

Original Research Article

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Effect of Phyllosphere Application of *Methylobacterium* on Growth and Yield of Barnyard Millet (*Echinochloa frumentacea* Var. COKV 2)

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ABSTRACT

Methylobacterium as a ubiquitous symbiont in phylloplane and its influence on crop growth at various stages. This Pink-pigmented facultative methylotrophic bacteria (PPFMs) strains isolated from the phylloplane of *Echinochloa frumentacea*. A field experiment was conducted to study the effect of *Methylobacterium* with combination of treatments viz., seed imbibition, soil application and phyllosphere spray in kudiraivali under dryland conditions. This treatment was imposed at 30 and 60 days after sowing. Observations on phylloplane microbial population, plant growth and yield characters were recorded to study the effect of the treatments. Results revealed that, treatment (T₆ – Seed Imbibition + 1% Foliar spray of *Methylobacterium* at 30 & 60 DAS) recorded the highest yield of 1251 Kgs/ha. This treatment also increased the yield parameters like number of productive tillers (8.3 Nos), plant height (183cm), days to 50 % flowering (48days), No of productive tillers (8.0 Nos), panicle length (23.6 cm) and days to maturity (33 days)) and straw yield (2863kg/ha) were also recorded in the treatment. This treatment is followed by T₁₂ (Seed Treatment + 1% Foliar spray of *Methylobacterium* at 30 & 60 DAS) 1128 Kg/ha.

Keywords

Barnyard millet,
Methylobacterium,
Phyllosphere spray,
Seed imbibitions,
Plant growth
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Introduction

Bio-fertilizer can be an important component of integrated nutrients management. Microorganisms that are commonly used as bio-fertilizer components include; nitrogen fixers (N-fixer), potassium and phosphorus solubilizers, growth promoting rhizobacteria

(PGPRs), endo and ecto mycorrhizal fungi, cyanobacteria and other useful microscopic organisms. The use of bio-fertilizers leads to improved nutrients and water uptake, plant growth and plant tolerance to abiotic and biotic factors. These potential biological fertilizers would play a key role in productivity and sustainability of soil and also

in protecting the environment as eco-friendly and cost effective inputs for the farmer (Itelima *et al.*, 2018).

Plant-associated beneficial microbes are recently attaining greater attention as they play an important role in enhancing the productivity of the crops and also providing resistance against the stress conditions and are known as plant growth promoting microbes (PGPMs) (Yadav and Yadav, 2018). Bacteria of the genus *Methylobacterium* are also called as, pink-pigmented facultative methylotrophic bacteria (PPFMs). They are strict aerobic, Gram-negative rods, able to grow on C1 compounds. The PGPMs contribute to mitigate the stress conditions by diverse mechanisms. The PGPMs directly enhancing the uptake of the micronutrients, through phytohormones production; fixing of atmospheric nitrogen; P, K, and Zn-solubilization or indirectly stimulating the immune system against various fungal pathogens by production of various compounds, enzymes, siderophores, antibiotics, osmolytes or improving either texture or structure of the soil (Sivakumar *et al.*, 2018).

Methylobacterium are ubiquitous in nature found in a variety of habitats. Methylotrophs have been reported to influence seed germination and seedling growth by producing plant growth regulators like zeatin and related cytokinins, auxins and to alter agronomic traits like branching, seedling vigour, rooting and heat/cold tolerance. These PPFMs are especially abundant on leaves of field-grown crops averaged about 10^6 cfu of PPFMs per leaflet, and typically >80% of the viable bacteria recovered from leaves were PPFMs. The occurrence of soil methylotrophs is probably related to the abundance of plant lignin and pectin in soils; these polymers are major potential sources of methanol (Subhaswaraj *et al.*, 2017).

Henceforth, inoculation of *Methylobacterium* isolates that are able to alleviate drought stress could be preferable in the context of environmentally sustainable agriculture. Keeping all the above points in view, it is proposed to conduct an experiment to study the effect of methylotrophs on growth and yield of kudiraivali.

