



# Influence of Soil Amendment on Growth, Yield, Chemical and Biological Properties in Tea Soil of North East India

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## Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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## ABSTRACT

Field experiment was conducted at Jorhat, Assam to evaluate the efficacy of microbial based soil amendment (manufactured by Cisbay) in combination with reduced doses of inorganic NPK fertilizers in tea soils and its effect on yield and soil chemical as well as biological properties. The experiment was conducted in a randomised block design with 3 replications and 6 treatments. Among various treatments, soils treated with 75% RDF + AGN soil amendment @ 14.4 L/ha/year recorded the highest (183.83) number of plucking points/m<sup>2</sup>/round of plucking over other treatments. The same treatment also produced 4.03 per cent more yield compared to 100% RDF (Control) though the treatment effect was found to be not significant. The highest percentage (47.95%) of fine shoots was also reported under this treatment. Regarding soil chemical properties, pH was

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significantly affected by the application of the soil amendment though there was no significant difference among control, 75% RDF + AGN soil amendment @ 14.4 L/ha/year and 100% RDF + AGN soil amendment @ 14.4 L/ha/year in top soil. In sub soil, pH of the soil was significantly increased in all treatments when compared to control. However, soil organic carbon in top soil was not affected by the treatments. Application of AGN soil amendment along with RDF had no negative impact on mold population in soil. However, population of bacteria was reduced at the end of the cropping period.

**Keywords:** *Camellia sinensis* (L) O. Kuntze; organic amendment; yield; soil pH; soil microbial community.

## 1. INTRODUCTION

Tea (*Camellia sinensis* (L) O. Kuntze) is the major cash crop of North East India particularly in Assam with high export potential. In Assam, it occupies an area of 3.48 lakh ha with a production of 688.70 Mkg (Anon., 2022). Being a leaf crop, it consumes a huge amount of nutrients throughout its growing period. Generally, in tea, inorganic fertilizers are mostly applied as a source of nutrients. But continuous application of synthetic chemical fertilizers resulted in degradation of soil health (Ebrahimi et al., 2021). Therefore, integrated nutrient management would be a viable strategy for sustaining soil health and yield as well as quality of crop (Aeron et al., 2011).

Plant growth-promoting (PGP) microbes including bacteria, fungi, actinomycetes, yeast and algae have profound effect on crop production through their biofertilization mechanisms with low impact on soil and environment. Plant growth promoting rhizobacteria (PGPR) directly stimulates growth when they colonised on root surfaces by solubilising nutrients, fixing of biological nitrogen, producing of phytohormones in appreciable quantities and inducing systemic resistance (Vejan et al. 2016) and indirectly by suppressing hazardous effects of biotic stresses (Backer et al., 2018) as well as antagonising pathogenic fungi by producing certain antimicrobial compounds (Lyu et al., 2020, Shahrajabian et al., 2023 and Sopheareth et al., 2013). Zahir as well as Arshad (2004) and Cakmakçi et al. (2006) reported that PGPRs act as biofertilizer, biostimulant, and bioprotectant. Moreover, there have been considerable efforts to find PGPR to minimise the application of synthetic fertilizers load in fertile soil conditions.

Bacillus, Pseudomonas, Azotobacter, Azospirillum etc. are the commonly used PGPRs. Out of these, the genus Bacillus is widely studied

as PGPR due to their ability to root colonisation and sporulation (Jamal et al., 2018). These bacteria are used as microbial pesticides, fungicides or fertilizers. Bohme and Bohme (2005), Baymukhambetova et al. (2017) and Castro et al. (2008) reported that Bacillus-based products are the most important class of microbial products for phytosanitary use available in the market. Applications of some of these PGPRs in tea for promotion of growth and suppression of disease have reported by Chakraborty et al. (2006, 2009, 2010 and 2013) and Choudhary as well as Johri (2009). Inoculation of *Bacillus* sp. into soil as PGPR can solubilise fixed soil phosphorus and thus enhance increase inorganic phosphorus availability to plants (Kumar and Narula, 1999) resulting in higher crop yields (Yadav and Dadarwal, 1997; Puente et al., 2004). Plant-associated *Bacillus amyloliquefaciens* has been shown to produce a variety of secondary metabolites (Chen et al., 2009) and enzymes such as chitinase involved in microbial antagonism (Niazi et al., 2014), thus supporting the plant growth indirectly by disease suppression. The positive effect of *Bacillus megaterium* on the growth and development of cotton was reported by (Bataeva et al., 2022). Spraying of *Bacillus megaterium* and along with yeast on availability of phosphorous, better growth and yield of onion was reported by Hussein and Hassan (2021). The significant drought tolerance mechanism and increased osmolyte accumulation of *Bacillus megaterium* was stated by Devarajan et al. (2021). These PGPRs effectively colonize soils and plant tissues and produce a wide range of biologically active compounds that are involved in promoting plant growth (Gray and Smith, 2005; Zhao et al., 2021; Yagmur and Gunes, 2021) and antiphytopathogenic activity (Nascimento et al., 2020).

