3. All the treatments received 100 % K. The treatment with biofertilizers is done at four stages viz., seed treatment during sowing, main field application before transplanting, seedling dipping before

**Table 2.** Experimental details of the present study

Date of sowing	25/06/04
Date of planting	18/07/04
Spacing	20 × 10 cm
Gross plot size	2.5 × 3.0 (7.5 m <sup>2</sup> )
Fertilizer (NPK)	150-60-60 kg/ha
Treatments	24
Replication	3
Design	Split plot design
Field area	0.25 acre

**Table 3.** Description of treatments

Main plot treatments	
M <sub>0</sub>	0 % N, P and 100 % K
M <sub>1</sub>	50 % N, P and 100 % K
M <sub>2</sub>	75 % N, P and 100 % K
M <sub>3</sub>	100 % N, P and 100 % K
Sub plot treatments	
S <sub>0</sub>	Uninoculated control
S <sub>1</sub>	Foliar spray of GA <sub>3</sub>
S <sub>2</sub>	<i>Methylobacterium</i> sp. PPFM-Os-07
S <sub>3</sub>	<i>Methylobacterium</i> sp. NPFM- Os-04
S <sub>4</sub>	<i>Azospirillum lipoferum</i> Az204 + <i>Bacillus megatherium</i> var <i>phosphaticum</i> PSB1
S <sub>5</sub>	<i>Azospirillum lipoferum</i> Az204 + <i>Bacillus megatherium</i> var <i>phosphaticum</i> PSB1 + PPFM-Os-07 + NPFM- Os-04

\*Also received phyllosphere spraying of PPFM isolates.

transplanting and foliar spray of PPFM during active tillering stage (15 DAT), panicle initiation stage (45 DAT) and flowering stage (60 DAT). GA<sub>3</sub> foliar spray was done on 0<sup>th</sup> day, 3<sup>rd</sup> day and 6<sup>th</sup> day during active tillering stage, panicle initiation stage and flowering stage. The method of application of biofertilizer is done as per the standard recommendation (21). For seed treatment with microbial consortia of biofertilizers 600 g of each biofertilizers were mixed with sufficient quantity of water separately or in combination where in the seeds are soaked overnight before sowing in the nursery bed. For seedling dipping one kg of each biofertilizers was mixed with 15 L of water separately or in combination as per the requirement and the roots of crop seedlings were dipped for 15 min and then transplanted. Soil application of biofertilizers is carried out by mixing 2 kg of each biofertilizers with 25 kg of FYM and 25 kg of soil separately or in combination as per the requirement and applied or spread uniformly before sowing or after transplanting. Phyllosphere spraying of PPFM were carried out by diluting standard inoculum of PPFM at 1:100 ratio with sterile water and sprayed with hand sprayer on the phyllosphere @ 500 litres per hectare at 6.00 A.M. in the morning or at 6.00 P.M. in the evening (5). An *in-vitro* experiment was conducted to find out the effect of microbial consortia of biofertilizers on growth, germination and vigour index of rice crop by germination tests (22). To observe the effect of microbial consortia of biofertilizers on growth and yield of hybrid rice crop the plant samples were collected following the standard protocol (23) and the following biometric observations were recorded. In the gross plot area, two rows all around the plot were left as border rows. Next to border rows on either side was used as sampling rows. Five plants in the sampling rows were tagged

and used to record growth parameters, chlorophyll content (24), yield parameters (number of productive tillers, panicle length, number of grains per panicle, spikelet fertility percentage, 1000 grain weight, grain yield), harvest index (25), microbial population studies and nutrient analysis (16-20) after harvest were recorded as per the standard procedure.

### Statistical analysis

The experiments data were statistically analysed using STATA software (Version 13) (26). For significant results the critical difference was worked out at 5 % probability level to draw statistical conclusions.

### Results and Discussion

The *in-vitro* experiment conducted to study the effect of different combinations of bioinoculants *Azospirillum*, phosphate solubilizing bacteria and *Methylobacterium* sp. on growth, germination percentage and vigour index of hybrid rice ADTRH1 are presented in Table 4. It was observed from the results that of all the treatments T<sub>5</sub> recorded maximum germination percentage (95.81 %) followed by T<sub>4</sub> (95.63 %), while lowest germination percentage was observed in T<sub>1</sub> (80.65 %). In case of shoot length T<sub>5</sub> recorded maximum shoot length of 13.23 cm followed by T<sub>4</sub> 12.77 cm, while lowest shoot length was observed in T<sub>1</sub> 11.35 cm. Whereas in case of root length T<sub>5</sub> recorded maximum root length of 14.62 cm, this was followed by T<sub>3</sub> 13.81 cm, while lowest root length was recorded in T<sub>1</sub> 9.00 cm. Similarly in case of other growth parameters viz., seedling length, number of lateral roots per plant, number of seminal root per plant and vigour index T<sub>5</sub> recorded maximum value of 27.85cm, 198.36, 16.33 and 2668 respectively followed by T<sub>3</sub> (25.76 cm, 196.84, 5.91 and 2463 respectively), while all the above growth parameters were lowest in T<sub>1</sub>. From the results of the above germination tests T<sub>5</sub> recorded maximum growth parameters, vigour index over other treatments and uninoculated control, which is due to the synergistic action of biofertilizers (27-29) viz., nitrogen fixing *Azospirillum*, phosphate solubilizing bacteria, plant growth promoting rhizobacteria NPFM and plant growth promoting phyllosphere bacteria PPFM. Similar results were observed by Vijaya Nirmala et al. (30) in Cumbu and Madhaiyan et al. (31, 32) in sugarcane and cotton.

