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Effect of *Methylobacterium* on seed germination, growth and yield of Barnyard Millet (*Echinochloa frumentacea* Var. COKV 2) under Rainfed Condition

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Abstract
Under rainfed conditions dependence on chemical fertilizers being quite expensive and making the cost of production high. In such a situation the biofertilizers play a major role and an alternative method for provide nutrients to plants. Hence, this study was carried at Arrupukottai during rainfall periods (A total of 273.1 mm of rainfall was received in 21 rainy days. A deficit rainfall of 25.7 per cent was recorded over normal 273.1 mm) out to determine the effect of *Methylobacterium* on the germination and yield components of *Echinochloa frumentacea.* A field experiment was conducted to study the effect of *Methylobacterium* on seed imbibition, on germination of kudiraivali under dryland conditions. Observations on Germination percentage, vigour index, microbial population, plant growth and yield characters were recorded to study the effect of the treatments. Results revealed that, the seed imbibitions of *Methylobacterium* 2% (T_5) showed higher germination percentage (97%), vigour index (2832) and population of bacteria (112.32 x 10^6 cfu/g), fungi (52.14 x 10^3 cfu/g) and actinobacteria (26.4 x 10^4 cfu/g) and the yield parameters like number of productive tillers (6.6 Nos), plant height (186 cm), days to 50% flowering (54 days), panicle length (22 cm) and days to maturity (33 days), grain yield (1838 kg/ha) and straw yield (3759kg/ha).

Keywords: *Echinochloa frumentacea*, *Methyl bacterium*, Microbial population Seed germination, Yield

Introduction
Microorganisms play a vital role in sustainable agriculture and are used in maintaining soil texture, health and fertility. To have sustainability in agriculture, it is necessary to establish a production system, which is efficient, profitable, eco-friendly, conserving or enhancing renewable sources. Biofertilizers are now well recognized as important component of sustainable agriculture.

Bioinoculants have been used as a commercial alternative to chemical fertilizers to reduce environmental effects and diseases and promote plant growth. Bioinoculants are microbial preparations of a single or consortia of living microorganisms. The methylotrophic bacteria are important bioinoculants (Raghavendra *et al*., 2019) [14].

The use of chemical fertilizers in combination with beneficial rhizobacteria is another approach to enhancing soil fertility as well as increasing crop yields without loss of nutrients. Methylotrophs such as some strains of *Methylobacterium* are known to play an important role in increasing crop yields and land fertility. Their phosphate acquisition and nitrogen fixation abilities make them promising candidates as biofertilizers. They are very effective when applied with a small amount of chemical fertilizer to a field. Development and commercial production of bioinoculants will be accepted by farmers and will help promote sustainable agriculture (Krishnaraj and Dahale 2014) [7]. The recent application of PPFM foliar spray along with a biofertilizer enhanced the microbial population in soil, making nutrients more available to the plants (Jeyajothi *et al.*, 2014) [6].

Methylotrophs are those microorganisms which are able to grow utilizing the reduced carbon compounds, like methanol (released during plant metabolism) enhance the plant growth by providing it with nitrogen. Whereas PPFMs colonize rhizosphsere as well as phyllosphere and enhance the plant growth by providing plant growth hormones like auxins, siderospore production and P-solubilization (Ivanova *et al.*, 2001) [5]. Methylobacteria may produce phytohormones such as cytokinin and auxins 25.

In addition, they can fix atmospheric nitrogen, bring about mineral phosphate solubilization regulate the ethylene level in rhizosphere by 1-aminocyclopropane-1-carboxylate deaminase and stimulate the resistance against plant pathogens (Madhaiyan *et al*., 2006). The present work was designed to study the responses of Methylobacterium on seed germination of kudiraivali under dryland conditions.

Materials and Methods

Field experiment was conducted under rained conditions with Barnyard millet variety Co-KV-2 at Regional Research station, Aruppukottai, Tamil Nadu Agricultural University, Virudhunagar district of Tamil Nadu during Kharif 2018-19. The experiment was designed in RBD with 5 treatments, and three replications. Standard cultural practices was 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

T_{1}- Un inoculated Control
T_{2}- Phosphobacteria +Azospirllum (Seed Treatment 20g/kg of seeds)
T_{3}- Seed imbibitions in water for 15 minutes
T_{4} - Seed imbibitions in 1% PPFM for 15 minutes
T_{5} - Seed imbibitions in 2% PPFM for 15 minutes

Seed imbibition with PPFM

Seeds of Barnyard millet were surface sterilized with 0.02% mercuric chloride for 5 min and rinsed thoroughly with sterile distilled water. Bacterization of the seeds was achieved by soaking 1g of seeds in 10 ml of Methylobacterial suspension, using 0.2% sterilized carboxy methyl cellulose (CMC) as sticker. for 12 h and shade dried before sowing as described by Holland and Polacco (1994) [3]. Seeds treated with sterile distilled water followed by CMC served as control

Observations

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

For germination test paper towel method was followed (ISTA,1993). Vigour index value was computed using the formula suggested by Abdul-Baki and Anderson (1973) [1] and was expressed as whole number.

