**Assessment of changes in soil biology under based agroforestry system**


## ABSTRACT

A field study was conducted at the Research Farm, Department of Forestry, College of Agriculture, JNKVV, Jabalpur (MP) for two consecutive years (2022–23 and 2023-24). The experiment consisted of three crop establishment methods of mustard ($M_1$: Broadcasting, $M_2$: Line sowing, and $M_3$: Transplanting) and four levels of boron ($B_0$: Control, $B_1$: 1 kg $ha^{-1}$ as basal $B_2$: 2 kg $ha^{-1}$ and $B_3$: ½ kg $ha^{-1}$ as basal + ½ kg $ha^{-1}$ as foliar spray) and these treatments were compared with the same treatments in open. The experiment was assessed in the double-split plot design with three replications. The objective of the study was to assess the changes in soil biology (total bacteria, Azotobacter, rhizobia, total fungiand actinomycetes) due to crop establishment methods and levels of boron under agroforestry ($S_1$) and open ($S_2$) systems. On an average, significantly higher counts of total bacteria (42.78 CFU x $10^6$), Azotobacter (31.37 CFU x $10^5$), rhizobium (21.99 CFU x $10^6$), total fungi (8.3 CFU x $10^5$) and actinomycetes (12.99 CFU x $10^5$) were higher under agroforestry system than the open system. Meanwhile higher colony counts of total bacteria, Azotobacter, rhizobium, total fungiand actinomycetes were found to be significantly superior in transplanting (20.28 CFU x $10^6$, 29.59 CFU x $10^5$, 37.52 CFU x $10^6$, 7.97 CFU x $10^5$ and 12.69 CFU x $10^5$, respectively) over both line an **Shisham** d broadcasting crop establishment methods. In case of boron levels, no significant changes occurred in the soil biology under both agroforestry and open systems.



**Key Words:** Agroforestry, Crop establishment methods, Boron levels, Mustard, Soil biology and Shisham.


## INTRODUCTION

Soil serves as the foundation for agriculture, and the presence of microorganisms is crucial for enhancing soil health and promoting robust crop growth. Microorganisms play a vital role in establishing an intricate interrelationship between plants and the soil. Soil microorganisms are an active and essential part of the soil, carrying out numerous functions within the soil system. Usually, a gram of soil containing over 90 million bacteria provide assistance to plants in absorbing nutrients by converting inaccessible elements into available form (Rao, 2007). The microbial biosphere is the most extensive reservoir of biodiversity on the planet (Vibha and Neelam, 2012). Indeed, microorganisms have the ability to recycle nutrients present in the soil (Javed *et al*., 2021). Soil microorganisms play a crucial role in enhancing the quality of soil and its maintenance within the soil system. Soil microorganisms

have a crucial role in the decomposition of organic matter, such as animal and plant remains. They also contribute to the formation of soil structure and govern the rate of biogeochemical cycling in the soil (Tate III, 2020).

The soil harbours a multitude of microorganisms that play a crucial role in augmenting soil fertility and stimulating plant growth (Basu *et al*., 2021). The physical and chemical characteristics of soil are influenced by the amount and composition of soil organic matter, pH levels, and the circumstances of redox potential. These factors have a substantial impact on the composition and behavior of the microscopic population, as well as the functioning of the soil (Sadeghi *et al*., 2023). Microbes are commonly perceived as pathogenic organisms. Nevertheless, the breakdown of organic matter will be facilitated by these microbes present in the soil (Raza *et al*. 2023). Moreover, it has been shown that numerous bacterial species have been employed for converting organic contaminants into minerals in soil, which is commonly known as bioremediation of soil pollutants (Sonune, 2021). The influence of soil biota in the soil profile is complex and challenging due to the fact that the same activity can have either detrimental or beneficial effects depending on its location (Trabelsi and Mhamdi, 2013). In contrast, plants exhibit a diverse array of interactions with soil-dwelling microbes, encompassing a wide range of biological possibilities such as competition, exploitation, neutrality, commensalism, and mutualism. However, based on the present circumstances, research has been conducted on the mitigation of harmful consequences, such as infection and herbivory (Grunseich *et al*., 2019).