### *In-vivo* experiments on the effect of *Azospirillum*, *Bacillus megatherium* var *phosphaticum* and *Methylobacterium* sp. on growth and yield of hybrid rice ADTRH1

#### Growth parameters

Based on the positive results of the *in-vitro* studies on the effect of *Azospirillum*, phosphate solubilizing bacteria and *Methylobacterium* sp. on germination percentage and vigour index of hybrid rice ADTRH1 the field experiment was designed to study the influence of different dosage of fertilizer, bioinoculant treatments and their interaction effects on various growth and yield parameters of hybrid rice ADTRH1. The growth parameters were recorded at active tillering stage, panicle initiation, flowering stages. The plant height increased up to 60 DAT in all the treatments. Biofertilizers and different level of chemical fertilizers had significant effect on plant height, dry matter production (DMP), while there is no significant interaction effect on number of leaves per plant and leaf area and the results were presented in Table 5. The maximum plant height was recorded in foliar spray of GA<sub>3</sub> on phyllosphere with 75 % NP and 100 % K (28.07 cm plant<sup>-1</sup>) followed by microbial consortia with 100 % NPK (27.83 cm plant<sup>-1</sup>). Similar results were observed for. plant height at 45 and 60 DAT. DMP increased from 15 DAT to 60 DAT and the maximum dry matter production was observed at 60 DAT at 75 % NP and 100 % K with GA<sub>3</sub> foliar spray (191.24 g/plant), followed by *Methylobacterium* sp. PPFM-Os-07 inoculation at 75 % NP with 100 % K (166.98 g/plant). while the lowest dry matter production was recorded in uninoculated control in 0 % NP with 100 % K (102.94 g/plant). The number of leaves per plants increased up to 45 DAT and thereafter no significant increase was observed. The maximum number of leaves per plant was observed at 45 DAT in case of microbial consortia with 75 % NP and 100 % K followed by 100 % NPK with GA<sub>3</sub> foliar spray and conventional biofertilizer application (10). At 15 DAT the maximum leaf area was observed in phyllosphere spray of GA<sub>3</sub> with 75 % NP and 100 % K (19.13 cm<sup>2</sup>) followed by microbial consortia with 100 % NPK (17.50 cm<sup>2</sup>). The lowest leaf area was observed in case of uninoculated control in 0 % NP with 100 % K (10.12 cm<sup>2</sup>). Similar results were observed at 45 and 60 DAT. The leaf area rapidly increased significantly up to 45 DAT and reaches the maximum at 60 DAT in 100 % NPK with GA<sub>3</sub> spray (39.87 cm<sup>2</sup>) followed by microbial consortia (29.00 cm<sup>2</sup>) as shown in Fig. 1. The increase in plant growth parameters due to microbial consortia of biofertilizer might be possible due to increased

**Table 4.** Effect of microbial consortia of biofertilizers on growth and vigour index of hybrid rice ADTRH1.

Treatment	Germination Percentage (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Lateral roots (No. /plant <sup>-1</sup> )	seminal roots (No plant <sup>-1</sup> )	Vigour index
T <sub>1</sub>	80.65	11.35	9.00	20.35	142	5.50	1641
T <sub>2</sub>	93.29	12.75	9.79	22.54	178.65	5.57	2103
T <sub>3</sub>	95.63	11.94	13.81	25.76	196.84	5.91	2463
T <sub>4</sub>	92.98	12.77	10.31	23.08	183.64	5.58	2146
T <sub>5</sub>	95.81	13.23	14.62	27.85	198.36	6.33	2668
SEd	0.9734	0.5034	0.3755	0.3555	0.5552	0.9200	0.4369
CD (0.05)	0.4369	11.1217	0.8366	0.8411	1.2372	32.0639	0.9734

**Notes:** Data is mean ± SD. T<sub>1</sub> - Uninoculated control, T<sub>2</sub> - *Methylobacterium* sp. PPFM-Os-07, T<sub>3</sub> - *Methylobacterium* sp. NPFM- Os-04, T<sub>4</sub> - *Azospirillum lipoferum* Az204 + *Bacillus megatherium* var *phosphaticum* PSB1, T<sub>5</sub> - *Azospirillum lipoferum* Az204 + *Bacillus megatherium* var *phosphaticum* PSB1 + *Methylobacterium* sp. PPFM-Os-07 + *Methylobacterium* sp. NPFM- Os-04.



availability of nitrogen by nitrogen fixing organisms *Azospirillum* (28, 39), increased uptake of phosphorous due to solubilization of insoluble form of phosphorous into soluble form in the presence of acid secreted by phosphobacteria which helps in stimulating the growth and development of roots thereby facilitating better nutrient absorption (30), production of plant growth promoting substances by facultative methylotrophic bacteria (31, 32). The synergistic effect of biosynthesis of plant growth promoting substances like phytohormones Cytokinin, indole acetic acid, gibberellic acid (7, 10), vitamins and antibiotic substances by the microbial inoculants (33) increased the growth and yield parameters of rice crop (34-39).