Materials and Methods

Field experiment was conducted with Barnyard millet variety Co-KV-2 at Regional Research station, Aruppukottai, Tamil Nadu Agricultural University, Virudhunagar district of Tamil Nadu during Kharif 2016-17. The experiment was designed in RBD with 12 treatments, and three replications. Standard cultural practices were followed as recommended by Tamil Nadu Agricultural University, Coimbatore, Tamilnadu. The data were statistically analyzed and critical differences determined by following Gomez and Gomez, 1984.

The treatment details

- T₁. Un inoculated Control
- T₂– Seed Treatment (ST) with Azos + PSB
- T₃ – Seed Imbibition (SI) with 1% PPFM (*Methylobacterium extorquens*)
- T₄ - SI + 1% Foliar spray of PPFM at 30 Days after Sowing (DAS)
- T₅ - SI + 1% Foliar spray of PPFM at 60DAS
- T₆ - SI + 1% Foliar spray of PPFM at 30 & 60 DAS
- T₇ - 1% Foliar spray of PPFM at 30DAS
- T₈ - 1% Foliar spray of PPFM at 60DAS
- T₉ - 1% Foliar spray of PPFM at 30 & 60 DAS
- T₁₀- SI + 1% Foliar spray of PPFM at 30 DAS
- T₁₁-ST + 1%Foliar spray of PPFM at 60DAS
- T₁₂-ST + 1% Foliar spray of PPFM at 30 & 60 DAS

Observations

The following observations *viz.*, microbial population, plant growth parameters were recorded at 30 DAS, 60 DAS, and at harvest. The yield parameters were recorded during harvest.

Enumeration of microbial population and PPFM in leaves

Enumeration of different microbial population (*viz.* Bacteria, Fungi, Actinomycetes) were done in their specific media, sterilized in an autoclave at 15 psi pressure and 121°C temperature for 20 minutes using serial dilution spread plating technique (at different intervals of time).

Leaf samples from different treatments were collected after spraying, 30 DAS, and 60 DAS for enumeration of PPFM. The phyllosphere population of PPFM was enumerated by employing serial dilution and plating technique Corpe and Rheem, 1989).

Results and Discussion

Microbial population of the rhizosphere of Kudraivali Co-(kv) 2

The microbial population of bacteria was significantly recorded highest at 30 and 60 DAS due to inoculation of bio-fertilizers. The maximum population of bacteria was recorded in the Seed treatment and 1% phyllosphere spraying of PPFM at 30 & 60 DAS (58.00 CFU x 10⁶ g⁻¹ and 111.37 CFU x 10⁶ g⁻¹) which was on par with T₆ and the minimum population was recorded in the control (Table 1). Increased bacterial population in the rhizosphere of kudraivali plants inoculated with bio-fertilizers is because of inoculation bacterial cultures that would have proliferated in rhizosphere. Hence, in all the stages of plant growth, the microbial population with respect to bacteria

has given significant values. In case of fungi and actinomycetes population, also same results obtained at both the stages of plant growth. However, with respect to fungi, highest population was recorded in T₁₂ Seed treatment and 1% phyllosphere spraying of PPFM at 30 & 60 DAS (35.69 × 10⁻³ cfu g⁻¹ soil and 61.8 × 10⁻³ cfu g⁻¹ soil) followed by treatment T₆ (34.45 × 10⁻³ cfu g⁻¹ soil and 45.4 × 10⁻³ cfu g⁻¹ soil) and minimum in control, with respect to actinomycetes highest population was recorded in Seed treatment and 1% phyllosphere spraying of PPFM at 30 & 60 DAS (16.5 × 10⁻³ cfu g⁻¹ soil and 29.7 × 10⁻³ cfu g⁻¹ soil). Increase in rhizosphere microflora may be attributed to the multiplication of the strains in the rhizosphere, utilizing root exudates produced by the plants (Mohanram and Kumar, 2019) and synergistic interactions between introduced microbial inoculants