Vigour index = Germination percent x (Root length + Shoot length).

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 and 121 ^{0}C temperature for 20 minutes using serial dilution spread plating technique (at different intervals of time).

Results and Discussion

Methylotrophs are associated with either the plant rhizosphere or phyllosphere or both and show no pathogenic activity. In seed coating or a seed inoculum, methylotrophs can be used to enhance seed germination (Meena *et al*., 2012) [12]. Methylotrophs applied as a seed inoculants or in a foliar spray alone or in combination with a phosphate-solubilizing bacterium (*Burkholderia pyrrocinia* CBPB-HOD) or nitrogen-fixing bacterium (Azospirillum brasilense CW903) were found to enhance plant growth measured in terms of increased shoot or root length (Madhaiyan *et al*., 2010) [10]. The present study was found that seed imbibitions in 1.0 per cent PPFM for 15 minutes and seed imbibitions in 2.0 per cent PPFM for 15 minutes recorded higher germination of 95% and 97% and Vigour index (2670 and 2832) respectively.

The rhizosphere soil samples were collected from all the treatments for analysis of Bacteria,fungi and actinobacteria population during 30 DAS, 60DAS and during harvest (90DAS). In general the microbial population were increased in all the treatments during 60 DAS and significantly decreased after 90 DAS. The bacterial populations were higher in the treatment containing Methylobacterial inoculants compared to other treatments containing Phosphobacteria and Azospirillum. However the seed imbibitions of Methylobacterium 1% and 2% (T_{4} &T_{5}) showed higher population of bacteria (112.32 x 10^{6} cfu/g), fungi (52.14 x 10^{3} cfu/g) and actinobacteria (26.4 x 10^{4} cfu/g) when compared to other treatments. The treatment T_{1} showed lower population of bacteria (26.25 x 10^{6} cfu/g), fungi (11.3 x 10^{3} cfu/g) and actinobacteria (3.2 x 10^{4} cfu/g) (Table 2). The first step of the plant-bacteria interaction is the recognition of plant exudates by the bacteria. Such exudates are composed mainly of sugars, amino acids, and organic acids as well as flavonoids (LeFevre *et al*., 2013; Li *et al.*, 2013) [8, 9] and they are able to attract specific and beneficial microorganisms, establishing an indwelling bacteria-plant interaction. Root exudates possibly influence host recognition, biofilm formation, and colonization by *Methylobacterium* spp. as endophytes.

The influence of Methylobacterium on plant growth parameters *viz*., plant height, 50% flowering, days to maturity, number of tillers, panicle length, and yield parameters was recorded at 90 DAS. The highest value of 186cm plant height, 6.6 numbers of productive tillers, grain yield was recorded with the treatment T_{4}. The yield parameters like number of productive tillers (6.6 Nos), plant height (186cm), days to 50% flowering (54 days), panicle length (22cm) and days to maturity (33 days), grain yield (1838 kg/ha) and straw yield (3759 kg/ha). The treatment T_{1} (Control) and T_{3} (Seed imbibitions in water for 15 minutes) recorded the lowest panicle length, number of productive tillers, grain yield and straw yield (17.3 &18 cm; 4.9 & 4.3 tillers; 1256kg/ha & 1412 kg/ha; 1582kg/ha & 2956kg/ha) (Table 3). Higher yields of rice and tomatoes have been reported following rhizospheric bioinoculation (*Methylobacterium suomiense* CBMB120) of crops (Poonguzhali *et al.*, 2008) [13]. Another study confirmed that *M. suomiense* colonization resulted in improved yields from red pepper plants (Yim *et al.*, 2012) [15].

Table 1: Effect of seed treatment with Methylobacterium on seed germination of Kudiraivali Co(kv) 2

Treatments	Root length	Shoot length	Germination percentage	Vigor index
T_{1}- Control	3.75	14.2	62	1085
T_{2}- PSB+ Azosp (20g/kg of seeds)	4.50	17.2	78	1696
T_{3}- SI in water for 15 minutes	4.50	16.3	65	1478

T_4 -SI in 1% PPFM for 15 min	7.00	21.1	95	2670
T_5 -SI in 2% PPFM for 15 min	8.50	21.6	97	2832
S.Ed	0.21	0.73		
CD (0.05%)	0.42	1.47		
CV%	7.97	8.63		

Table 2: Effect of seed treatment with *Methyl bacterium* inoculation on microbial population in the rhizosphere of Kudiraivali Co(kv) 2