#### Physiological parameters

In general, the chlorophyll content increases up to 45 DAT, reaches the maximum value and thereafter decreased. The chlorophyll content increased with increase in the level of nitrogen application and reached the maximum value at 100 % NPK. Regarding the effect of bioinoculant treatment the chlorophyll content was maximum in microbial consortia followed by individual biofertilizer application. Significant interaction effect was observed between different dosage of fertilizer application and bioinoculant treatment on total chlorophyll content of rice leaves. The results of chlorophyll content in the phyllosphere of rice leaf are presented in Table 5. The maximum chlorophyll content was observed at 60 DAT

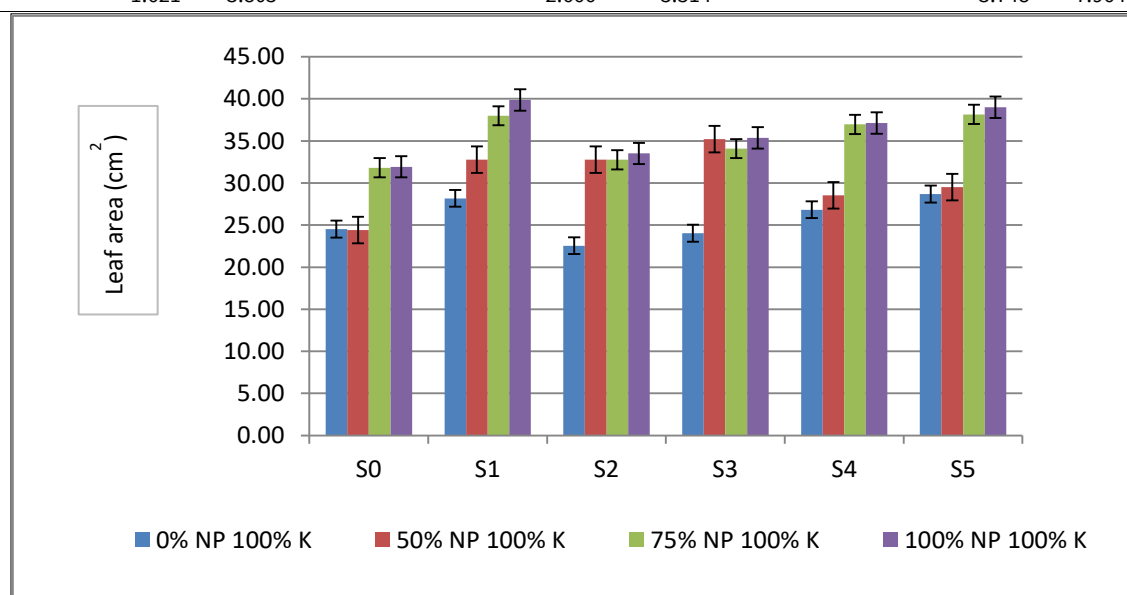
as shown in Fig. 2. in microbial consortia with 100 % NPK and 75 % NP with 100 % K (3.82 and 3.75 mg/g of leaf tissue respectively), followed by *Methylobacterium* sp. PPFM-Os-07 at 75 % NP with 100 % K and 100 % NPK (3.66 and 3.63 mg g<sup>-1</sup> of leaf tissue respectively) over uninoculated treatment in 75 % NP and 100 %K and 100 % NPK which recorded 2.97 and 3.17 mg g<sup>-1</sup> of leaf tissue respectively. Microbial consortia significantly increased the growth parameters, physiological parameters like chlorophyll content, urease activity and ultimately the photosynthate mobilization was found to be evidenced by the increased grain yield (31, 32).

#### Yield and yield parameters

Different dosage of fertilizer application and bioinoculant treatment had significant interaction effect on productive tiller per hill, panicle length, number of grains per panicle, 1000 grain weight, fertility percentage, grain yield and harvest index are presented in Table 6. Significant interaction effect was observed on number of productive tillers per hill and panicle length, while no significant interaction effect on other yield parameters and harvest index. The maximum number of productive tillers per hill was recorded in microbial consortia with 100 % NPK (11.33) over other treatment and uninoculated control with 0 % NP with 100 % K (8.44). The maximum panicle length was observed in GA<sub>3</sub> foliar spray with 100 % NPK (26.67 cm) followed by microbial consortia in 100 % NPK (26.28 cm) while the lowest panicle length was

**Table 5.** Effect of seed treatment with microbial consortia of biofertilizers and chemical fertilizers on growth parameters on ADTRH1 under *in-vivo* condition at 60 DAT

