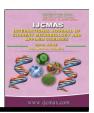


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Studies on Microbial Dynamics in Little Millet (*Panicum sumatrense* L.) based Intercropping System under Rainfed Condition

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ABSTRACT

Keywords

Little millet, intercropping, Bacteria, Fungi, Actinobacteria, Azospirillum, Dehydrogenase enzyme activity

Article Info

Accepted: 07 May 2019 Available Online: 10 June 2019 A field experiment was conducted at Agricultural College and Research Institute, Killikulam during *rabi* season (October 2018 - January 2019) to evolve suitable little millet (*Panicum sumatrense* L) based intercropping system under rainfed condition. The experiment was laid out in randomized block design with eight treatments and replicated thrice. The population of soil bacteria, fungi, actinobacteria, *Azospirillum* and dehydrogenase enzyme activities were recorded under different little millet based intercropping system as a measure of soil microbial dynamics. Among the treatments, soil microbial population was higher in little millet intercropped with both pulses and legumes. Also, the dehydrogenase enzyme activity was more in intercropping compared to mono-cropping and non-legume intercropping system.

Introduction

India is the largest producer of variety of millets, which are often referred as coarse cereals. Realizing the nutrient richness of these grains they are now considered as "Nutri-cereals". In India, little millet holds an area of 2.25 lakh ha with a production of 1.59 lakh tonnes and productivity of 707 kg/ha under small millet (Anonymous, 2016). Little millet is a good source of protein for vegetarians. Little millet is used to cure

cancer, diabetes, asthma, high blood pressure and provides relief from constipation, bloating and cramps. Little millet is used mainly as rice or bread, which serve as the poor man's food. The area under millets are shrinking over years due to replacement of little millet crop with more remunerative crops. Intercropping system has been recognized as beneficial cropping system under rain-fed condition and intercropping with suitable crops provide a better option for farmers. Cultivation of small millets in

general and more specifically little millet can be made more remunerative by addition of certain high value intercrops.

Intercropping increases soil microbial, soil nutrients, soil enzymes and biochemical properties. Ahmad et al., 2013 recorded enhanced soil microbial activity in pepper with green garlic intercropping system. Li et al., 2016 reported increased soil enzymatic activities in Maize + peanut intercropping system. Intercropping of Baby corn + okra system enhanced the actinomycetes population (Rekha et al., 2017). Enhanced soil microbial diversity was observed in Trifolium-cucumber and mustard-cucumber system (Li et al., 2018). Mulberry + alfalfa intercropping enhanced soil bacterial community, due to increasing soil total carbon, available phosphate and available potassium (Zhang et al., 2017). Bt cotton + fodder cowpea intercropping system increased bacterial population and Bt cotton + enhanced fungus moth actinomycetes population (Daisy et al., 2018).

Intercropping system not only the influences soil characteristics but also have positive impact on economics. Dubey *et al.*, 2001 reported little millet intercropped with pigeon pea gives higher benefit cost ratio of 1.83.

Little millet + green gram resulted in a benefit cost ratio of 2.42 (Sharmili and Manoharan, 2018). Higher returns and benefit cost ratio of 3.73 were recorded in foxtail millet + pigeon pea intercropping system (Himasree *et al.*, 2017).

In Tamirabarani command area, rice followed by pulses is the practice commonly followed and intercropping system is not prevalent. Also, suitable intercropping system is not evolved for Tamirabarani command area. Since, cultivation of legumes and pulses, improves soil physical properties, soil nitrogen content, microbial activity and restores organic matter content; vegetables form part of daily diet, intercropping of little millets with legumes, pulses and vegetables were chosen for the study to have nutritional security and to generate additional income to the farmers of Tamirabarani command area.

