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Impact of Application of Biofertilizers and Pink Pigmented Facultative Methylotrophs (PPFM) Bacteria on the Soil Properties under Rabi Sorghum Cultivation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: A field experiment was conducted to study the effect of application of biofertilizers and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria on the soil properties.

Study Design: The experiment was laid out in randomised block design with seven treatments that are allocated randomly and replicated thrice.

Place and Duration of Study: Rabi, 2023-24 at Agricultural College Farm, Bapatla.

Methodology: The treatments were $\mathbf{T_1}$ - Control (100 % RDF - 100 : 60 : 40 NPK kg ha^{-1}), $\mathbf{T_2}$ - T_1 + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}), $\mathbf{T_3}$ - T_2 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}, $\mathbf{T_4}$ - T_2 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application, $\mathbf{T_5}$ - 75 % RDF (75: 45: 30 NPK kg ha^{-1}) + Soil application of Biofertilizers (*Azospirillum*, PSB and KRB each @ 1250 ml ha^{-1}), $\mathbf{T_6}$ - T_5 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}, $\mathbf{T_7}$ - T_5 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application.

Results: The data on soil pH, EC and OC as influenced by application of Biofertilizers and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria after harvest of sorghum crop was found to be non-significant. Sorghum crop grown on the treatment $\mathbf{T_3}$ (T_2 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}) showed higher soil available N (265 kg ha^{-1}), P_2O_5 (81.4 kg ha^{-1}) and K_2O (490 kg ha^{-1}) status after harvest of the crop. The treatment $\mathbf{T_4}$ (T_2 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application) showed higher Nitrogen (Grain - 59.7, Stover - 39.2 and Total - 98.9 kg ha^{-1}) Phosphorus (Grain - 16.9, Stover - 10.3 and Total - 27.2 kg ha^{-1}) and Potassium (Grain - 26.7, Stover - 60.5 and Total - 87.1 kg ha^{-1}) uptakes by sorghum at harvest.

Conclusion: Sorghum crop grown on the treatment $\mathbf{T_3}$ (T_2 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}) showed higher soil available N, P_2O_5 and K_2O status after harvest of the crop. The treatment $\mathbf{T_4}$ (T_2 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application) showed higher Nitrogen, Phosphorus and Potassium uptakes by sorghum at harvest.

Keywords: Biofertilizers; foliar application; PPFM bacteria; sorghum.

1. INTRODUCTION

"Sorghum (*Sorghum bicolor* L. Moench) is an important grain and feed crop in the world. It is ranked fifth among the most important grain crops in the world after wheat, rice, maize and barley" (FAOSTAT, 2021). This crop is distinctive due to its drought tolerance capacity, improved water and nutrient use efficiency. It is the main food for the inhabitants of the arid and semi-arid regions of the world. The sorghum is characterized by its tolerance of environmental conditions that are not suitable for the production of other summer crops, especially extreme temperatures, drought and soil salinization. Sorghum is considered as the crop of choice for dry regions and areas with unreliable rainfall because of its drought resistance and wide range of ecological adaptation. Recently, sorghum has been used in the production of biofuels and has become the second largest after maize despite its various uses for food and manufacturing. However, the average production per unit area is very low due to the farmers reluctance to cultivate it and the tendency to grow more cash-profitable crops.

"The inorganic fertilizer could supply only one, two or three nutrients but integrated use of inorganic fertilizers, biocompost and biofertilizer would provide macro and micronutrient to plant, soil and resist occurrence of multiple deficiencies. Bio-fertilizers are also one of the modern methods that lead to increasing crop productivity and improving their quality" (Taey et al., 2019). "Living microbes are used as biofertilizers, inoculants, which are soil amendments intended to promote plant development. They boost crop output by 10-30 % and are incredibly economical. They can partially replace or complement chemical fertilizers by upto 25 %. They promote soil biological activity and plant growth. In addition to protecting against certain soil-borne illnesses, they replenish the natural fertility of the soil" (Yazeid et al., 2007). "Among biofertilizers, *Azospirillum* and phosphate solubilizing bacteria (PSB) are commonly used in crops like sorghum. *Azospirillum* is known to fix considerable quantity of nitrogen in range of 20 - 40 kg ha^{-1} in the rhizosphere in non-leguminous plants such as cereals, millets, oil seeds, cotton etc. The potassium in the soil was either complexed or chelated to insoluble form as in mica or illite which could be solubilized by potassium releasing bacteria by the secretion of organic acids and convert the insoluble potassium (K) into a soluble form which can be readily available to the plants" (Aleksandrov et al., 1967).

