

Original Research Article

Microbial diversity of bacterial species (*Rhizobium* and *Azospirillum*) under different land used systems

ABSTRACT

This investigation associated with microbial diversity of different agroforestry system. The study interpreted the changes of bacterial population during duration of experiments. This study was analyzed by CRBD (Complete Randomized Block Design) with 5 treatments (*i.e.* agroforestry systems) and 4 replications. The research was conducted on cropping systems at JNKVU, Jabalpur, and Forest Research Farm during the Rabi season of 2021-22 and 2022-23. Soil collection was carried out at a depth of Rhizosphere soil (0 to 15 cm) in different agroforestry systems, letter on the soil was tested through using of serial dilution methods. The result revealed that the *Rhizobium* species population was found in maximum T₁-*D. sissoo*-wheat (66.28 and 66.96 X 10⁷ cfu g⁻¹) in respective year of 2021-22 and 2022-23 followed the sequence of population of *Rhizobium* spp. had T₃ (*M. pinnata*-Wheat) > T₂ (*G. arborea*-Mustard) > T₅ (*M. indica*-linseed) > T₄ (*A. nilotica* -Wheat) estimated under agroforestry systems. The *Azospirillum* species population increases year to year under agroforestry system. T₁ (*D. sissoo*- wheat) T₃ (*M. pinnata*-wheat) > T₂ (*G. arborea*-mustard) > T₅ (*M. indica*-linseed) > T₄ (*A. nilotica* -wheat) obtained under agroforestry systems.

Keyword: *Azospirillum* spp, *Rhizobium* spp, Agroforestry system, Microbial *etc.*

INTRODUCTION

Soil microbial communities are the diverse communities of microorganisms that inhabit soil, including bacteria, fungi, algae, etc. These microbes play a vital role in soil health and function by influencing nutrient cycling, greenhouse gas emissions, and other processes. Soil microbes are essential for plant growth, nutrient mineralization, and the stability of agricultural systems. Various systems are included in the category of agroforestry in umbrella. Agroforestry systems include roadside planting systems, shelterbelts, pastures, and reforestation. In temperate zones, cropping systems have become increasingly popular because they can maintain or increase productivity while being more environmentally friendly than conventional agricultural systems (Foley *et al.*, 2005). The trees and crop elements of these systems are often planted in alternating rows with a north-south orientation to minimize shading. The spatial proximity of trees and crops allows for a variety of interspecific interactions in these systems, such as competition for resources and complementary use of resources (Jose *et al.*, 2004). Plant health and productivity and nutrient cycling are strongly influenced by soil organisms, particularly microorganisms (Berendsen *et al.*, 2012). In recent decades, many soil organisms have been studied in temperate agroforestry systems, ranging from soil macro-fauna (Cardinael *et al.*, 2019) against microorganisms (Guillot *et al.*, 2021). Also the impact of agroforestry systems on soil fauna and their functions has been extensively studied recently (Marsden *et al.*, 2020), Agroforestry structures boom soil microbial populace sizes, and that this useful impact can expand steadily into the crop rows. Additionally, fungi can also additionally advantage greater than micro-organism, as numerous research indicated a boom with inside the fungi micro-organism ratio. Agroforestry structures boom soil microbial variety

especially thru the status quo of a tree–row affiliation microbiome this is compositionally wonderful from the microbiome of the crop rows (*i.e.*, improved beta variety inside agroforestry structures) in preference to improved alpha variety. Agroforestry structures that have to be taken into consideration while analyzing soil micro-organisms. Soil microorganisms in temperate agroforestry structures throughout time.