Materials and Methods

The experimental field is geographically located in the Southern part of Tamil Nadu at 8°46′ N latitude and 77°42′ E longitude with an altitude of 40 m above MSL. The experiment was laid out in randomized block design with three replications with the following treatments

- T₁ Little millet sole crop
- T₂ Little millet + Blackgram (4:1)
- T₃ Little millet + Greengram (4:1)
- T_4 Little millet + Cowpea (4:1)
- T₅ Little millet + Groundnut (4:1)
- T_6 Little millet + Horsegram (4:1)
- T_7 Little millet + Small onion (4:1)
- T_8 Little millet + Coriander (4:1)

Soil sampling

Soil samples of black gram, green gram, cowpea, groundnut, horse gram, small onion and coriander were collected during different

plant growth period as a 20 cm² by 30 cm depth of soil. Plants and soil samples were immediately transferred in polyethylene bags to avoid excessive desiccation during transport and were stored thereafter at 4°C.

Each sample was separated into two fractions: bulk soil (NRS) and rhizosphere soil (RS).

In order to collect bulk soil, plants were vigorously shaken by hand for ten minutes (Figure 1). Rhizosphere soil was afterwards collected by hand shaking roots for 10 minutes in one litre of sterile 0.9% NaCl solution to remove the adhering soil (Figure 2).

Enumeration of total bacteria, fungi and actinobacteria

The soil samples were serially diluted to enumerate the total bacteria, fungi and actinobacteria of both rhizosphere and non-rhizosphere soil. The population of each group was calculated and expressed as cfu per g of dry weight soil.

Serial dilution and plating technique

One gram of soil sample was weighed and added to 100 ml water blank. These samples were shaken for 15 minutes to get complete dispersion (This gives 10^{-2} dilution). One ml of the suspension were transferred to nineml water blank (This gives 10^{-3} dilution). Transferred serially upto 10⁻⁸ dilution or required dilution. One ml of appropriate dilutions was transferred to petri dishes. Three replications for each dilution maintained. Media was melted and cooled of about 15-20 ml was poured and mixed well by rotating clock wise and anti-clock wise for 3 or 4 times and allowed it for complete solidification. The plates were incubated in inverted position at room temperature or in an incubator for 2-7 days. The bacteria, fungi and actinobacteria colonies were observed after 2 days, 3-4 days and 7 days respectively. The colonies per plate were counted and the soil microbial population was calculated. Each colony is referred as colony forming units (cfu).

(Note: Normally 10^{-7} or 10^{-8} dilution can be used for bacteria; 10^{-3} or 10^{-4} dilution for actinobacteria and 10^{-4} or 10^{-5} dilution for fungi can be used if soil is used for enumeration).

Enumeration of *Azospirillum*

The population of *Azospirillum* was enumerated from soil using Most Probable Number (MPN) method. This method relies upon the pattern of positive and negative growth of *Azospirillum* in the tubes inoculated with a consecutive series of dilutions of the soil / root sample. Based on the number of positive tubes, the population estimate is arrived with the help of MPN table.

N-free semi solid malic acid medium was prepared in tubes of five mL quantity and sterilize. Ten gram of representative sample (root/rhizosphere soil) was taken and mixed thoroughly in 90 mL sterile water blank to get 10⁻¹ dilution from which serial dilutions are prepared up to 10⁻⁴ by transferring one mL to nine mL water blank. The dilutions 10^{-2} , 10^{-3} and 10⁻⁴ were selected for enumeration. One ml suspension from each of the dilution was transferred aseptically to each of the five tubes containing the sterilized medium. One set of tubes (5 Nos.) as control without inoculation were maintained and incubated at room temperature 28±2°C for a period of 4 to 6 days.