"Plant growth-promoting microorganisms (PGPMs) supports plant to survive extreme environmental condition moreover to improving plant growth by establishing beneficially interaction with plants. Pink Pigmented Facultative Methylotrophs (PPFM) bacteria are responsible for diverse beneficial effects on plant includes enhance seed germination and seedling growth, accelerate vegetative growth by producing phytohormones, increase leaf area index and chlorophyll content, earliness in flowering, fruit set, maturation, improves fruit quality, colour, seed weight, yield increase of up to 10 % and mitigate drought. PPFMs promote plant growth by producing an enzyme 1-aminocyclopropane - 1 - carboxylate (ACC) deaminase is responsible for drought management during the beneficial interaction with plants" (Chinnadurai et al., 2009).

2. MATERIALS AND METHODS

The experiment was conducted during *rabi*, 2023-24 at Agricultural College Farm, Bapatla under Acharya N. G. Ranga Agricultural University. The experimental site was situated at an altitude of 5.49 meters above mean sea level (MSL), 15^0 54' North latitude, 80^0 25' East longitude and about 7 km away from the Bay of Bengal in the Krishna Agro Climatic Zone of Andhra Pradesh state of India. The experimental soil was sandy clay in texture, slightly alkaline in reaction, EC was non saline in nature and below the critical point, Soil is medium in organic carbon, low available nitrogen (220 kg ha^{-1}), high available phosphorous (62.1 kg ha^{-1}) and available potassium contents (460 kg ha^{-1}). The experiment was laid out in randomised block design with seven treatments and replicated three times. The treatments include **T_1** - Control (100 % RDF - 100 : 60 : 40 NPK kg ha^{-1}), **T_2** - T_1 + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}), **T_3** - T_2 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}, **T_4** - T_2 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application, **T_5** - 75 % RDF (75: 45: 30 NPK kg ha^{-1}) + Soil application of Biofertilizers (*Azospirillum*, PSB and KRB each @ 1250 ml ha^{-1}), **T_6** - T_5 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}, **T_7** - T_5 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application. The recommended dose of fertilizers (RDF) was in two different doses they are 100 % RDF (100:60:40 NPK kg ha^{-1}) and 75 % RDF (75:45:30 NPK kg ha^{-1}). In case of biofertilizers *viz., Azospirillum*, PSB (Phosphate Solubilizing Bacteria), KRB (Potassium Releasing Bacteria) and PPFM (Pink Pigmented Facultative Methylotrophs) bacteria were thoroughly mixed with FYM and applied in experimental plots as per the treatments which was done one day prior to sowing and was thoroughly mixed in the soil with the help of spade to form a homogeneous blend with the soil. Nitrogen, phosphorus and potassium were supplied through urea, Single Super Phosphate (SSP) and Muriate of Potash (MoP). Nitrogen was applied in two equal split doses *viz*., first dose applied at basal and second dose applied at 30 DAS. Entire quantity of phosphorus and potassium was applied at basal as per the treatments.

Soil samples were collected initially and after harvest of the crop up to 30 cm depth and were shade dried, grounded with a wooden pestle and mortar, passed through 2 mm sieve and finally stored in labelled cloth bags for laboratory analysis. Processed soil samples were used for analyzing various parameters and various nutrients. Soil reaction from the experimental plot was determined in 1:2.5 soil, water suspension using combined glass electrode method (Jackson, 1973). The soluble salt content of soil samples was determined in 1:2.5 soil, water suspension using digital electrical conductivity meter (Jackson, 1973). A known weight (0.5 g) of 0.2 mm sieved soil was treated with a known volume of chromic acid ($K_2Cr_2O_7$ + H_2SO_4). After oxidation of organic carbon, the unreacted $K_2Cr_2O_7$ left in the contents was back titrated against standard ferrous ammonium sulphate solution using diphenylamine indicator (Walkley and Black, 1934). Soil available N content in the soil was determined by alkaline potassium permanganate method (Subbiah and Asija, 1956). Soil available phosphorus in the soil samples was estimated with 0.5 M $NaHCO_3$ of pH 8.5 and the phosphorus in the extract was estimated calorimetrically with ascorbic acid method by using spectrophotometer at 660 nm by Olsen's method (Olsen et al., 1954). Per cent P was multiplied with 2.29 and expressed as P_2O_5. Soil available potassium in the soil was estimated by using neutral normal ammonium acetate method (Jackson, 1973) and potassium in the extract was determined using flame photometer. Per cent K value was multiplied with 1.21 and presented as K_2O.