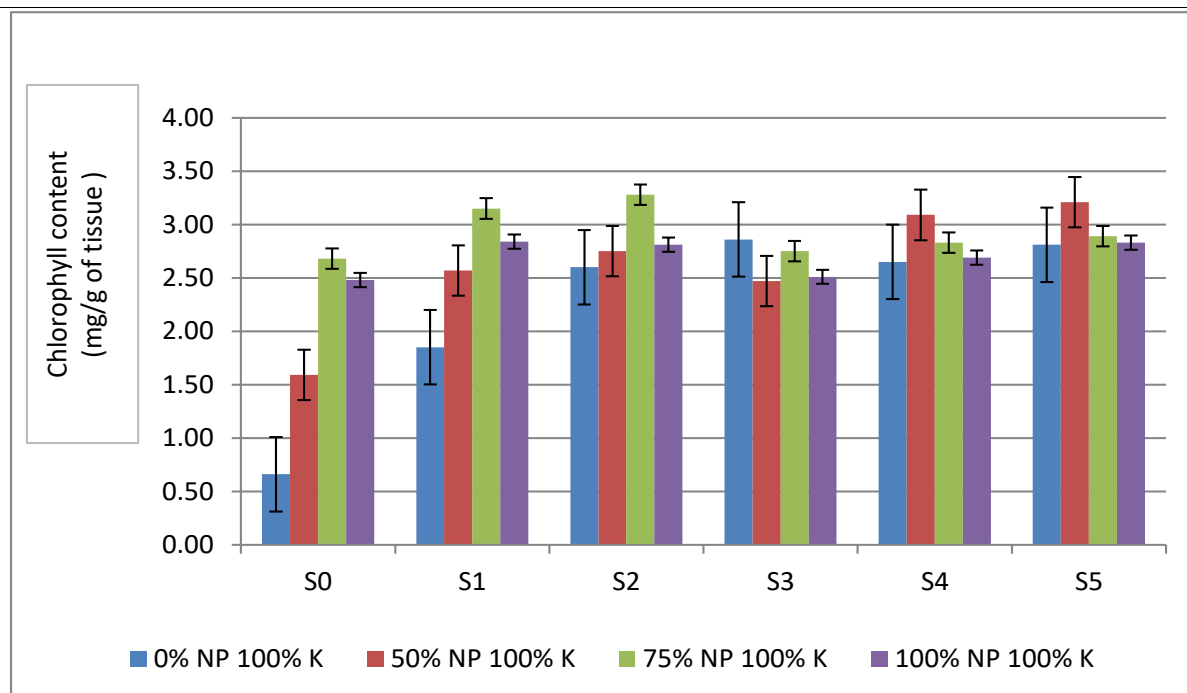
Treatment	Plant height (cm)				DMP (g/plant)				Number of leaf/plant			
	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>
S <sub>0</sub>	56.54	70.00	72.60	77.10	102.94	133.12	149.89	142.65	22.00	27.00	31.33	32.00
S <sub>1</sub>	70.07	80.27	81.25	84.10	142.48	153.71	191.24	158.31	27.33	30.00	33.67	35.00
S <sub>2</sub>	59.79	73.81	76.97	77.63	124.09	148.49	166.98	118.49	26.67	27.33	31.00	31.67
S <sub>3</sub>	65.67	71.5	79.52	80.02	124.92	129.45	157.08	129.70	24.33	25.67	31.67	34.33
S <sub>4</sub>	67.80	79.54	79.98	83.48	126.84	139.69	152.97	152.12	30.33	28.67	31.67	35.00
S <sub>5</sub>	69.17	80.48	81.22	84.14	148.64	150.14	162.32	154.30	31.67	30.33	33.33	37.00
	SEd		CD (0.05)		SEd		CD (0.05)		SEd		CD (0.05)	
M	1.419		3.471		0.886		2.168		1.85		4.527	
S	0.43		0.869		1.377		2.784		1.785		3.609	
M × S	1.621		3.805		2.666		5.514		3.748		7.964	



**Fig. 1.** Effect of different level of chemical fertilizers and microbial consortia of biofertilizers on leaf area of hybrid rice ADTRH1.

**Table 6.** Effect of seed treatment with microbial consortia of biofertilizers and chemical fertilizers on yield parameters of hybrid rice ADTRH1

Treatment	Number of productive tillers per hill				Fertility percentage				Grain yield (kg/ha)			
	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>
S <sub>0</sub>	8.44	8.44	8.91	9.71	63.47	75.8	77.82	63.71	3594	5569	5759	5891
S <sub>1</sub>	9.11	9.42	10	10.34	79.28	70.73	77.99	89.65	4141	6430	7483	7576
S <sub>2</sub>	8.48	8.74	9.62	9.63	65.02	75.99	85.58	80.96	3732	6398	6452	5880
S <sub>3</sub>	8.84	7.67	8.78	9.96	71.21	75.62	77.48	70.75	3839	5385	5865	5994
S <sub>4</sub>	8.81	7.89	9.44	10.18	66.87	75.51	76.27	80.02	4099	5599	6452	6505
S <sub>5</sub>	9.6	8.56	10.71	11.33	68.29	71.71	76.6	80.29	4898	6446	7371	7627
	SEd		CD (0.05)		SEd		CD (0.05)		SEd		CD (0.05)	
M	0.325		0.596		0.008		0.02		487		1199	
S	.230		0.465		0.023		0.047		286		578	
M × S	0.532		1.159		0.043		0.087		714		1585	