Estimation of dehydrogenase activity

Twenty gram of air dried soil (<2mm) and 0.2 gram of CaCO₃ was thoroughly mixed and place six gram of this mixture in each of three test tubes. Add one mL of 3% aqueous solution of triphenyl tetrazolium chloride and 25 mL of distilled water to each tube. This amount of liquid should be sufficient that a small amount of free liquid appears at the surface of the soil after mixing. The contents

of each tube with a glass rod were mixed and stopper the tube and incubated it at 37°C. The stopper was removed after 24 hours, add ten mL of methanol and stopper the tube and shake it for 1 minute. Tube was unstopper and filtered the suspension through a glass funnel plugged with absorbent cotton into a 100 mL volumetric flask. Washed the tube with methanol and quantitatively transferred the soil to the funnel, then additional methanol (in 10 mL portions) was added to the funnel until the reddish color has disappeared from the cotton plug. Filtrate was diluted to a 100 mL volume with methanol. The intensity of the reddish color was measured by using a spectrophotometer at a wavelength of 485 nm and a one cm cuvette with methanol as a blank. The amount of TPF produced was calculated by reference to a calibration graph prepared from TPF standards. To prepare standard graph, diluted ten mL of Triphenyl Formazon (TPF) standard solution to 100 mL with methanol (100 mg of TPF mL⁻¹), made up the volume with methanol and mixed thoroughly. The intensity of the red color of TPF as described for the samples was measured. Absorbance readings were plotted against the amount of TPF in the 100 mL standard solutions.

Results and Discussion

Effect of different intercropping system on population of soil bacteria

The different intercropping systems significantly influenced the population of bacteria in both rhizosphere and non rhizosphere soil at 30, 60 DAS and at harvest stage.

At 30 DAS, Little millet + blackgram (4:1) recorded significantly higher bacteria population of 205x 10⁷ cfu g⁻¹ dry soil and 173 x 10⁷ cfu g⁻¹ dry soil in both rhizosphere soil and non-rhizosphere soil, respectively. It

was on par with little millet + groundnut (4:1) and little millet + greengram (4:1).

Little millet + groundnut (4:1) recorded significantly higher bacteria population of 301 x 10⁷ cfu g⁻¹ dry soil and 257 x 10⁷ cfu g⁻¹ dry soil in both rhizosphere soil and non-rhizosphere soil, respectively at 60 DAS. It was on par with little millet + blackgram (4:1) and little millet + greengram (4:1).

At harvest stage, Little millet + groundnut (4:1) recorded significantly higher bacteria population of 228 x 10⁷ cfu g⁻¹ dry soil and 202 x 10⁷ cfu g⁻¹ dry soil in both rhizosphere soil and non-rhizosphere soil, respectively. It was on par with little millet + blackgram (4:1) and little millet + greengram (4:1).

The lowest bacteria population were recorded in sole little millet crop in both rhizosphere soil and non-rhizosphere soil at 30, 60 DAS and at harvest stage (Table 1).

Effect of different intercropping system on population of soil fungi

The different intercropping systems also significantly influenced the population of fungi in both rhizosphere and non rhizosphere soil at 30, 60 DAS and at harvest stage.

At 30 DAS, little millet + blackgram (4:1) recorded significantly higher fungi population of 67x 10⁴ cfu g⁻¹ dry soil and 64 x 10⁴ cfu g⁻¹ dry soil in both rhizosphere soil and non-rhizosphere soil, respectively. It was on par with little millet + groundnut (4:1) and little millet + greengram (4:1).

However, Little millet + groundnut (4:1) had significant fungi population of 75 x 10⁴ cfu g⁻¹ dry soil and 71 x 10⁴ cfu g⁻¹ dry soil in both rhizosphere soil and non-rhizosphere soil, respectively at 60 DAS. It was on par with little millet + blackgram (4:1) and little

millet + greengram (4:1). At harvest stage, Little millet + groundnut (4:1) recorded significantly higher fungi population of 70 x 10^4 cfu g⁻¹ dry soil and 67 x 10^4 cfu g⁻¹ dry soil in both rhizosphere soil and non-rhizosphere soil respectively at 60 DAS. It was on par with little millet + blackgram (4:1) and little millet + greengram (4:1).

The lowest fungi population were recorded in sole little millet crop in both rhizosphere soil and non-rhizosphere soil at 30, 60 DAS and at harvest stage (Table 2).