For uptake the plant samples collected at harvest were washed with dilute 0.1 N HCl and then with distilled water. The samples were shade dried initially and then oven-dried at 65 ^{0}C temperature and powdered in Willey mill. The nitrogen content in sorghum plants was estimated by the micro Kjeldahl distillation method (Piper, 1966) and the results are expressed in percentage. Preparation of acid extract by wet digestion - Take one gram of powdered plant sample was taken in 150 mL Erlenmeyer flask and digested with di-acid mixture of HNO_3 and $HClO_4$ (9:4 ratio). The sample digest was filtered through Whatman No.42 filter paper by washing the residue with double-distilled water till chloride-free and made up to 100 mL volume and the clear extract was used for the determination of P and K. Phosphorus in the di-acid extract of plant samples was estimated by Vanadomolybdo phosphoric yellow colour method using a spectrophotometer at 420 nm wavelengths as described by Tandon (2009) and the results expressed in percentage. Potassium in the di-acid extract of plant samples was determined using a flame photometer as per the method described by Tandon (2009) and Nitrogen, phosphorus, and potassium contents were expressed as percentages on a dry weight basis.

Nutrient uptake - From the chemical analytical data, uptake of the macronutrients at harvest of the crop were calculated and expressed by using the formulae. Nutrients uptake was expressed as kg ha^{-1}.

> Nutrient Uptake (kg ha-1) = Nutrient concentration (%) x Dry weight of grains/stover (kg ha-1) / 100

The data obtained on the different parameters were analyzed statistically by the method of analysis of variance as per the procedure outlined for randomised block design given by Gomez and Gomez (1984). Statistical significance was tested by F value at 0.05 level of probability and critical difference was worked out where ever the effects were significant.

3. RESULTS AND DISCUSSION

3.1 Soil pH

The effect of biofertilizers and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria on soil pH after harvest was found to be non-significant. Similar findings were documented by Mohan and Sharma (2013), Goutami et al. (2015) and Sharma and Thakur (2016).

3.2 Soil EC (dS m^{-1})

The effect of biofertilizers and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria on soil EC after harvest was found to be non-significant. These findings are in accordance with those of Pothare et al. (2007) and Goutami et al. (2015).

3.3 Soil OC (%)

An increase in soil organic carbon content over the initial soil status was observed. However, the influence of biofertilizers and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria on soil organic carbon after harvest was found to be non-significant. The results were in conformity with the findings of Kumar et al. (2023).

3.4 Available Soil N (kg ha^{-1})

Higher available soil nitrogen (265 kg ha^{-1}) was found with the application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Soil application of PPFM bacteria @ 2500 ml ha^{-1}) (T_3), but it was at par with treatments supplied with 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application (T_4) (254 kg ha^{-1}) and application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) (T_2) (248 kg ha^{-1}). Significantly lower soil nitrogen (228 kg ha^{-1}) was recorded with the application of 75 % RDF + Soil application of Biofertilizers (*Azospirillum*, PSB and KRB each @ 1250 ml ha^{-1}) (T_5).

It might be due to microbes harbouring rhizosphere of crops provide benefits to crops through better nutrient availability by way of atmospheric N_2 fixation or solubilizing fixed mineral forms of nutrients (Pandey et al., 2010). *Azospirillum* and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria both are can fixed the nitrogen from atmospheric nitrogen (N_2)

into ammonia (NH_3) through the process of biological nitrogen fixation. The results were in conformity with the findings of Sy et al. (2001), Patidar and Mali (2004) and Reddy et al. (2023).

3.5 Available Soil P_2O_5 (kg ha^{-1})

Significantly higher available soil P_2O_5 (81.4 kg ha^{-1}) was found with the application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Soil application of PPFM bacteria @ 2500 ml ha^{-1}) (T_3), but it was at par with treatments supplied with 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application (T_4) (79.5 kg ha^{-1}) and application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) (T_2) (75.8 kg ha^{-1}). Significantly lower soil phosphorus (65.5 kg ha^{-1}) was recorded with the application of 75 % RDF + Soil application of Biofertilizers (*Azospirillum*, PSB and KRB each @ 1250 ml ha^{-1}) (T_5).