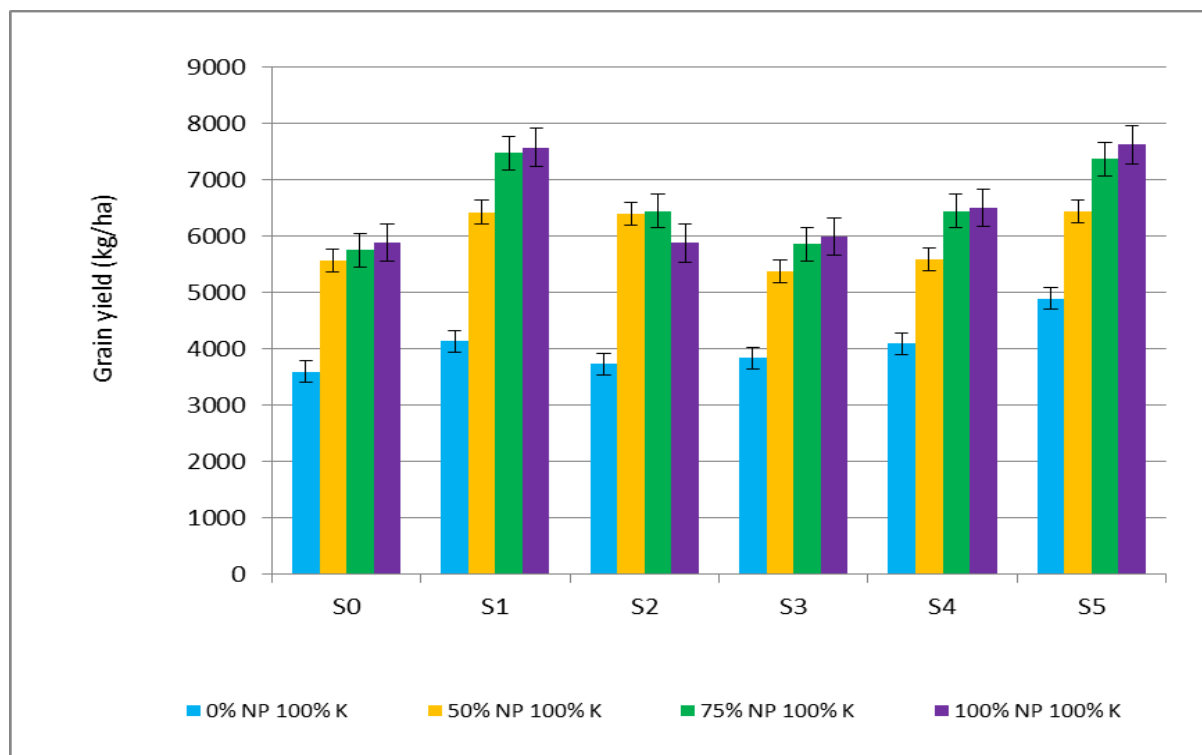
**Fig. 2.** Effect of different level of chemical fertilizers and microbial consortia of biofertilizers on chlorophyll content of hybrid rice ADTRH1.

observed in uninoculated control in 0 % NP with 100 % K (20.19 cm). Though the interaction effect was not statistically significant, the maximum number of grains per panicle was observed in microbial consortia with 100 % NPK (197.29) followed by conventional biofertilizer application with 100 % NPK (196.67) followed by GA<sub>3</sub> foliar spray with 100 % NPK (193.32). The lowest number of filled grains per panicle was recorded in uninoculated control in 0 % NP with 100 % K (128.38). PPFM spraying induces uniform flowering and earliness in flowering (10) as shown in Plate 1. The maximum fertility percentage were recorded in GA<sub>3</sub> spray in 100 % NPK (89.65 %) followed by *Methylobacterium* sp. PPFM-Os-07 in 75 % NP with 100 % K (85.58 %). Though the interaction effect was not statistically significant, the maximum 1000 grain weight was observed in case of 100 % NPK with GA<sub>3</sub> foliar spray (34.30 g) followed by microbial consortia with 100 % NPK (34.27 g). The lowest 1000 grain weight was recorded in uninoculated control with 0 % NP with 100 % K (28.00 g). It could be observed from the results that the maximum grain yield of 7627 kg/ha was recorded in case of treatment of seeds with microbial consortia of biofertilizers at 100 % NPK, followed by PPFM spraying (Fig. 3), followed by GA<sub>3</sub> foliar spray in 100 % NPK (7576 kg/ha). The lowest grain yield of 3594 kg/ha was obtained in uninoculated control in 0 % NP

with 100 % K. The harvest index was the maximum at 100 % NPK with microbial consortia and GA<sub>3</sub> foliar spray (0.43) followed by 75 % NP with 100 % K with microbial consortia and GA<sub>3</sub> foliar spray (0.42). The lowest harvest index was recorded in *Methylobacterium* sp. PPFM-Os-07 at 0 % NP and 100 % K (0.37). The overall effect may be due to combined effect of the PPFM activity in phyllosphere (31, 32), *Azospirillum* (29), phosphate solubilizing bacteria (30) and facultative methylophilic bacteria activity in the rhizosphere soil (31, 32) influences microbial population in soil, thereby enhancing nutrient uptake and nutrient use efficiency by crop plants (38). There is no significant difference in yield, between 75 % NP and 100 % NP fertilizer doses with microbial consortia of biofertilizer treatments which clearly indicate the saving of 25 % cost of NP fertilizers by substituting with combined inoculants thereby reduced the cost of cultivation and increasing the farmer's income and profit.