2. MATERIAL AND METHODS

The experimental trail carried out in agroforestry field of Forestry Research Farm, Department of Forestry, College of agriculture, JNKVV, Jabalpur. These studies conducted during *Rabi* season of 2021-22 and 2022-23. The systems soil collected in surface soil (0 to 15 cm depth) during investigation year different land used systems. Tree age was found under different agroforestry system *i.e.* *D.sissoo* (24-25 year), *G.arborea* (6-7 year), *M. piñata* (14-15 year), *A.nilotica* (24-25 year) and *M. indica* (4-5 year). The soil sample was packed in tightly in polythene after that the estimation of bacterial population through serial dilution methods

2.1 Serial dilution method for *Rhizobium*

Soil samples were collected periodically for microbial study and processed for serial dilution by suspending 1g of soil sample in 9 ml sterilized water in test tube and shaken thoroughly which resulted 10^{-1} dilution. Subsequent serial dilutions were made up to 10^{-9} dilution for plating purpose.

2.1.1 Plating (pour plate method): Plating in sterilized Petri plates was done by taking 1 ml each of 10^{-6} to 10^{-9} dilutions as required for rhizobia counts in rhizospheric soil treatment wise. Plating was performed in triplicate for each dilution. The plating's were done as per the composition of growth media for the respective microorganism *viz.* YEMA (*Rhizobium*). The serial dilutions obtained from soil samples collected at initial and harvest of the chickpea crop were used for plating adopting pour plate method because more surface area covered as the sample is spread throughout the media than other plate method. Soil dilution (10^{-6} to 10^{-9}) aliquot of 1 ml was taken in a Petri plate, to this 15 ml of the melted medium was poured within the aseptic environment of laminar air flow chamber. After pouring the growth medium, plates were rotated gently clockwise and anti- clockwise to mix the soil dilution with the medium. After solidification of medium the plates were incubated upside down at 27 ± 2 °C for 3-6 days. The colonies were counted with specific growth characteristics (Table 1)

Table 1 Composition media used for population counts of *Rhizobium* and *Azospirillum* species.

REAGENTS	YEMA (<i>Rhizobium</i>)	<i>Azospirillum</i> Spp.
CaCO ₃	1 g	-
MgSO ₄ .7 H ₂ O	0.2 g	-
MgSO ₄	-	0.2 g
MnSO ₄	-	-
MnSO ₄ .7 H ₂ O	-	0.01 g
CaCl ₂	-	0.02 g
KOH (for pH)	-	4.5 g
NH ₄ Cl	-	1.0 g
FeSO ₄	-	0.01 g
FeSO ₄ 7H ₂ O		
NaCl	0.1 g	0.1 g
KCl	-	-
C ₆ H ₁₂ O ₆	-	-
Yeast Ext.	1 g	0.02 g
Agar	20 g	20 g
K ₂ HPO ₄	0.5 g	0.5 g
Malic Acid	-	5.0 g
Mannitol	10 g	-
Bromothymol blue 5% solution	-	2.0 ml

pH	-	6.6-7.0
Distilled Water	1000 ml	1000 ml

2.2 Serial dilution method for *Azospirillum* Spp.

A sequential method was used to estimate the soil microbial population. Soil samples were collected regularly according to the planned microbial research program. Serial dilutions were made by suspending 10 g of soil sample in 90 ml of sterile water in a bottle. The suspension was effectively disrupted, which was a ratio of 10^{-1} . The next series was increased to 10^{-1} to determine the target. Plating (Pour plate methods): The aliquots of 1 ml each 10^{-2} to 10^{-8} dilutions were poured in sterilized petri plates to determine the rhizobial population count in soil, each dilution was plated in triplicate. The composition of growth medium for respective microorganisms tabulated in Table 1. The pour plate method was used to plate serial dilutions obtained from soil samples collected after harvest of wheat, mustard and linseed. In petri plate, a 1 ml aliquot of soil dilution (10^{-2} to 10^{-8}) was taken and 15 ml of sterilized melting medium was poured to evenly mix the soil dilution with the medium. Once the medium had solidified three plates were incubated upside down at 28 ± 2 °C for 3-7 days

$\text{Viable Cells (Cfu g}^{-1}\text{ Soil)} = \frac{\text{Number of colonies}}{\text{Oven dry weight of soil (1g)}} \times \text{X Dilution factor}$
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3. RESULT AND DISCUSSION

3.1. Population of *Rhizobium* spp

The data about population of *Rhizobium* spp. in the surface soil (0 to 15 cm depth) during investigation year 2021-22 and 2022-23 under different land used systems are presented in Table 2 and Fig 1.