The increased in available P_2O_5 content in soil due to application of P fertilizer, phosphate solubilizing bacteria application and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria might be contributed to direct P addition as well as native P solubilization via release of various organic acids during microbial process. Organic acids and CO_2 liberated during decomposition may have formed complex substances with metal ions, increasing the phosphorus concentration in soil solution. Similar results were reported by Rather and Sharma (2009), Bhavya et al. (2018), Jakhar et al. (2018) and Kumar et al. (2023).

3.6 Available Soil K_2O (kg ha^{-1})

Significantly higher available soil K_2O (490 kg ha^{-1}) was found with the application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Soil application of PPFM bacteria @ 2500 ml ha^{-1}) (T_3), but it was at par with treatments supplied with 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application (T_4) (488 kg ha^{-1}) and application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) treatments (T_2) (484 kg ha^{-1}) and 100 % RDF (T_1) (469 kg ha^{-1}). Significantly lower soil potassium (428 kg ha^{-1}) was recorded with the application of 75 % RDF + Soil application of Biofertilizers (*Azospirillum*, PSB and KRB each @ 1250 ml ha^{-1}) (T_5). Aleksandrov et al. (1967) and Bennet et al. (1988) stated that the potassium in the soil was either complexed or chelated to insoluble form as in mica or illite which could be solubilized by potassium releasing bacteria by the secretion of citric acid and oxalic acids both can convert the insoluble potassium (K) into a soluble form which can be readily available to the plants. Similar findings were reported by Vijayalakshmi et al. (2020).

3.7 Nitrogen Uptake (kg ha^{-1})

Significantly higher nitrogen uptake in grain, stover and total (59.7, 39.2 and 98.9 kg ha^{-1}, respectively) was found with the application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application (T_4). However, it was statistically at par with application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Soil application of PPFM bacteria @ 2500 ml ha^{-1}) (T_3) (55.2, 35.6 and 90.8 kg ha^{-1}, respectively) and 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) (T_2) (52.0, 34.1 and 86.1 kg ha^{-1}, respectively). Significantly lower nitrogen uptake in grain, stover and grain (39.2, 23.0 and 62.2 kg ha^{-1}, respectively) was recorded with the application of 75 % RDF + Soil application of Biofertilizers (*Azospirillum*, PSB and KRB each @ 1250 ml ha^{-1}) (T_5). The higher nitrogen uptake of nutrient was noticed at harvest, it might be due to increase in biomass production. It is well emphasized that with increasing rate of nitrogen improved over all growth of crop in terms of dry matter production. Bio inoculant application enhances the root growth coupled with increased availability of nutrients in the rhizosphere by N_2 fixation which leads to increased N uptake by the plants (Rekha et al., 2018). Furthermore, enhancing the production of higher stover yield and higher nutrient availability and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria can increased the phytohormones production which in turn increased root growth and proliferation leads to higher nutrient uptake. Holland and Polacco (1992) reported that foliar spraying of PPFM enhanced N and P uptake in blackgram, as they also produced Cytokinin, Indole acetic acid and Gibberellic Acid. These findings are in accordance with those of Jat et al. (2013), Kushwaha et al. (2014) and Bhumika et al. (2020).

Table 1. Soil pH, EC (ds m^{-1}) and OC (%) of sorghum influenced by the application of biofertilizers and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria

Treatments	pH	EC (ds m^{-1})	OC (%)
T_1 - Control (100 % RDF 100: 60: 40 NPK kg ha^{-1})	7.47	0.41	0.63
T_2- T_1 + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1})	7.42	0.39	0.65
T_3 - T_2 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}	7.37	0.37	0.66
T_4 - T_2 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application	7.39	0.38	0.67
T_5 - 75 % RDF (75: 45: 30 NPK kg ha^{-1}) + Soil application of Biofertilizers (*Azospirillum*, PSB and KRB each @ 1250 ml ha^{-1})	7.34	0.36	0.60
T_6 - T_5 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}	7.28	0.35	0.61
T_7 - T_5 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application	7.31	0.35	0.62
SEm±	0.28	0.02	0.03
CD (p = 0.05)	NS	NS	NS
CV (%)	6.70	7.33	7.60
Initial	7.69	0.41	0.59