Soil microorganisms establish a connection between soil and roots, facilitate nutrient recycling, decompose organic materials, and respond quickly to changes in soil ecology. They serve as reliable indicators for specific functions in the soil environment (Bardgett & Caruso, 2020). Global agricultural practices today incorporate a variety of non-symbiotic bacteria (such as Azotobacter, Azospirillum, Bacillus, and *Klebsiella sp*.) as well as symbiotic bacteria (specifically Rhizobium sp.) to enhance plant productivity (Jalal *et al*., 2022). Meanwhile, microorganisms play a crucial role in mitigating the issues associated with the use of chemical fertilizers and pesticides. They are widely employed in natural agricultural land and organic farming to address these concerns (Bokade *et al*., 2023). Thus, this research elucidates the function of soil bacteria in agricultural crop development.

**Comment [CQJ1]:** It is necessary to relate agroforestry with the functioning of the soil in a more specific way so that it leads you towards the objective

**Comment [CQJ2]:** This objective is very general and endless. It must be specific based on the work carried out.

## MATERIAL AND METHODS

### Details of the Experimental site

A field experiment took place under *Dalbergia sissoo* agroforestry model and open system at Research Farm, Department of Forestry, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh. The experimental site is positioned at an elevation of 391 meters above sea level. It is situated at a latitude of 23° 12' 50" north and a longitude of 79° 57' 56" east in the Kymore Plateau and Satpura Hills, agroclimatic zones of Madhya Pradesh. The climate is characterized by scorching, arid summers with an average maximum temperature of 46°C and frigid, arid winters with an average minimum temperature of 4°C. Jabalpur experiences an average annual rainfall of 1350 mm. The majority of rain received during mid-June to the end of September. The remaining months, particularly from December to February, receive just 75 mm of rainfall due to the influence of westerly winds. The region is renowned for its elevated relative humidity levels, ranging from 80 to 90%, 60 to 75% and 20 to 23% during the rainy, summer and winter, respectively.

**Details of the Experiment**

The experiment arranged in a split-split plot design, with systems ($S_1$: agroforestry system and $S_2$: open system) as the main factors. Three sub factors were crop establishment methods ($M_1$: broadcasting, $M_2$: line sowing, and $M_3$: transplanting) and four sub-sub factors consisted of boron levels ($B_0$: 0 kg ha$^{-1}$, $B_1$: 1 kg ha$^{-1}$ as basal, $B_2$: 2 kg ha$^{-1}$ as basal and $B_3$: ½ kg ha$^{-1}$ as basal + ½ kg ha$^{-1}$ as foliar just before flowering) during the *Rabi* seasons in the years 2022–23 and 2023–24. Hence, a total number of 24 different treatment combinations ($S_1M_1B_0$, $S_1M_1B_1$, $S_1M_1B_2$, $S_1M_1B_3$, $S_1M_2B_0$, $S_1M_2B_1$, $S_1M_2B_2$, $S_1M_2B_3$, $S_1M_3B_0$, $S_1M_3B_1$, $S_1M_3B_2$, $S_1M_3B_3$, $S_2M_1B_0$, $S_2M_1B_1$, $S_2M_1B_2$, $S_2M_1B_3$, $S_2M_2B_0$, $S_2M_2B_1$, $S_2M_2B_2$, $S_2M_2B_3$, $S_2M_3B_0$, $S_2M_3B_1$, $S_2M_3B_2$, $S_2M_3B_3$) were replicated thrice. The objective of implementing the agrisilviculture system with *Dalbergia sissoo* (tree component) and *Bassica juncea* (crop component) was to optimize productivity across different boron concentrations and crop establishment methods. Mustard was planted using three different spacing methods for crop establishment: 45 x 15 cm for transplanting, 30 x 10 cm for line sowing, and unevenly scattered for broadcasted in the plots. Crop was sown in plots measuring 3.6 x 15 m in the alley, with *Dalbergia sissoo* trees with the RDF (80:40:40 N:P:K kg ha$^{-1}$, respectively). The trees were arranged in a square pattern with a spacing of five meters in both directions.