Treatments	30 DAS			60 DAS			90 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}	Bacteria CFU x 10^6 g^{-1}	Fungi CFU x 10^3 g^{-1}	Actino-bacteria CFU x 10^4 g^{-1}
T_1- Control	32.5	20.50	10.47	48.70	25.97	14.78	26.25	11.35	3.75
T_2- PSB+ Azosp (20g/kg of seeds)	44.2	21.14	12.48	78.35	23.35	20.14	45.12	12.34	10.15
T_3- SI in water for 15 minutes	31.5	28.47	9.47	53.78	26.47	12.47	38.56	12.9	6.78
T_4 -SI in 1% PPFM for 15 min	65.7	34.21	15.36	105.73	47.50	23.0	59.64	28.14	12.78
T_5 -SI in 2% PPFM for 15 min	68.4	36.0	14.78	112.32	52.14	26.41	52.64	23.47	13.62

Table 3: Effect of seed treatment of *Methyl bacterium* on plant biometric and yield parameters of Kudiraivali 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- Control	56	35	174.3	4.9	17.3	1256	1582
T_2- PSB+ Azosp (20g/kg of seeds)	55	36	177.7	5.6	21.0	1596	3265
T_3- SI in water for 15 min	56	35	157.7	4.3	18.0	1412	2956
T_4 -SI in 1% PPFM for 15 min	54	34	186.0	6.6	22.0	1838	3759
T_5 -SI in 2% PPFM for 15 min	54	33	185.3	6.5	22.7	1843	3763
SEd	0.27	0.37	2.70	0.23	0.85	3.67	4.02
CD (0.05%)	0.55	0.74	5.41	0.47	1.70	7.83	9.34
CV%	1.07	2.25	3.25	9.00	8.94	9.23	9.45

References

1. Abdul- Baki AA, Anderson JD. Vigour determination in soybean seed by multiple criteria. Crop Sci. 1973; 13:630-632.
2. Gomez KA, Gomez AA. Statistical procedure for agricultural research. John Wiley and Sons, New York, USA, 1984, 680
3. Holland MA, Polacco JC. PPFMs and other covert contaminants: Is there more to plant physiology than just plant? Annu. Rev. Plant Physiol. Plant Mol. Biol. 1994; 45:197-209.
4. ISTA. Proceeding of the International Seed Testing Association: International Rules for Seed Testing. Seed Sci. Technol. 1993; 21:25-30.
5. Ivanova EG, Doronina NV, Trotsenko YA.Aerobic methylobacteria are capable of synthesizing auxins. Microbiol. 2001; 70:392-397.
6. Jeyajothi R, Subbalakshmi L, Nalliah D. Effect of PPFM application on microbial population in transplanted Tice. Trends Biosci. 2014; 7:3573-3574
7. Krishnaraj PU, Dahale S. Mineral phosphate solubilization: concepts and prospects in sustainable agriculture. Proc Indian Natl Sci Acad. 2014; 80:389–405
8. LeFevre GH, Hozalski RM, Novak PJ. Root exudates enhanced contaminant desorption: an abiotic contribution to the rhizosphere effect, Environ Sci Technol. 2013; 47(20):11545–11553.
9. Li XG, Zhang TL, Wang XX, Hua K, Zhao L, Han ZM. The composition of root exudates from two different resistant peanut cultivars and their effects on the growth of soil borne pathogen, Int. J Biol Sci. 2013; 9(2):164-173.
10. Madhaiyan M, Poonguzhali S, Kang BG, Lee YJ, Chung JB, Sa TM Effect of co-inoculation of methylotrophic *Methylobacterium oryzae* with *Azospirillum brasilense* and *Burkholderia pyrrocinia* on the growth and nutrient uptake of tomato, red pepper and rice. Plant Soil. 2010; 328:71-82
11. Madhaiyan M, Poonguzhali S, Senthilkumar M, Sundaram SP. Nodulation and plant growth promotion by methylotrophic bacteria isolated from tropical legumes. Microbiol. Res. 2009; 164(1):114-120
12. Meena KK, Kumar M, Kalyuzhnaya MG, Yandigeri MS, Singh DP, Saxena AK *et al*. Epiphytic pink-pigmented methylotrophic bacteria enhance germination and seedling growth of wheat (*Triticum aestivum*) by producing phytohormone. Antonie Van Leeuwenhoek. 2012; 101:777–786
13. Poonguzhali S, Madhaiyan M, Yim WJ, Kim KA, Sa TM. Colonization pattern of plant root and leaf surfaces visualized by use of green-fluorescent-marked strain of *Methylobacterium suomiense* and its persistence in rhizosphere. App Microbiol Biotechnol. 2008; 78:1033–1043
14. Raghavendra J, Santhosh G, Mahadevaswamy P, Shubha S, Ravi MV. *In vitro* Screening of Pink Pigmented Facultative Methylotrophs Based on their Functional Characteristics. Int. J Pure App. Biosci. 2019; 7(1):553-562.
15. Yim W, Woo S, Kim K, Sa T. Regulation of ethylene emission in tomato (*Lycopersicon esculentum* Mill.) and red pepper (*Capsicum annuum* L.) inoculated with ACC deaminase producing *Methylobacterium* spp. Korean J Soil Sci Fert. 2012; 45:37–42