The population of *Rhizobium* spp in the surface soil (0 to 15 cm depth) 2021-22 in ranged from 33.36 to 66.28 cfu g⁻¹ with an average of 49.25. cfu g⁻¹. T₁- *D. sissoo* – wheat exhibited significantly maximum response with 66.28 which was 50.33 % more over that of T₄ – *A. nilotica*- wheat (33.36 cfu g⁻¹). This was followed by the response of T₃- *M. Pinnata*- wheat with 65.23 cfu g⁻¹.

The population of *Rhizobium* spp in the surface soil (0 to 15 cm depth) at 2022-23 ranged from 34.08 to 66.96 with an average of 50.31 cfu g⁻¹. T₁- *D. sissoo* – wheat exhibited significantly maximum response with 66.96 cfu g⁻¹. Which was 50.89 % more over that of T₄ – *A. nilotica*- wheat (34.08 cfu g⁻¹). This was followed by the response of with T₃- *M. Pinnata*-wheat (34.08 cfu g⁻¹).

On the other hand, pooled data shows similar pattern followed. Where the maximum found in T₁ (66.62 cfu g⁻¹) were significantly superior to T₂ (42.07 cfu g⁻¹), T₃ (33.72 cfu g⁻¹) and T₅ (40.78 cfu g⁻¹) and at par with T₅ (65.72 cfu g⁻¹). Data on difference between years to year found non significantly under different agroforestry systems. The data was similar to the noted by Marco *et al.*, 2023 *i.e.* the diversity of root-nodulating rhizobia, morphology and formation of legume and augmented to Janati *et al.* 2021 moreover like to *Rhizobium* and *Bradyrhizobium* are associated with leguminous plants.

Table 2 *Rhizobium* spp (X 10⁷ cfu g⁻¹ soil) in soil under different land used systems

Treatments	2021-22	2022-23	Pooled
T ₁ - <i>D.Sissoo</i> -Wheat	66.28	66.96	66.62
T ₂ - <i>G. arborea</i> -Mustard	41.12	43.02	42.07
T ₃ - <i>M. Pinnata</i> -Wheat	65.23	66.22	65.72
T ₄ - <i>A. nilotica</i> -Wheat	33.36	34.08	33.72
T ₅ - <i>M. Indica</i> -Linseed	40.27	41.28	40.78
Mean	49.25	50.31	49.78
Sem±	2.88	4.12	2.25

CD	10.70	15.34	8.83
SEm±(Year)	1.59	SEm±(YXT)	3.56
CD(Year)	6.24	CD(YXT)	13.96

3.2 Population of *Azospirillum spp* (cfu g⁻¹ soil)

The data about population of *Azospirillum spp.* in the surface soil (0 to 15 cm depth) during investigation year 2021-22 and 2022-23 under different land used systems are presented in Table 3 and Fig 2

The population of *Azospirillum spp* in the surface soil (0 to 15 cm depth) 2021-22 in ranged from 5.32 log cfu (2.08 x10⁵ cfu g⁻¹) to 9.38 log cfu (2.29 x10⁹ cfu g⁻¹) with an average of 7.40 log cfu (2.53 x10⁷ cfu g⁻¹). T₁- *D. sissoo* – wheat exhibited maximum response with 9.38 log cfu (2.39 x10⁹ cfu g⁻¹) which was 56.72 % more over that of T₄ – *A. nilotica*- wheat 5.32 log cfu (2.08 x10⁵ cfu g⁻¹). This was followed by the response of T₃ - *M. Pinnata*- wheat with 8.16 log cfu (1.45 x10⁸ cfu g⁻¹).