**Comment [CQJ3]:** For readers outside the region this term may not be common, in parentheses the time period and a synonym if possible

**Comment [CQJ4]:** Expand and place this idea in the introduction

**Soil Sampling and Analysis**

**Soil Sampling**

Prior to planting the crop in a test field, a comprehensive soil samples were collected in order to assess the initial soil condition. The purpose of obtaining a representative sample was to gather initial data on soil biology before implementing experimental interventions. In order to get spatial diversity, five distinct sampling locations were chosen at random within each treatment plot and using an auger device from the root zone 0-15 cm deep. Once the soil samples were gathered and subjected to determination of microbial population.

**Preparation of culture media**

The required quantity of media was measured and mixed with the prescribed volume of distilled water in a conical flask. The components were completely dissolved in the distilled water through boiling. The autoclave was utilized to sterilize the media for a duration of 15 minutes at a temperature of 121.6°C at a pressure of 15 pounds.

The media used for the investigation and their composition are as follows:

**Comment [CQJ5]:** If the culture media are commonly used, it is not necessary to detail them in a table. You can write this section briefly and use the original citations from the authors.

**Table 1: Culture medias for rhizospheric bacterial count.**

| **Total Bacteria (Thornton's medium)** | | **Azotobacter (BuRK's medium)** | | **Rhizobium (Yeast extract Mannitol Agar medium)** | |
|---|---|---|---|---|---|
| **Ingredients** | **Quantity** | **Ingredients** | **Quantity** | **Ingredients** | **Quantity** |
| $K_2HPO_4$ | 1 g | Sucrose | 20 g | Mannitol | 10 g |
| $MgSO_4$ | 0.2 g | $K_2HPO_4$ | 0.64 g | $K_2HPO_4$ | 0.5 g |
| $CaCl_2$ | 0.1 g | $KH_2PO_4$ | 0.16g | $MgSO_4.7H_2O$ | 0.2 g |
| NaCl | 0.1 g | $MgSO_4.7H_2O$ | 0.2 g | NaCl | 0.1 g |
| $FeCl_3$ | 0.001 g (Trace) | NaCl | 0.2 g | $CaCO_3$ | 1 g |
| $KNO_3$ | 0.5 g | $Na_2MoO_4$ | 0.001 g (trace) | Yeast extract | 1 g |
| Asparagine | 0.5 g | $FeSo_4$ | 0.003 g (trace) | Congored (1:400) | 2 mL |
| Mannitol | 1 g | $CaCO_3$ | 1 g | Distilled water | 1000 mL |
| Yeast extract | Very small amount | Agar-Agar | 15-18 g | Agar-Agar | 15-18 g |
| Agar-Agar | 15-18 g | Distilled water | 1000 mL | | |
| Distilled water | 1000 mL | | | | |

**Table 2: Culture medias for rhizospheric fungal and actinomycetes count**

| Total Fungi (Martin's Rose Bengal Agar medium) | | Actinomycetes (Actinomycete Isolation Agar medium) | |
|---|---|---|---|
| **Ingredients** | **Quantity** | **Ingredients** | **Quantity** |
| Glucose | 10 g | Sodium propionate | 4.0 g |
| Peptone | 5 g | Sodium caseinate | 2.0 g |
| $KH_2PO_4$ | 4 g | L-Asparagine | 0.1 g |
| $MgSO_4$ | 0.5 g | Dipotassium phosphate | 0.5 g |
| Rose Bengal | 0.033 g | Magnesium sulphate | 0.1 g |
| Agar-Agar | 15-18 g | Ferrous sulphate | 0.001 g |
| Distilled water | 1000 mL | Agar-agar | 15.0 g |
| | | Distilled water | 1000 mL |

A concentrated cell culture diluted to a more manageable concentration through serial dilution.