Effect of different intercropping system on population of soil actinobacteria

The different intercropping systems significantly shown the similar trends in population of actinobacteria both rhizosphere and non rhizosphere soil at 30, 60 DAS and at harvest stage. little millet + blackgram (4:1) recorded significantly higher actinobacteria population of 73 x 10⁵ cfu g⁻¹ dry soil and 69 x 10⁵ cfu g⁻¹ dry soil in both rhizosphere soil and non-rhizosphere soil, respectively.

It was on par with little millet + groundnut (4:1) and little millet + greengram (4:1) at 30 DAS, At 60 DAS, Little millet + groundnut (4:1) shows significantly higher actinobacteria population of 79 x 10⁵ cfu g⁻¹ dry soil and 73 x 10⁵ cfu g⁻¹ dry soil in both rhizosphere soil and non-rhizosphere soil respectively. It was on par with little millet + blackgram (4:1) and little millet + greengram (4:1).

At harvest stage, Little millet + groundnut (4:1) recorded significantly higher actinobacteria population of 76 x 10⁵ cfu g⁻¹ dry soil and 70 x 10⁵ cfu g⁻¹ dry soil in both rhizosphere soil and non-rhizosphere soil respectively at 60 DAS. It was on par with little millet + blackgram (4:1) and little millet

+ greengram (4:1). The lowest fungi population were recorded in little millet sole crop in both rhizosphere soil and non-rhizosphere soil at 30, 60 DAS and at harvest stage (Table 3).

Daisy et al., (2018) reported that the microbial population such as bacteria, fungi and actinobacteria were significantly superior under legume intercropped systems than sole crop of little millet. Similar findings were reported by Li et al., (2016); Zhang et al., (2017); Rekha et al., (2017); Li and Wu (2018); Li et al., (2018). This might be due to a greater root biomass and soil C content of intercropping systems than that of sole crop. Another potential indirect effect in the rhizosphere of intercropped species is enhanced nutrient mineralization due to the change in soil organic matter decomposition rates, resulting from the addition of fresh organic matter (Khan et al., 2014).

Becker and Ladha (1995) and Peoples *et al.*, (1995) reported that the incorporation of legume stubbles under intercropping systems contributed large amount of nitrogen and organic matter to the soil and also favoured for the better proliferation of the soil microorganisms (Table 4).

Effect of different intercropping system on dehydrogenase enzyme activity of soil bacteria

Dehydrogenase enzyme plays a vital role in the biological oxidation of soil organic matter by transferring hydrogen from organic substrates to inorganic acceptors (Zhang *et al.*, 2010). It is a measure of soil microbial activity (Trevors, 1984; Garcia and Hernandez *et al.*, 1997). Among all enzymes in soil environment, the dehydogenase enzyme activity is commonly used as an indicator of biological activity in soils (Burns, 1978).

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Table.1 Effect of different intercropping system on bacteria population in both rhizosphere soil and non-rhizosphere soil at 30, 60 DAS and at harvest stage