The *Azospirillum spp* population in the surface soil (0 to 15 cm depth) 2022-23 in ranged from 5.64 log cfu (4.34 x10⁵ cfu g⁻¹) to 9.47 log cfu (2.97 x10⁹ cfu g⁻¹) with an average of 7.88 log cfu (7.51 x10⁷ cfu g⁻¹). T₁- *D. sissoo* – wheat exhibited maximum response with 9.47 log cfu (2.97 x10⁹ cfu g⁻¹) which was 56.71 % higher than that of T₄ – *A. nilotica*- wheat 5.64 log cfu (4.34 x10⁵ cfu g⁻¹). This was followed by the response of T₃ - *M. Pinnata*- wheat with 8.48 log cfu (3.02 x10⁸ cfu g⁻¹).

The *Azospirillum spp* population in the surface soil (0 to 15 cm depth) pooled in varied from 5.48 log cfu (2.81 x10⁷ cfu g⁻¹) to 9.42 log cfu (9.42 x10⁹ cfu g⁻¹) with an average of 7.64 log cfu (4.36 x10⁷ cfu g⁻¹). 2021-22 exhibited maximum response with 9.42 log cfu (2.64 x10⁹ cfu g⁻¹) which was 58.17 % higher than that of T₄ – *A. nilotica*- wheat 5.48 log cfu (3.00 x10⁵ cfu g⁻¹). This was followed by the response of T₃ - *M. Pinnata*- wheat with 8.32 log cfu (2.10 x10⁷ cfu g⁻¹). The free-living bacteria include Cyanobacteria (blue-green algae), *Azotobacter*, *Azolla*, *Azospirillum*, *Agrobacterium*, *Clostridium*, *Gluconobacter*, *Flavobacterium*, and *Herbaspirillum*. They are habitually associated with non-legumes Mott *et al.*, 2007. *Azospirillum* species and *Frankia* are associated with cereal grasses and certain dicotyledonous species Marco *et al.*, 2023.

Table 3 *Azospirillum spp* (cfu g⁻¹ soil) under different land used systems

Treatments	2021-22	2022-23	Pooled
T ₁ - <i>D.Sissoo</i> -Wheat	9.38 (2.39X10 ⁹)	9.47 (2.97X10 ⁹)	9.42 (2.64X10 ⁹)
T ₂ - <i>G. arborea</i> -Mustard	7.22 (1.65X10 ⁷)	7.84 (6.92X10 ⁷)	7.53 (3.38X10 ⁷)
T ₃ - <i>M. Pinnata</i> -Wheat	8.16 (1.45X10 ⁸)	8.48 (3.02X10 ⁸)	8.32 (2.10X10 ⁷)
T ₄ - <i>A. nilotica</i> -Wheat	5.32 (2.08X10 ⁵)	5.64 (4.34X10 ⁵)	5.48 (3.00X10 ⁵)
T ₅ - <i>M. Indica</i> -Linseed	6.94 (8.76X10 ⁶)	7.96 (9.02X10 ⁷)	5.48 (2.81X10 ⁷)
Mean	7.40 (2.53X10 ⁷)	7.88 (7.51X10 ⁷)	7.64 (4.36X10 ⁷)
Sem±	0.45	0.52	0.31
CD	1.66	1.95	1.21
SEm±(Year)	0.22	SEm±(YXT)	0.49
CD(Year)	0.85	CD(YXT)	1.91

4. CONCLUSION

Thus, agroforestry system increase the bacterial population (*Azospirillum* and *Rhizobium spp.*) with duration but it not significantly variation found the time of investigation between years. The result was estimated that the soil bacterial poultaiion in aspect to

Rhizobium spp was found in *T₁-D.sissoo-wheat* (Tree crop combination) based system followed by *M. Pinnata*-Wheat based system and the list population estimated in rhizosphere soil *A. nilotica*-Wheat. Furthermore in case of *Azospirillum spp* population similar trend estimated in the 2021-22 and 2022-23 as well as statically pooled analysis data.