To prepare solidified agar plates for the purpose of selectively separating different microorganisms, 100 µl of the appropriate dilution were applied onto the plates. The BOD incubator was maintained at a temperature of 37±2°C in order to facilitate the incubation of culture plates. Observations were recorded after a 48-hour period to enumerate the colonies. The number of colony-forming units per gram of soil was counted by tallying each type of microorganisms.

**Comment [CQJ6]:** You should comment on how the data and statistical analyses were performed. Clarify the significance criteria and whether p-value corrections were used.

## RESULTS AND DISCUSSION

### Effect of Systems

The data presented in Table 3 and 4 revealed that microbial counts significantly changed due to change of system. The highest counts of Azotobacter (31.37 CFU x $10^5$), rhizobium (21.99 CFU x $10^6$), total bacteria (42.78 CFU x $10^6$), total fungi (8.30 CFU x $10^5$) and actinomycetes (12.98 CFU x $10^5$) were recorded under Agroforestry system. The higher counts of microbes under Agroforestry system might be due to the presence of abundant quantity of organic matter and moisture favouring the proliferation of microbes. These findings are in accordance with Beule *et al.* (2019); Beule *et al.* (2022); Lori *et al*. (2022) and López□Ramírez, *et al.* (2023).

**Effect of Crop Establishment Methods**

According to data in relation to soil microbial population (Table 3 and 4), the population of soil microbes was significantly influenced due to crop establishment methods. Broadcasting method accommodated to significantly higher counts of Azotobacter, rhizobia, total bacteria, total fungi and actinomycetes (29.59 CFU x $10^5$, 20.28 CFU x $10^6$, 37.52 CFU x $10^6$, 7.97 CFU x $10^5$ and 12.68 CFU x $10^5$, respectively) than in the line sowing and transplanting. This might be due to the spatial configuration of the crop in different crop establishment methods had different densities of plants which regulate the evaporation of moisture as well as interception of sunlight and ultimately the population of microbes. Similar conclusions were reached by Romdhane *et al.* (2019); Kim *et al.* (2020) and dos Santos Cordeiro *et al.* (2021).

**Effect of the Boron Levels**

Data presented in Table 3 and 4 pertaining to soil microbial population, revealed that the levels of boron did not show any significant impact on the rhizobia, Azotobacter, other bacterial population, total fungiand actinomycetes.

**Table 3: Changes in population of Azotobacter and Rhizobium and Total Bacterial count in rhizospheric soil under different treatments.**