Table 2. Soil available N (kg ha^{-1}), P_2O_5 (kg ha^{-1}) and K_2O (kg ha^{-1}) status after harvest as influenced by application of biofertilizers and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria

Treatment	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})
T_1 - Control (100 % RDF 100: 60: 40 NPK kg ha^{-1})	236	70.4	469
T_2 - T_1 + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1})	248	75.8	484
T_3 - T_2 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}	265	81.4	490
T_4 - T_2 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application	254	79.5	488
T_5 - 75 % RDF (75: 45: 30 NPK kg ha^{-1}) + Soil application of Biofertilizers (*Azospirillum*, PSB and KRB each @ 1250 ml ha^{-1})	228	65.5	428
T_6 - T_5 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}	231	69.8	444
T_7 - T_5 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application	229	68.0	443
SEm±	7.7	3.4	14.3
CD (p = 0.05)	23.6	10.6	44.2
CV (%)	5.5	8.2	5.4
Initial	220	62.1	460

Table 3. Nitrogen uptake (kg ha^{-1}) by sorghum at harvest as influenced by application of biofertilizers and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria

Treatments	Grain	Stover	Total
T_1 - Control (100 % RDF 100: 60: 40 NPK kg ha^{-1})	47.7	28.3	76.0
T_2 - T_1 + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1})	52.0	34.1	86.1
T_3 - T_2 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}	55.2	35.6	90.8
T_4 - T_2 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application	59.7	39.2	98.9
T_5 - 75 % RDF (75: 45: 30 NPK kg ha^{-1}) + Soil application of Biofertilizers (*Azospirillum*, PSB and KRB each @ 1250 ml ha^{-1})	39.2	23.0	62.2
T_6 - T_5 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}	41.8	24.8	66.6
T_7 - T_5 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application	45.2	26.1	71.3
SEm$\pm$	2.6	1.8	3.1
CD (p = 0.05)	8.1	5.5	9.8
CV (%)	9.3	10.2	7.0

3.8 Phosphorus Uptake (kg ha^{-1})

Significantly higher phosphorus uptake in grain, stover and total (16.9, 10.3 and 27.2 kg ha^{-1}, respectively) was found with the application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application (T_4). However, it was statistically at par with application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Soil application of PPFM bacteria @ 2500 ml ha^{-1}) (T_3) (15.1, 9.7 and 24.8 kg ha^{-1}, respectively) and application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) (T_2) (14.3, 9.3 and 23.6 kg ha^{-1}, respectively). Significantly lower phosphorus uptake in grain, stover and total (10.4, 5.7 and 16.1 kg ha^{-1}, respectively) was recorded with the application of 75 % RDF + Soil application of Biofertilizers (*Azospirillum*, PSB and KRB each @ 1250 ml ha^{-1}) (T_5). Sharma et al. (2012) found that significant increase of phosphorus uptake by wheat with increasing levels of phosphorus up to 60 kg P_2O_5 ha^{-1}. Application of phosphate solubilizing bacteria and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria might have improved the soil environment, which encouraged proliferous root system resulting in better absorption of water and nutrients from lower layers and thus resulting in higher yield and nutrient uptake and greater mobilization of insoluble inorganic phosphate and mineralization of organic phosphorus and thus stimulated better root growth and favoured absorption of phosphorus. Similar findings were documented by Dadheech, (2001), Roul and Sarawgi (2005), Abdullahi et al. (2014), Kumar et al. (2016) and Vijayalakshmi et al. (2020).

3.9 Potassium Uptake (kg ha^{-1})

Significantly higher potassium uptake in grain, stover and total (26.7, 60.5 and 87.1 kg ha^{-1}, respectively) was found with the application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application (T_4). However, it was statistically at par with application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Soil application of PPFM bacteria @ 2500 ml ha^{-1}) (T_3) (24.4, 55.6 and 80.0 kg ha^{-1}, respectively) and application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) (T_2) (23.5, 53.0 and 76.5 kg ha^{-1}, respectively). Significantly lower potassium uptake in grain, stover and total (13.9, 33.0 and 46.9 kg ha^{-1}, respectively) was recorded with the application of 75 % RDF + Soil application of Biofertilizers (*Azospirillum*, PSB