#### Microbial population studies

Different dosage of fertilizer application, bioinoculant treatments and their interaction effects had significant effect on PPFM population in the phyllosphere and *Azospirillum*, phosphate solubilizing bacteria, facultative methylophilic



**Fig. 3.** Effect of different level of chemical fertilizers and microbial consortia of biofertilizers on grain yield of hybrid rice ADTRH.

bacteria activity in the rhizosphere soil. In general, the population of *Azospirillum*, phosphate solubilizing bacteria and facultative methylotrophic bacteria increased significantly towards the maturity stage of the crop and thereafter the population decreased. The phyllosphere spraying of PPFMs significantly influenced the *Methylobacterium* population in the phyllosphere of rice crop (38). The maximum PPFM population were recorded after three weeks of PPFM spray, thereafter, declines and again increases one week after PPFM spray. Phyllosphere spraying of PPFM induces uniform flowering (Plate 1). The microbial population of *Azospirillum*, phosphate solubilizing bacteria in soil at 45 DAT, PPFM, NPFM population in soil at 60DAT and PPFM population in phyllosphere at 45 DAT were presented in Table 7. The maximum PPFM population in phyllosphere was observed in (Fig. 4) 100 % NPK with microbial consortia ( $216.12 \times 10^6$  cfu/g fresh weight of leaf tissue) over other treatments *Methylobacterium* sp. PPFM-Os-07, GA<sub>3</sub> foliar spray and uninoculated control (201.99, 187.60 and  $181.90 \times 10^6$  cfu/g fresh weight of leaf tissue respectively). The maximum *Azospirillum* population in rhizosphere soil was recorded in microbial consortia with 100 % NPK ( $5.92 \times 10^6$  cfu/g dry weight of soil) followed by conventional biofertilizer application in 75 % NP with 100 % K ( $5.62 \times 10^6$  cfu/g dry weight of soil). The lowest *Azospirillum* population in rhizosphere soil was recorded in uninoculated control at 0 % NP with 100 % K ( $3.50 \times 10^6$  cfu/g dry weight of soil). The maximum phosphate solubilizing bacteria population in rhizosphere soil was recorded in microbial consortia with 100 % NPK ( $8.95 \times 10^6$  cfu/g dry weight of soil). The lowest phosphate solubilizing bacteria population in rhizosphere soil was recorded in uninoculated control in 0 % NP with 100 % K ( $7.55 \times 10^6$  cfu/g dry weight of soil respectively). The highest population of PPFM and the NPFM in rhizosphere soil was recorded at 60 DAT with microbial consortia at 100 % NPK (32.73 and  $47.80 \times 10^3$  dry weight of soil respectively) The lowest *Methylobacterium* population in



A. Effect of PPFM spraying on early flowering in hybrid rice



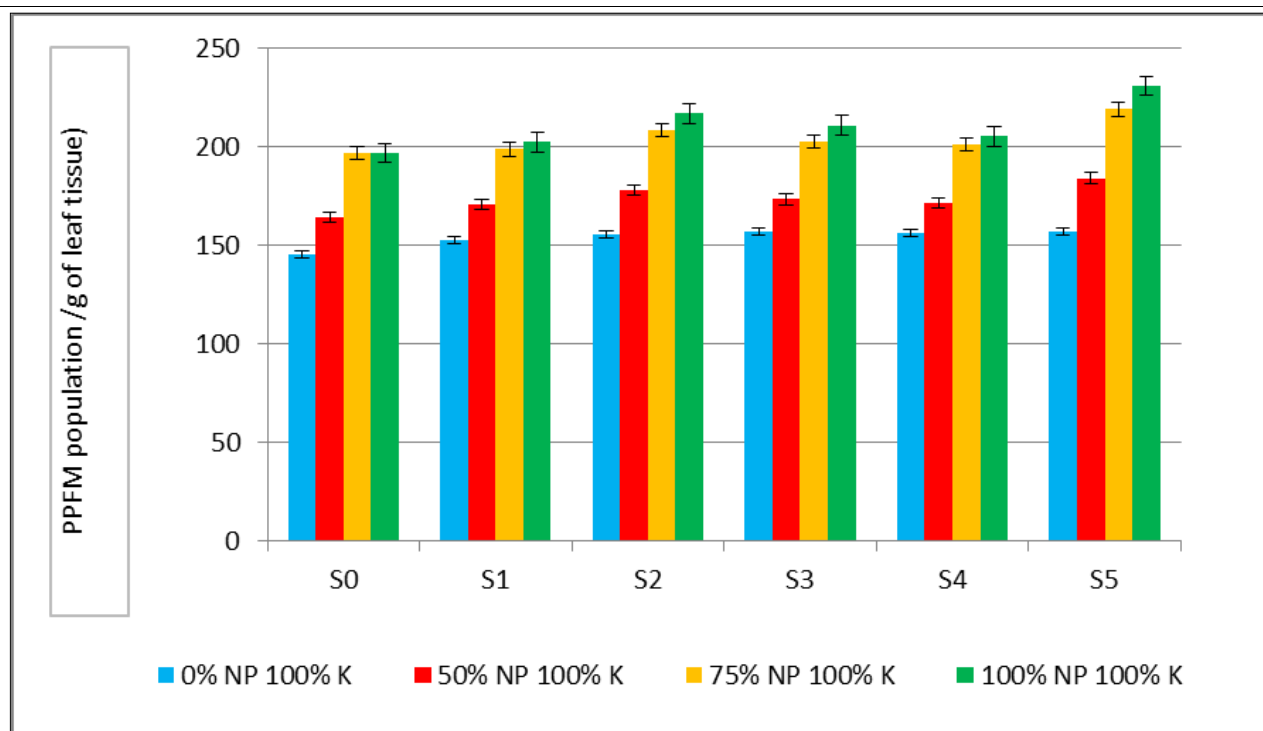
B. Overall view of the experimental field