	Treatments	Crop	Population cfu \times 10 ⁷ g ⁻¹ of dry weight of soil								
			30 DAS			60 DAS			At Harvest		
			RS	NRS	R:S	RS	NRS	R:S	RS	NRS	R:S
T ₁	Little millet Sole crop	Little millet	68 (1.833)	36 (1.556)	1.89:1	164 (2.215)	120 (2.079)	1.37:1	91 (1.959)	65 (1.813)	1.40:1
T ₂	Little millet + Black gram (4:1)	Black gram	205 (2.312)	173 (2.238)	1.18:1	280 (2.447)	236 (2.373)	1.19:1	207 (2.316)	181 (2.258)	1.14:1
T ₃	Little millet + Green gram (4:1)	Green gram	173 (2.238)	141 (2.149)	1.23:1	269 (2.430)	225 (2.352)	1.20:1	196 (2.292)	170 (2.230)	1.15:1
T ₄	Little millet + Cowpea (4:1)	Cowpea	142 (2.152)	110 (2.041)	1.29:1	238 (2.377)	184 (2.265)	1.29:1	165 (2.217)	139 (2.143)	1.19:1
T ₅	Little millet + Groundnut (4:1)	Groundnut	184 (2.265)	152 (2.182)	1.21:1	301 (2.479)	257 (2.410)	1.17:1	228 (2.352)	202 (2.305)	1.13:1
T ₆	Little millet + Horse gram (4:1)	Horsegram	121 (2.236)	89 (1.949)	1.36:1	217 (2.336)	138 (2.410)	1.57:1	144 (2.158)	118 (2.072)	1.22:1
T ₇	Little millet + Small onion (4:1)	Small onion	89 (1.949)	57 (1.756)	1.56:1	185 (2.267)	132 (2.121)	1.40:1	112 (2.049)	82 (1.914)	1.37:1
T ₈	Little millet + Coriander (4:1)	Coriander	92 (1.964)	60 (1.778)	1.53:1	188 (2.274)	126 (2.100)	1.49:1	115 (2.061)	85 (1.929)	1.35:1
	SEd			0.084		0.065	0.063		0.060	0.057	
	CD (p=0.05)			0.184		0.140	0.135		0.130	0.124	

Figure in parenthesis are logarithmic transformed values.

RS-Rhizosphere Soil; NRS- Non-Rhizosphere Soil; R:S- Ratio of Rhizosphere Soil and Non-Rhizosphere Soil

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Table.2 Effect of different intercropping system on fungi population in both rhizosphere soil and non-rhizosphere soil at 30, 60 DAS and at harvest stage

	Treatments	Crop	Population cfu \times 10 ⁴ g ⁻¹ of dry weight of soil								
			30 DAS			60 DAS			At Harvest		
			RS	NRS	R:S	RS	NRS	R:S	RS	NRS	R:S
T ₁	Little millet Sole crop	Little millet	13 (1.114)	10 (1.000)	1.30:1	21 (1.322)	15 (1.176)	1.40:1	16 (1.204)	13 (1.114)	1.23:1
T ₂	Little millet + Black gram (4:1)	Black gram	67 (1.826)	64 (1.806)	1.05:1	72 (1.857)	70 (1.845)	1.03:1	67 (1.826)	62 (1.792)	1.08:1
T ₃	Little millet + Green gram (4:1)	Green gram	55 (1.740)	53 (1.724)	1.04:1	61 (1.785)	69 (1.839)	0.88:1	62 (1.792)	61 (1.785)	1.02:1
T ₄	Little millet + Cowpea (4:1)	Cowpea	43 (1.633)	40 (1.602)	1.08:1	51 (1.708)	45 (1.653)	1.13:1	46 (1.663)	43 (1.633)	1.07:1
T ₅	Little millet + Groundnut (4:1)	Groundnut	60 (1.778)	57 (1.756)	1.05:1	75 (1.875)	71 (1.851)	1.06:1	70 (1.845)	67 (1.826)	1.04:1
T ₆	Little millet + Horse gram (4:1)	Horsegram	40 (1.602)	37 (1.568)	1.08:1	48 (1.681)	42 (1.623)	1.14:1	43 (1.633)	40 (1.602)	1.08:1
T ₇	Little millet + Small onion (4:1)	Small onion	14 (1.146)	11 (1.041)	1.27:1	22 (1.342)	16 (1.204)	1.38:1	17 (1.230)	14 (1.146)	1.21:1
T ₈	Little millet + Coriander (4:1)	Coriander	12 (1.176)	12 (1.079)	1.25:1	26 (1.415)	17 (1.203)	1.53:1	19 (1.247)	15 (1.176)	1.27:1
	SEd			0.038		0.044	0.042		0.042	0.041	
CD (p=0.05)			0.088	0.083		0.096	0.091		0.092	0.088	

Figure in parenthesis are logarithmic transformed values.