Table 4. Phosphorus uptake (kg ha^{-1}) by sorghum at harvest as influenced by application of biofertilizers and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria

Treatments	Grain	Stover	Total
T_1 - Control (100 % RDF 100: 60: 40 NPK kg ha^{-1})	13.2	8.1	21.2
T_2 - T_1 + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1})	14.3	9.3	23.6
T_3 - T_2 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}	15.1	9.7	24.8
T_4 - T_2 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application	16.9	10.3	27.2
T_5 - 75 % RDF (75: 45: 30 NPK kg ha^{-1}) + Soil application of Biofertilizers (*Azospirillum*, PSB and KRB each @ 1250 ml ha^{-1})	10.4	5.7	16.1
T_6 - T_5 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}	11.9	6.3	18.2
T_7 - T_5 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application	12.2	7.0	19.2
SEm$\pm$	0.9	0.4	0.9
CD (p = 0.05)	2.7	1.2	2.9
CV (%)	11.2	8.7	7.6

Table 5. Potassium uptake (kg ha^{-1}) by sorghum at harvest as influenced by application of biofertilizers and Pink Pigmented Facultative Methylotrophs (PPFM) bacteria

Treatments	Grain	Stover	Total
T_1 - Control (100 % RDF 100: 60: 40 NPK kg ha^{-1})	19.4	45.6	65.1
T_2- T_1 + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1})	23.5	53.0	76.5
T_3 - T_2 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}	24.4	55.6	80.0
T_4 - T_2 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application	26.7	60.5	87.1
T_5 - 75 % RDF (75: 45: 30 NPK kg ha^{-1}) + Soil application of Biofertilizers (*Azospirillum*, PSB and KRB each @ 1250 ml ha^{-1})	13.9	33.0	46.9
T_6 - T_5 + Soil application of PPFM bacteria @ 2500 ml ha^{-1}	14.4	36.3	50.7
T_7 - T_5 + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application	16.4	40.2	56.6
SEm$\pm$	1.0	2.5	2.5
CD (p = 0.05)	3.2	7.6	7.7
CV (%)	9.1	9.3	6.6

and KRB each @ 1250 ml ha^{-1}) (T_5). This might be due to potassium releasing bacteria can forms the biofilms on root surfaces, that can increase the contact between plant roots and solubilized potassium. Pink Pigmented Facultative Methylotrophs (PPFM) bacteria increases the gibberellic acid this acid can increased the root growth leads to increased nutrient uptake. Jeyajothi and Pazhanivelan (2017) concluded that application of 125% RDF through WSF + *Azophosmet* under drip irrigation and foliar spray of 1% PPFM with variety of Co (Rg)7 can be recommended for higher yield and nutrient uptake of pigeonpea. Similar results were reported by Jat et al. (2013), Kushwaha et al. (2014), Kumar et al. (2016) and Vijayalakshmi et al. (2020).

4. CONCLUSION

Significantly higher N (265 kg ha^{-1}), P_2O_5 (81.4 kg ha^{-1}) and K_2O (490 kg ha^{-1}) status of the soil recorded with the application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Soil application of PPFM bacteria @ 2500 ml ha^{-1}) (T_3), but it wssas at par with the application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application (T_4) (N-254 kg ha^{-1}, P_2O_5 -79.5 kg ha^{-1} and K_2O-488kg ha^{-1}) and with application of

100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) (N-248 kg ha^{-1}, P_2O_5 -75.8 kg ha^{-1} and K_2O-484 kg ha^{-1}) (T_2).

Significantly higher nitrogen uptake in grain, stover and total (59.7, 39.2 and 98.9 kg ha^{-1}, respectively) was found with the application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, Phosphate Solubilizing Bacteria and Potassium Releasing Bacteria each @ 1250 ml ha^{-1}) + Foliar application of PPFM bacteria at 30 and 45 DAS @ 1250 ml ha^{-1} for each application (T_4), but it was at par with application of 100 % RDF + Soil application of Biofertilizers (*Azospirillum*, PSB and KRB each @ 1250 ml ha^{-1}) + Soil application of PPFM bacteria @ 2500 ml ha^{-1} (T_3) (55.2, 35.6 and 90.8 kg ha-1, respectively). Almost similar trend was observed in respect to uptakes of P and K.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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