**Plate 1.** Effect of foliar spraying of PPFM on early flowering in hybrid rice and overall view of experimental field.

rhizosphere soil was recorded in uninoculated control in 0 % NP with 100 % K ( $15.59$  and  $17.78 \times 10^3$  cfu/g dry weight of soil respectively). Thus, it was hypothesized that the increase in the microbial population was correlated with increasing yield and physiologically it was due to higher photosynthate mobilization (31) which may be due to the production of growth hormones and results in the maintenance of better soil fertility by the added biofertilizers. This elucidates that

**Table 7.** Dynamics of *Azospirillum*, PSB, PPFM and NPFM population in rhizosphere soil at 45 and 60 DAT

Treatment	Microbial population in rhizosphere soil ( $\times 10^6$ cfu g <sup>-1</sup> dry weight of rhizosphere soil)								Microbial population in rhizosphere soil ( $\times 10^3$ cfu g <sup>-1</sup> dry weight of rhizosphere soil)							
	Azospirillum (45 DAT)				PSB (45 DAT)				PPFM (60 DAT)				NPFM (60 DAT)			
	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>
S <sub>0</sub>	3.50	0.91	4.05	4.36	7.55	7.58	8.07	8.14	15.59	19.63	22.00	22.92	17.78	20.83	21.62	24.37
S <sub>1</sub>	3.56	4.58	4.99	4.59	7.74	7.96	8.06	8.71	27.36	27.91	20.66	30.57	20.10	25.92	28.98	40.10
S <sub>2</sub>	3.77	4.83	5.01	5.22	8.06	8.11	8.18	8.20	26.79	26.66	26.66	26.88	19.61	23.11	27.8	36.45
S <sub>3</sub>	3.68	4.70	4.81	4.87	8.12	8.18	8.3	8.92	24.16	25.28	24.82	26.03	21.87	24.00	29.84	39.57
S <sub>4</sub>	3.88	5.22	5.3	5.50	8.29	8.30	8.51	9.00	27.23	28.00	29.02	29.30	27.00	27.33	32.86	37.37
S <sub>5</sub>	4.41	5.28	5.62	5.92	8.45	8.62	8.75	8.95	27.23	29.19	29.82	32.73	29.05	20.02	36.44	47.80
SEd	CD (0.05)				SEd				CD (0.05)				SEd			
M	0.018				0.019				0.301				0.240			
S	0.023				0.010				0.478				0.430			
M $\times$ S	0.046				0.027				0.923				0.820			

**Fig. 4.** Effect of different level of chemical fertilizers and microbial consortia of biofertilizers on PPFM Population in phyllosphere of hybrid rice ADTRH.

the soil nutrient levels alone may not be sufficient for the increasing the yield, but the sufficient microbial population in the rhizosphere soil is essential for minimal nutrient mining from the soil and helps to maintain the soil fertility status and increase the yield of crop plants (31, 32, 34).

#### Nutrient analysis

The nutrient analysis available N was estimated by Alkaline permanganate method, available P was estimated by Colorimetry and available K was estimated by Neutral normal ammonium acetate flame photometry method (16-20). The results of nutrient analysis nitrogen, phosphorus and potassium uptake are presented in Table 8. The result revealed that the nutrient uptake varied significantly with the treatment. The different dosage of fertilizer application, biofertilizer treatment and their interaction effect had significant influence on nutrient uptake. The maximum nitrogen, phosphorus and potassium uptake was recorded in microbial consortia of biofertilizers at 100 % NPK (6.92, 9.12

62.18 g/plant respectively) followed by 100 % NPK with GA<sub>3</sub> foliar spray for nitrogen uptake, whereas 100 % NPK with conventional biofertilizer application for higher phosphorus and potassium uptake (8.98 and 59.71 g/plant respectively) over uninoculated control at 0 % NP with 100 % K (4.20, 6.31 and 43.16 g/plant respectively). In case of nutrient use efficiency, the nitrogen, phosphorus and potassium use efficiency varied with the treatments. The nitrogen use efficiency was significantly influenced by different dosage of fertilizer application and bioinoculant treatment (Table 9), while no significant interaction effect was observed. The maximum nitrogen and phosphorous use efficiency was recorded in microbial consortia in 50 % NP with 100 % K (85.95 and 214.88) followed by GA<sub>3</sub> spray with 50 % NP with 100 % K (85.74 and 214.35). The lowest nitrogen and phosphorous use efficiency was recorded in *Methylobacterium* sp. PPFM-Os-07 with 100 % NPK (39.20 and 97.99). Though the interaction effect is not statistically