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ABBREVIATIONS

Dalbergia sissoo = *D. sissoo*, *Millettia pinnata* = *M. pinnata*, *Gmelina arborea* = *G. arborea*, *Acacia nilotica* = *A. nilotica*, CEC = Cation exchange capacity, spp = species

Fig 1. *Rhizobium spp* ($\times 10^7$ cfu g^{-1} soil) in rhizosphere soil under different land used systems

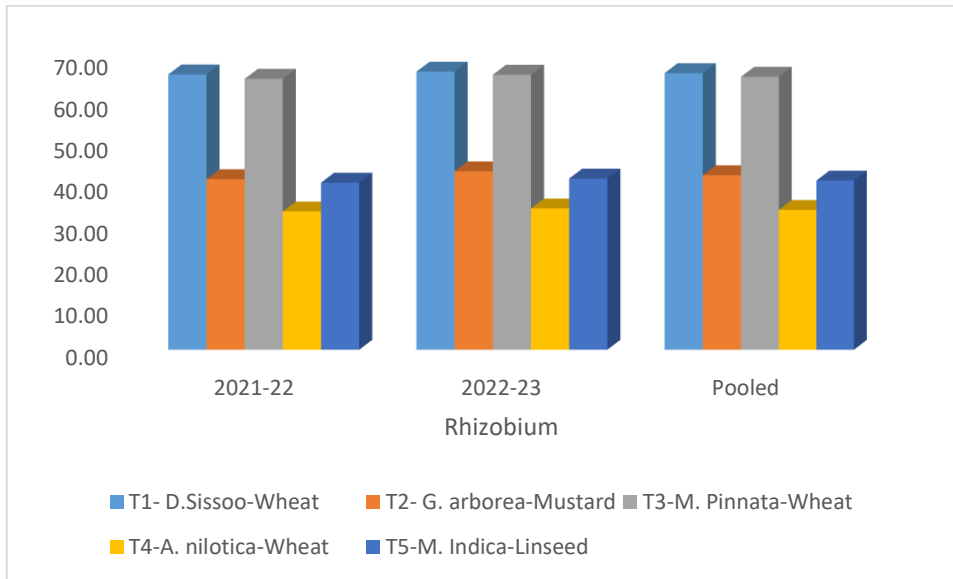
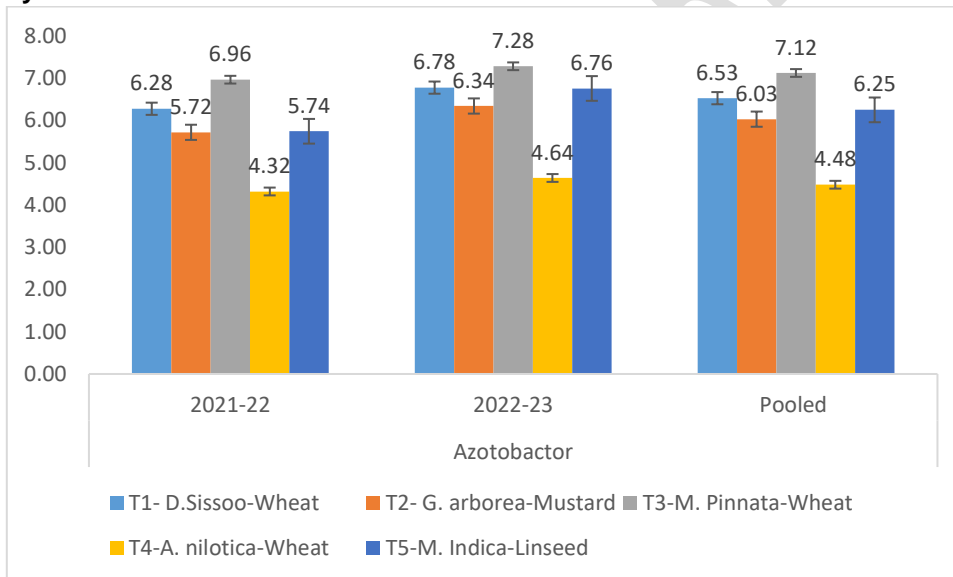


Fig 2. *Azospirillum* spp (cfu g⁻¹ soil) in rhizosphere soil under different land used systems



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