| Microbes | Azotobacter (CFU x $10^5$ $g^{-1}$ soil) | | | Rhizobium (CFU x $10^6$ $g^{-1}$ soil) | | | Total Bacteria (CFU x $10^6$ $g^{-1}$ soil) | | |
|---|---|---|---|---|---|---|---|---|---|
| | $Y_1$ | $Y_2$ | Pooled | $Y_1$ | $Y_2$ | Pooled | $Y_1$ | $Y_2$ | Pooled |
| **Systems** | | | | | | | | | |
| $S_1$ | 30.93 | 31.80 | 31.37 | 21.17 | 22.80 | 21.99 | 43.70 | 41.85 | 42.78 |
| $S_2$ | 24.10 | 25.21 | 24.66 | 14.43 | 16.02 | 15.23 | 24.84 | 23.12 | 23.98 |
| SEm± | 0.50 | 0.40 | 0.45 | 0.31 | 0.32 | 0.28 | 0.59 | 0.51 | 0.55 |
| C. D. (*P*=0.05) | 3.03 | 2.45 | 2.7 | 1.91 | 1.96 | 1.70 | 3.58 | 3.13 | 3.35 |
| **Crop Establishment Methods** | | | | | | | | | |
| $M_1$ | 29.19 | 29.98 | 29.59 | 19.48 | 21.08 | 20.28 | 38.45 | 36.59 | 37.52 |
| $M_2$ | 27.33 | 28.56 | 27.94 | 17.66 | 19.25 | 18.45 | 34.26 | 32.58 | 33.42 |
| $M_3$ | 26.03 | 26.97 | 26.50 | 16.27 | 17.91 | 17.09 | 30.09 | 28.29 | 29.19 |
| SEm± | 0.40 | 0.48 | 0.38 | 0.28 | 0.31 | 0.26 | 0.33 | 0.40 | 0.36 |
| C. D. (*P*=0.05) | 1.31 | 1.58 | 1.25 | 0.91 | 1.01 | 0.83 | 1.09 | 1.32 | 1.18 |
| **Boron Levels (kg $ha^{-1}$)** | | | | | | | | | |
| $B_0$ | 26.96 | 27.91 | 27.43 | 17.30 | 19.29 | 18.30 | 34.13 | 32.05 | 33.09 |
| $B_1$ | 27.31 | 28.19 | 27.75 | 17.58 | 19.35 | 18.47 | 34.24 | 32.37 | 33.3 |
| $B_2$ | 27.70 | 28.80 | 28.25 | 17.96 | 19.46 | 18.71 | 34.31 | 32.61 | 33.46 |
| $B_3$ | 28.10 | 29.11 | 28.61 | 18.37 | 19.54 | 18.96 | 34.39 | 32.93 | 33.66 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SEm± | 0.41 | 0.39 | 0.31 | 0.28 | 0.37 | 0.24 | 0.41 | 0.33 | 0.27 |
| C. D. (P=0.05) | NS | NS | NS | NS | NS | NS | NS | NS | NS |

**$Y_1$ – 2022-23**

**$Y_2$ – 2023-24**

Comment [CQJ7]: Add it to the table description, this and any other details you think are necessary.

**Table 4: Changes in population ofTotal fungi and Actinomycetes count in rhizospheric soil under different treatments.**

| Microbes | Total Fungi (CFU x $10^5$ $g^{-1}$ soil) | | | Actinomycetes (CFU x $10^5$ $g^{-1}$ soil) | | |
|---|---|---|---|---|---|---|
| **Year** | **$Y_1$** | **$Y_2$** | **Pooled** | **$Y_1$** | **$Y_2$** | **Pooled** |
| **Systems** | | | | | | |
| $S_1$ | 6.90 | 9.70 | 8.30 | 11.99 | 13.97 | 12.98 |
| $S_2$ | 4.16 | 7.07 | 5.62 | 9.10 | 11.08 | 10.09 |
| SEm± | 0.11 | 0.16 | 0.13 | 0.18 | 0.20 | 0.19 |
| C. D. (*P*=0.05) | 0.65 | 0.98 | 0.8 | 1.08 | 1.23 | 1.15 |
| **Crop Establishment Methods** | | | | | | |
| $M_1$ | 6.61 | 9.34 | 7.97 | 11.69 | 13.66 | 12.68 |
| $M_2$ | 5.41 | 8.30 | 6.85 | 10.40 | 12.38 | 11.39 |
| $M_3$ | 4.59 | 7.50 | 6.04 | 9.55 | 11.55 | 10.55 |
| SEm± | 0.06 | 0.09 | 0.07 | 0.15 | 0.26 | 0.17 |
| C. D. (*P*=0.05) | 0.21 | 0.31 | 0.22 | 0.48 | 0.86 | 0.57 |
| **Boron Levels (kg $ha^{-1}$)** | | | | | | |
| $B_0$ | 5.40 | 8.26 | 6.83 | 10.41 | 12.37 | 11.39 |
| $B_1$ | 5.50 | 8.33 | 6.91 | 10.49 | 12.49 | 11.49 |
| $B_2$ | 5.56 | 8.43 | 6.99 | 10.59 | 12.57 | 11.58 |
| $B_3$ | 5.67 | 8.52 | 7.09 | 10.69 | 12.68 | 11.69 |
| SEm± | 0.10 | 0.11 | 0.08 | 0.24 | 0.19 | 0.17 |
| C. D. (*P*=0.05) | NS | NS | NS | NS | NS | NS |