**Table 8.** Effect of microbial consortia of biofertilizers and chemical fertilizers on nutrient uptake of hybrid rice ADTRH1 at 60 DAT

Treatment	Nutrient uptake (g/plant)											
	N				P				K			
	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>
S <sub>0</sub>	4.20	5.24	5.55	5.75	6.31	7.45	7.64	8.65	43.16	44.63	50.15	51.00
S <sub>1</sub>	5.87	6.10	6.23	6.74	6.45	6.98	7.75	8.70	47.12	48.43	51.11	52.66
S <sub>2</sub>	5.04	5.86	5.75	6.38	6.75	7.14	7.98	8.71	48.13	49.63	52.73	54.63
S <sub>3</sub>	5.24	6.11	6.12	5.82	6.98	7.32	8.26	8.85	49.13	50.83	52.99	55.18
S <sub>4</sub>	5.68	6.05	6.23	5.98	7.12	7.42	8.62	8.98	51.33	52.83	53.79	59.71
S <sub>5</sub>	5.98	6.20	6.35	6.92	7.42	7.68	8.75	9.12	53.13	53.75	54.63	62.18
	SEd		CD (0.05)		SEd		CD (0.05)		SEd		CD (0.05)	
M	0.001	0.004			0.001	0.004			0.113	0.276		
S	0.001	0.002			0.001	0.003			0.18	0.363		
M × S	0.002	0.005			0.003	0.006			0.347	0.717		

**Table 9.** Effect of microbial consortia of biofertilizers and chemical fertilizers on nutrient use efficiency of hybrid rice ADTRH1 at 60 DAT

Treatment	Nutrient use efficiency (%)											
	N				P				K			
	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>0</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>
S <sub>0</sub>	0	74.26	51.19	39.28	0	185.64	127.97	98.19	59.90	92.80	96.00	98.20
S <sub>1</sub>	0	85.74	66.52	50.51	0	214.35	166.30	126.27	69.00	107.20	124.70	126.30
S <sub>2</sub>	0	85.31	57.35	39.20	0	213.28	143.37	97.99	62.20	106.60	107.50	98.00
S <sub>3</sub>	0	71.80	52.14	39.96	0	179.51	130.34	99.90	64.00	89.80	97.80	99.90
S <sub>4</sub>	0	74.65	57.35	43.37	0	186.62	143.37	108.42	68.30	93.30	107.50	108.40
S <sub>5</sub>	0	85.95	65.52	50.84	0	214.88	163.81	127.11	81.60	107.40	122.90	127.10
	SEd		CD (0.05)		SEd		CD (0.05)		SEd		CD (0.05)	
M	5.155	14.310			12.959	35.980			8.116	19.859		
S	3.938	8.043			9.851	20.118			4.770	9.640		
M × S	8.084	18.923			20.261	47.465			11.904	26.415		

significant the maximum potassium use efficiency was recorded in microbial consortia with 100 % NPK (127.1) followed by GA<sub>3</sub> foliar spray (126.3). The lowest potassium use efficiency was observed in case of control with 0 % NP and 100 % K (59.9). The availability of nutrients to the rice plant is increased partly due to enhanced microbial population, thereby enhanced microbial activity in the phyllosphere and rhizosphere soil (5, 38), nitrogen fixation by PPFMs (31, 32), production of plant growth promoting substances like cytokinin, IAA, GA and vitamins especially B<sub>12</sub> (5, 7, 38) and antibiotic substances which exert antagonistic activity against plant pathogens (33, 38). Biofertilizers is essential for minimal nutrient mining from the soil, helps to maintain the soil fertility status and increased the yield of crop plants (3, 30). In hybrid rice uniform flowering is a problem and to avoid the incompatibility of male and female parent the GA<sub>3</sub> spray is done to increase the spikelet fertility percentage (12, 13), increases the early vigour, good crop growth, increased the number of flowering clusters, number of flowers per plant and ultimately increased the yield of the crop plants. Even though it increases fertility percentage and yield of rice crop it is an expensive technique and cannot be afforded by all farmers. Hence Seed treatment with microbial consortia of biofertilizers followed by phyllosphere spraying of PPFM was found to be the economic and best alternative for GA<sub>3</sub> sprays (30-32).

## Conclusion

Seed treatment with facultative methylophilic bacteria along with conventional biofertilizer *Azospirillum* and phosphate

solubilizing bacteria followed by phyllosphere spraying of PPFM increases the growth and yield of rice crop. The results of the present investigation are the first report in tropical country as potent microbial consortia for increasing crop production especially on hybrid rice crop ADTRH1 under *in-vivo* condition. It might therefore be appropriate to target these bacteria for improving the performance of the crop plants. The continued research on facultative methylophilic bacteria is warranted to further elucidate to make facultative methylophilic bacteria as a valuable partner in future agriculture. The potential uses of facultative methylophilic bacteria in world food production are great and may someday have a tremendous impact.

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## Authors' contributions

GT carried out the *in-vitro*, *in-vivo* experiments and drafted the manuscript. SPS participated in the design of the study, conceptualization, supervision and editing. KRK performed the statistical analysis. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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