AGN is a concentrated soil amendment manufactured by Cisbay contains 30% plant

growth promoting microbes (*Bacillus amyloliquefaciens*:  $5.85 \times 10^8$  cfu/ml and *Bacillus megaterium*:  $3.20 \times 10^8$  cfu/ml), 5% nitrogen, 35% Humic acid with 30% non ionic penetrants. The Humic acid present in the product acts as carrier and provides amino acids and proteins to increase the soil microbial load in soil which ultimately boost the healthy plant growth. The penetrant which facilitates movement of water uniformly into the soil system both vertically and horizontally thus reduces the evaporation loss of water. Increased nutrients use efficiency and improvement of crop yield as well as quality, after application of Humic acid was also reported by Bera et al. (2024).

Considering the importance of PGPRs and Humic acid on tea growth, yield and sustaining soil health, the present study was undertaken.

## 2. MATERIALS AND METHODS

Field experiment was conducted by Department of Agronomy, Tocklai Tea Research Institute, Jorhat, Assam, India at Tocklai Tea Estate to study the effect of integration of microbial soil amendment with Recommended dose of fertilizer (RDF) on yield, soil microbial properties and reduction of fertiliser dose in tea. The section was planted in 1998 with TV-23 clone in single hedge planting pattern at a spacing of 120 cm x 70 cm. The topography of the area was flat with good drainage system. The soil profile of the experimental area was well developed and no impervious layer in sub soil. The soil of the experimental site was sandy loam in texture and acidic in reaction (pH: 4.54) as well as medium in organic carbon (0.89%). Status of shade tree in the experimental area was moderate. The experiment was laid out in a Randomised Block Design with three replications and six numbers of treatment. Thus, 18 plots were prepared and each plot consisted of 60 tea bushes. Two rows of tea as guard rows surrounding each plot were maintained.

The treatment details are given below:

- T<sub>1</sub>: 100% RDF + AGN soil amendment @ 14.4 L/ha/year
- T<sub>2</sub>: 75% RDF + AGN soil amendment @ 14.4 L/ha/year
- T<sub>3</sub>: 50% RDF + AGN soil amendment @ 14.4 L/ha/year
- T<sub>4</sub>: 100% N & K + AGN soil amendment @14.4 L/ha/year
- T<sub>5</sub>: 100% RDF + AGN soil amendment @ 24.0 L/ha/year

T<sub>6</sub>: 100% RDF only (Control)

N.B. Treatments were applied in the month of June, July, August, September and October in T<sub>1</sub> – T<sub>4</sub>. (@ 4.8 L/ha in June and @ 2.4 L/ha each in July, August, September and October).

In T<sub>5</sub>, treatment was applied in June, August and September (10.0 L/ha in June + 10.0 L/ha in August + 4.0 L/ha/year during end of September).

After mixing of the product with water thoroughly, the spray solution was applied to the ground close to the collar zone of the tea bushes at definite time interval as per treatment. Knapsack sprayer (ASPEE make) was used for spraying with a spray volume of 400 L/ha. Recommended dose of NPK was applied @ 120-30-120 kg NPK/ha on ground as per treatment. The doses of fertiliser were broadcasted in two equal splits in the month of June and September. Urea, Single Super Phosphate and Muriate of Potash were the sources for N, P and K respectively. The crop was grown as rainfed. The total rainfall received during the period of study was 1252 mm. The monthly average maximum and minimum temperature ranged from 25.76 – 34.24 °C and 10.88 – 25.38 °C, respectively throughout the experimental period (June-December). Standard agronomic practices as well as plant protection measures were done during the study period. Among the insect-pests, infestation of Tea mosquito bug (*Helopeltis theivora* Water house) and Red spider mite (*Oligonychus coffeae* Nietner) was observed. Infestation was same for all the treatments. Two rounds of insecticides were sprayed in the month of August and October against Tea mosquito bug. Spot application of one round of acaricide was applied to control against Red spider mite in the month of September.