RS-Rhizosphere Soil; NRS- Non-Rhizosphere Soil; R:S- Ratio of Rhizosphere Soil and Non-Rhizosphere Soil

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Table.3 Effect of different intercropping system on actinobacteria population in both rhizosphere soil and non-rhizosphere soil at 30,60 DAS and at harvest stage

	Treatments	Crop	Population cfu \times 10 ⁵ g ⁻¹ of dry weight of soil								
			30 DAS			60 DAS			At Harvest		
			RS	NRS	R:S	RS	NRS	R:S	RS	NRS	R:S
T ₁	Little millet Sole crop	Little millet	19 (1.279)	15 (1.176)	1.27:1	25 (1.398)	19 (1.279)	1.32:1	22 (1.342)	16 (1.204)	1.38:1
T ₂	Little millet + Black gram (4:1)	Black gram	73 (1.863)	69 (1.839)	1.06:1	76 (1.881)	71 (1.851)	1.07:1	73 (1.863)	68 (1.833)	1.07:1
T ₃	Little millet + Green gram (4:1)	Green gram	59 (1.771)	56 (1.748)	1.05:1	58 (1.763)	59 (1.771)	0.98:1	55 (1.470)	56 (1.748)	0.98:1
T ₄	Little millet + Cowpea (4:1)	Cowpea	49 (1.690)	45 (1.653)	1.09:1	55 (1.740)	49 (1.690)	1.12:1	52 (1.716)	46 (1.663)	1.13:1
T ₅	Little millet + Groundnut (4:1)	Groundnut	66 (1.820)	62 (1.792)	1.06:1	79 (1.898)	73 (1.863)	1.08:1	76 (1.881)	70 (1.845)	1.09:1
T ₆	Little millet + Horse gram (4:1)	Horsegram	46 (1.663)	42 (1.623)	1.10:1	52 (1.716)	46 (1.663)	1.13:1	49 (1.690)	43 (1.663)	1.14:1
T ₇	Little millet + Small onion (4:1)	Small onion	20 (1.301)	16 (1.204)	1.25:1	26 (1.415)	20 (1.301)	1.30:1	23 (1.362)	17 (1.230)	1.35:1
T ₈	Little millet + Coriander (4:1)	Coriander	24 (1.380)	17 (1.203)	1.41:1	30 (1.477)	21 (1.322)	1.43:1	27 (1.431)	18 (1.255)	1.50:1
	SEd			0.043		0.046	0.044		0.045	0.042	
CD (p=0.05)			0.094	0.092		0.099	0.094		0.094	0.091	

Figure in parenthesis are logarithmic transformed values.

RS-Rhizosphere Soil; NRS- Non-Rhizosphere Soil; R:S- Ratio of Rhizosphere Soil and Non-Rhizosphere Soil

Table.4 Effect of different intercropping system on postharvest soil fertility status

	Treatments	Available N	Available P	Available K	
T	Little millet Sole crop	216.8	17.8	250.2	
T ₂	Little millet + Black gram (4:1)	237.7	21.7	274.5	
T ₃	Little millet + Green gram (4:1)	236.5	20.7	272.3	
T ₄	Little millet + Cowpea (4:1)	235.5	20.5	271.8	
T ₅	Little millet + Groundnut (4:1)	234.2	20.2	264.2	
T ₆	Little millet + Horse gram (4:1)	234.9	20.1	268.7	
T ₇	Little millet + Small onion (4:1)	216.3	17.3	246.3	
T ₈	Little millet + Coriander (4:1)	215.1	16.2	241.6	
	SEd	9.43	0.79	10.8	
	CD (p=0.05)	20.38	1.71	23.2	

Fig.1&2 Bulk soil - Hand shaking vigorously for 10 min and Rhizosphere soil hand shaking 1L NaCl 0.9% for 10 min