Methylobacterium population of the phyllosphere of Kudraivali Co-(kv) 2

Phyllosphere harbors diverse group of microorganisms and the interaction between the plant and microbes greatly affect the physiological activities of the plant. Pink pigmented facultative methylotrophs (PPFM) are the prime inhabitants of phyllosphere region of wide variety of plant species (Ochsne *et al.*, 2015; Raja *et al.*, 2008). The present study gives a result suggesting that the Seed imbibition and 1% phyllosphere spraying of PPFM at 30 & 60 DAS maximize the phyllosphere prevalence of PPFM recording an increased population on the leaf at 30DAS (18.42 X10⁵ cfu ml⁻¹), and 60 DAS (29.7 X10⁵ cfu ml⁻¹) followed by Seed treatment and 1% phyllosphere spraying of PPFM at 18.12 X10⁵ cfu ml⁻¹), and 60 DAS (24.8 X10⁵ cfu ml⁻¹). PPFMs are highly diversified group of microorganisms abundantly present on the phyllosphere region of several plant species and enhance the plant growth by producing a wide variety of

phytohormones (Meena *et al.*, 2012; Mizuno *et al.*, 2013). Methylobacterium are symbiotically associated with host plant and regulates host plant growth promotion by

producing a number of phytohormones like IAA, cytokinins (Madhaiyan *et al.*, 2012; Dourado *et al.*, 2015) (Table 2).

Table.1 Effect of phyllosphere spray of Methylobacterium on microbial population of the rhizosphere of Kudiraivali Co-(kv) 2

Treatments	30 DAS			60 DAS		
	Bacteria CFU x 10 ⁶ g ⁻¹	Fungi CFU x 10 ³ g ⁻¹	Actino- bacteria CFU x 10 ⁴ g ⁻¹	Bacteria CFU x 10 ⁶ g ⁻¹	Fungi CFU x 10 ³ g ⁻¹	Actino- bacteria CFU x 10 ⁴ g ⁻¹
T ₁ - Un inoculated Control	21.21	17.50	8.9	49.64	22.2	12.6
T ₂ - ST with Azos + PSB	34.72	20.36	13.1	72.11	25.6	19.4
T ₃ – SI with 1% PPFM	48.83	33.10	11.4	64.68	32.4	15.0
T ₄ - SI + 1% Foliar spray of PPFM at 30DAS	34.42	19.46	14.7	75.45	28.5	22.0
T ₅ - SI + 1% Foliar spray of PPFM at 60DAS	48.12	23.11	13.1	87.49	46.5	16.7
T ₆ - SI + 1% Foliar spray of PPFM at 30 & 60 DAS	52.60	34.45	14.9	97.66	45.4	26.2
T ₇ - 1% Foliar spray of PPFM at 30DAS	34.00	23.60	10.7	52.02	36.1	18.5
T ₈ - 1% Foliar spray of PPFM at 60DAS	25.00	27.54	10.7	41.57	39.3	10.4
T ₉ - 1% Foliar spray of PPFM at 30 & 60 DAS	37.00	33.10	14.7	44.64	52.5	24.8
T ₁₀ - SI + 1% Foliar spray of PPFM at 30 DAS	49.00	34.24	14.1	100.99	46.0	23.4
T ₁₁ -ST + 1%Foliar spray of PPFM at 60DAS	42.00	32.68	12.0	99.40	58.0	22.3
T ₁₂ -ST + 1% Foliar spray of PPFM at 30 & 60 DAS	58.00	35.69	16.5	111.37	61.8	29.7
SEd	3.06	2.079	1.806	4.095	2.515	2.210
CD (0.05%)	6.42	4.37	3.79	8.60	5.28	4.64

Table.2 Effect of phyllosphere spray of *Methylobacterium* on population of Kudraivali Co-(kv) 2

Treatments	Methylobacterium CFU x 10 ⁵ g ⁻¹	
	30 DAS	60 DAS
T ₁ - Un inoculated Control	8.52	12.5
T ₂ - ST with Azos + PSB	8.67	13.1
T ₃ – SI with 1% PPFM	10.12	17.5
T ₄ - SI + 1% Foliar spray of PPFM at 30DAS	17.23	22.5
T ₅ - SI + 1% Foliar spray of PPFM at 60DAS	10.45	23.2
T ₆ - SI + 1% Foliar spray of PPFM at 30 & 60 DAS	18.42	29.7
T ₇ - 1% Foliar spray of PPFM at 30DAS	17.45	21.2
T ₈ - 1% Foliar spray of PPFM at 60DAS	9.47	20.2
T ₉ - 1% Foliar spray of PPFM at 30 & 60 DAS	11.12	24.1
T ₁₀ - SI + 1% Foliar spray of PPFM at 30 DAS	16.47	22.2
T ₁₁ -ST + 1%Foliar spray of PPFM at 60DAS	8.47	25.1
T ₁₂ -ST + 1% Foliar spray of PPFM at 30 & 60 DAS	18.12	24.8
SEd	1.37	4.77
CD (0.05%)	2.88	12.5