**$Y_1$ – 2022-23**

**$Y_2$ – 2023-24**

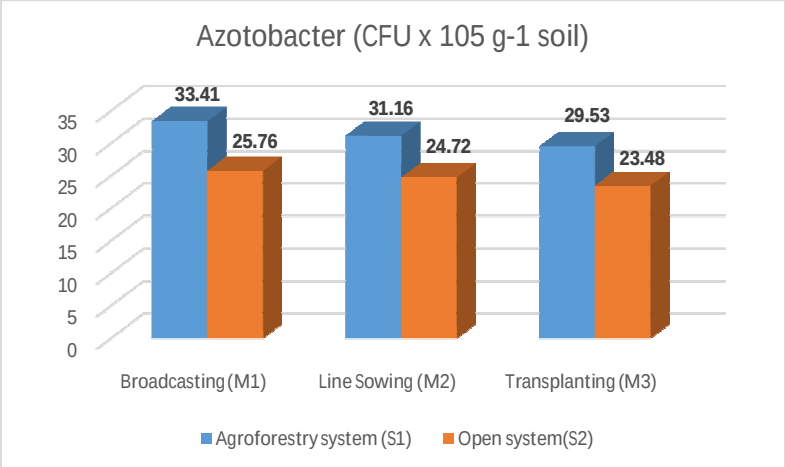


**Fig 1: Azotobacter count as affected by crop establishment methods under different systems.**

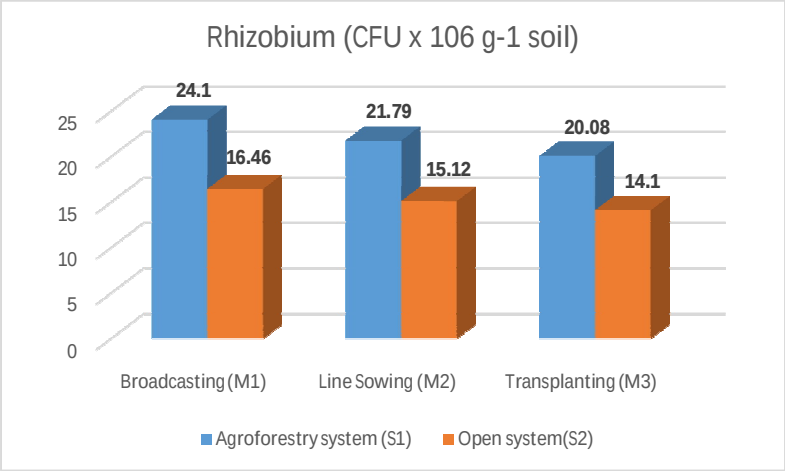


**Fig 2: Rhizobium count as affected by crop establishment methods under different systems.**

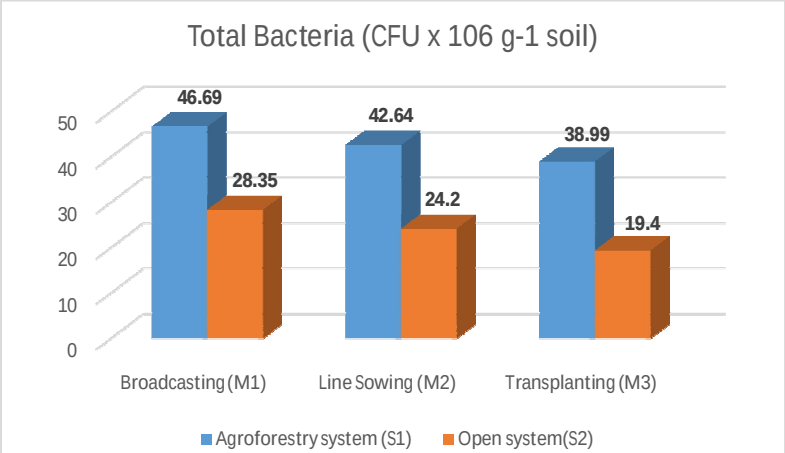


**Fig 3: Total Bacteriacount as affected by crop establishment methods under different systems.**

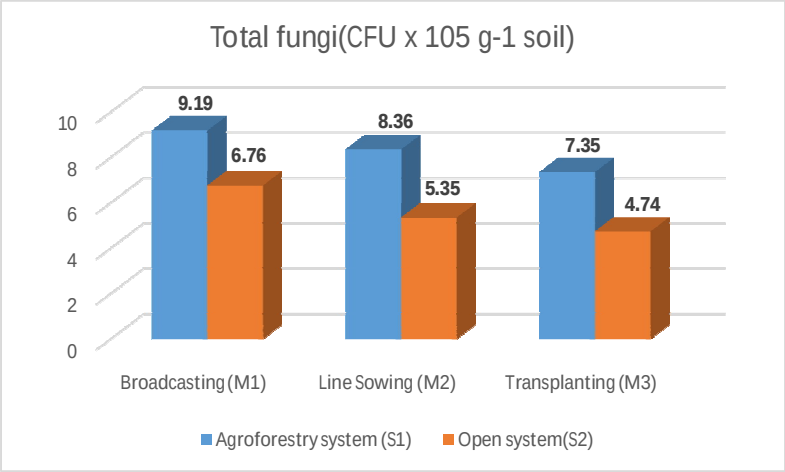


**Fig 4: Total fungicount as affected by crop establishment methods under different systems.**

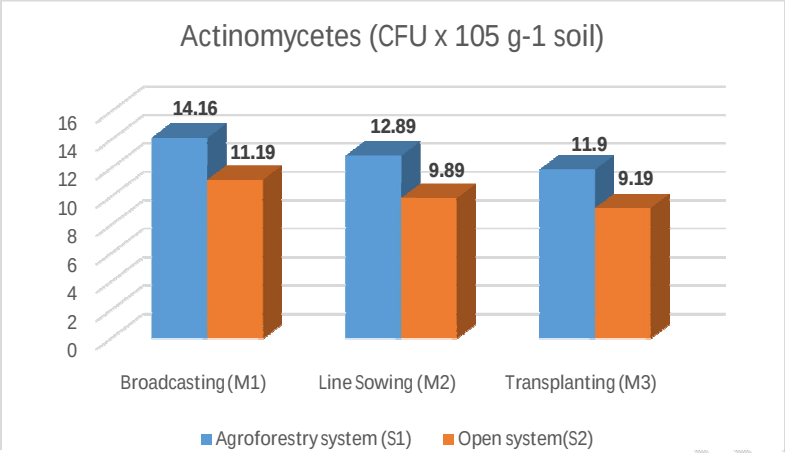


**Fig 5: Actinomycetes count as affected by crop establishment methods under different systems.**

Comment [CQJ8]: Choose a summary criterion or tables or figures. Figures are shown but not referred to in results and discussion

## CONCLUSION

Based on two year study it could be concluded that the Agroforestry system proved to be more congenial for the proliferation of rhizospheric microbes as compared to the open. Further, the microbial population counts varied with crop establishment methods beneath the *Dalbergia sissoo*. The marked higher microbial population was found under the broadcasting method over the other methods of crop establishment.

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