Fig.3

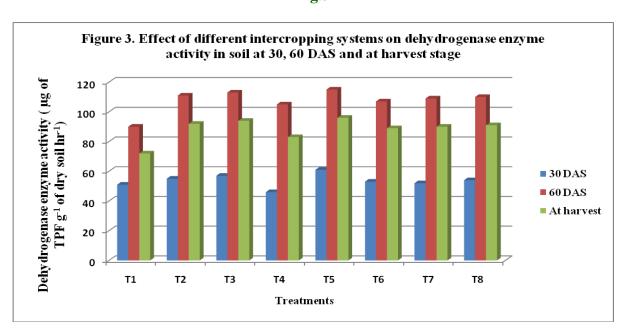
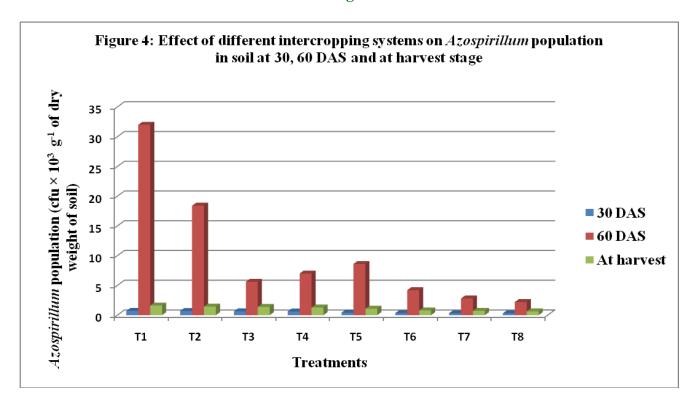


Fig.4



The various intercropping systems significantly influenced the dehydrogenase enzyme activity in soil at 30, 60 DAS and harvest stage.

In general, little millet + groundnut (4:1) recorded higher dehydrogenase enzyme activity of 61, 115 and 96 µg of TPF g⁻¹ of dry soil hr⁻¹ at 30, 60 DAS and harvest stage, respectively. The lowest dehydrogenase enzyme activity of 51, 90 and 72 µg of TPF g⁻¹ of dry soil hr⁻¹at 30, 60 DAS and harvest stage, respectively were observed in sole little millet (Figure 3).

Jiang *et al.*, (2010) who found that intercropping system could improve the soil enzyme activity. Similar findings were also reported by (Xingang *et al.*, 2011). Improvement in soil quality by the green manure legumes was reported by Shah *et al.*, (2011). Cai *et al.*, (2010) who reported that

enzyme activities were enhanced significantly after intercropping. These findings are also supported by Yang and Fz (2011) and Han *et al.*, (2013).

Effect of different intercropping system on population of soil *Azospirillum*

On the controversy of above results, Azospirillum population showed a reverse trends. Little millet sole crop recorded higher *Azospirillum* population of 0.72, 32 and 1.62 MPN (x 10³ cells g⁻¹ of dry soil) at 30, 60 DAS and at harvest stages, respectively and it was followed by the association of little millet + blackgram (4:1). The lowest values on *Azospirillum* population (0.31, 2.2 and 0.64 MPN) (x 10³ cells g⁻¹ of dry soil) at 30, 60 DAS and harvest stages, respectively were evidenced in little millet + coriander (4:1) (Figure 4). The *Azospirillum* population were higher in little millet sole crop than the

intercropping system. These findings were reported by Mohammed Rafi and Charyulu (2010).

In conclusion, the microbial population of soil bacteria, fungi, actinobacteria and dehydrogenase enzyme activity were higher in the little millet based legume intercropping system as it correlated with higher postharvest soil fertility status like available nitrogen, phosphorus and potassium compared to sole crop of little millet. Hence, compared to sole crop of little millet, intercropping of little millet + groundnut (4:1), little millet + black gram (4:1) and little millet + green gram (4:1) were selected as best intercropping system for Tamirabarani command area.

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