Table.3 Effect of phyllosphere spray of *Methylobacterium* on plant biometric and yield parameters of Kudraivali Co-(kv) 2

Treatments	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of productive tillers	Panicle length (cm)	Grain yield Kg/ha	Straw yield Kg/ha
T ₁ - Un inoculated Control	56	36	131	4.2	14.9	660	2175
T ₂ - ST with Azos + PSB	56	36	138	5.6	16.4	680	2543
T ₃ – SI with 1% PPFM	56	36	147	4.3	17.2	685	1958
T ₄ - SI + 1% Foliar spray of PPFM at 30DAS	54	34	142	5.8	18.1	780	1934
T ₅ - SI + 1% Foliar spray of PPFM at 60DAS	54	34	158	5.5	18.6	812	1906
T ₆ - SI + 1% Foliar spray of PPFM at 30 & 60 DAS	48	33	183	8.3	23.6	1251	2863
T ₇ - 1% Foliar spray of PPFM at 30DAS	55	35	136	6.3	20.3	943	1613
T ₈ - 1% Foliar spray of PPFM at 60DAS	53	34	146	6.7	19.0	962	1698
T ₉ - 1% Foliar spray of PPFM at 30 & 60 DAS	52	34	147	7.0	19.5	997	1972
T ₁₀ - SI + 1% Foliar spray of PPFM at 30 DAS	52	34	152	7.0	20.2	985	1701
T ₁₁ -ST + 1%Foliar spray of PPFM at 60DAS	53	35	162	5.3	20.8	1021	2016
T ₁₂ -ST + 1% Foliar spray of PPFM at 30 & 60 DAS	50	33	172	8.0	22.1	1128	2675
SEd	3.528	2.83	5.40	0.96	1.821	3.123	10.345
CD (0.05%)	7.412	5.94	11.34	2.02	3.827	6.342	22.458

Plant growth and yield

The phyllosphere spraying of PPFM with 1% concentration at 30 and 60 DAS increased the plant growth and grain yield. As regards to the 2016-2017 trial, the highest yield was recorded with the application of T₆ - 75 % RCF + SI and Phyllosphere spray of PPFM (1251 Kgs/ha) followed by T₁₂ of 1128 Kg/ha. High yield contributing characters like number of productive tillers (8.3 Nos), plant height (183cm), days to 50 % flowering (48days), No of productive tillers (8.0 Nos), panicle length (23.6 cm) and days to maturity (33 days)) and straw yield (2863 kg/ha) followed by T₁₂- RCF + ST and PPFM 1% 30 DAS and 60 DAS recorded the next higher values while control recorded lower yield of number of productive tillers (8.0 Nos), plant height (172cm), days to 50 % flowering (50 days), No of productive tillers (8.0) panicle length (22.1 cm) days to maturity (33 days) and straw yield (2675 kg/ha) (Table 3).

Madhaiyan *et al.*, (2005) has brought out the beneficial role of phyllosphere PPFM in augmenting the plant productivity. In the present study, increases in the number of PPFMs were positively correlated with the yield increases in kudiraivali.

The beneficial role of PPFMs, when applied as seed inoculant in enhancing germinability, storability or vigour of the seeds was already attributed to their ability to produce the plant growth regulators like Indole acetic acid and ultimately to increased plant growth and yield and also having biocontrol ability (Poorniammal, 2010; Ivanova *et al.*, 2000).

The foliar application of Methylobacterium had significantly improved the overall performance of the tomato plant growth and yield over uninoculated plants (Senthilkumar and Krishnamoorthy, 2017).

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