### 2.1 Observations

For counting of density of plucking points (No./m<sup>2</sup>/round of plucking), one representative sample bush selected randomly in the centre of individual plot was tagged and counted the number of fresh plucking points just after plucking by using a bamboo grid of 1.0 m x 1.0 m (100 equal size holes) at monthly interval and the average values were calculated out to express as yearly averages.

For estimation of fine shoot percentage in every month, hundred (100) g shoots were taken from

the plucker's basket plucked from each plot and separated out the un-bruised fresh small two leaves and a bud, single leaf and a bud and also single soft *banji*. Separation of fine portion from larger shoots was also done, where necessary, by breaking back the two leaves and a bud and soft *banji* from the plucked shoots which was added to fine shoots. Weighing of fine shoots was done separately for each treatment and replication and their masses were expressed as percentage of fine shoots.

Softness of harvested shoots was recorded on the basis of pluckers' observation at the time of plucking. Visual observation on effect of treatments in leaf colour was recorded at monthly interval during the period of experimentation.

Weight of green shoots plucked in each net plot in each round from June – December maintaining a plucking round of 7 days was recorded. At the end of the year, cumulative yield of fresh shoot per plot was converted to kilogram made tea ha<sup>-1</sup> multiplying with a standard factor of 0.225.

\*Recovery percentage of tea is 22.5% (Anon., 2016).

For estimation of pH, organic carbon, moisture and microbial status of soil, a representative soil sample from all the experimental plots was taken by soil auger from 0 – 15 cm and 15 – 30 cm at the end of the flushing period. The pH of the soil samples was estimated by pH meter method (1:2.5 :: soil : water) as suggested by Jackson (1973), organic carbon was estimated by Wet digestion method (Walkey & Black, 1934). For determination of moisture status of soil, the collected soil samples were brought to the laboratory to record their fresh weight. Then the samples were dried in oven at 105°C for 24 hours and the moisture content of each sample was determined gravimetrically. The formula used for this purpose was as follows:

$$\text{Soil moisture content (\%)} = \frac{W_1 - W_2}{W_2 - W_3} \times 100$$

Where,

W1 = Weight of wet soil + box (g)

W2 = Weight of dry soil + box (g)

W3 = Weight of empty box (g)

The microbial population count (Colony Forming Unit, CFU) was ascertained by the method suggested by Johnson and Curl (1972). The

different media used for detecting and enumerating the microbial population in soil are noted below:

1. Nutrient Agar (NA) media: for Bacteria
2. Rose Bengal Chloramphenicol Agar (RBCA) media: for Mold / Fungi
3. Yeast Malt Agar: for Yeast
4. Kneknigh Agar media: for Actinomycetes

Data pertaining to the different parameters wherever needed was statistically analysed as per Randomized Block Design (RBD) described by Panse and Sukhatme (1985). Critical differences (CD) at 5% probability level were calculated only when the F value been found out to be significant.

### 3. RESULTS AND DISCUSSION

#### 3.1 Effect on Density of Plucking Points (No. m<sup>-2</sup> Round of Plucking<sup>-1</sup>)

Density of plucking points per unit area is one of the yield determinants in tea. It is apparent from the data presented in Table 1 indicated that the values under the treatments did not differ significantly from each other due to application of AGN soil amendment. However, the highest value (183.83) was observed in T<sub>2</sub> (75% RDF + AGN soil amendment @ 14.4 L/ha) followed by T<sub>1</sub> (100% RDF + AGN soil amendment @ 14.4 L/ha) with a value of 180.17 No./m<sup>2</sup>. Application of AGN soil amendment resulted 2.88 – 6.06 per cent more plucking points m<sup>-2</sup> over control. It might be due to the plant growth promoting activity of the microbes which enhanced the availability and uptake of nitrogen in soil along with other nutrients. Increase in plucking point density due to nitrogen was also reported by (Ranganathan, 2015).

#### 3.2 Effect on Fineness of Shoots (% g 100 g<sup>-1</sup> Shoot)

The quality of harvested shoots is assessed in terms of fineness of shoots. It is evident from the Table 1 that the fine shoot percentage also increased after application of AGN soil amendment. The maximum value (47.95%) was recorded under T<sub>2</sub> though the treatment effects were not found to be statistically significant. An increase of 3.22 per cent fine shoot was observed after application of AGN soil amendment @ 14.4 L/ha along with 75% RDF over control. Increase in fine shoot after

application of AGN soil amendment may be due to availability of nutrients in soil and ample supply of nutrients particularly nitrogen which lowered the *banji* percentage in the sample. Reduction of *banjhiness* by nitrogen was also reported by Ranganathan (2015).

### 3.3 Effect on Yield (kg Made Tea ha<sup>-1</sup>)

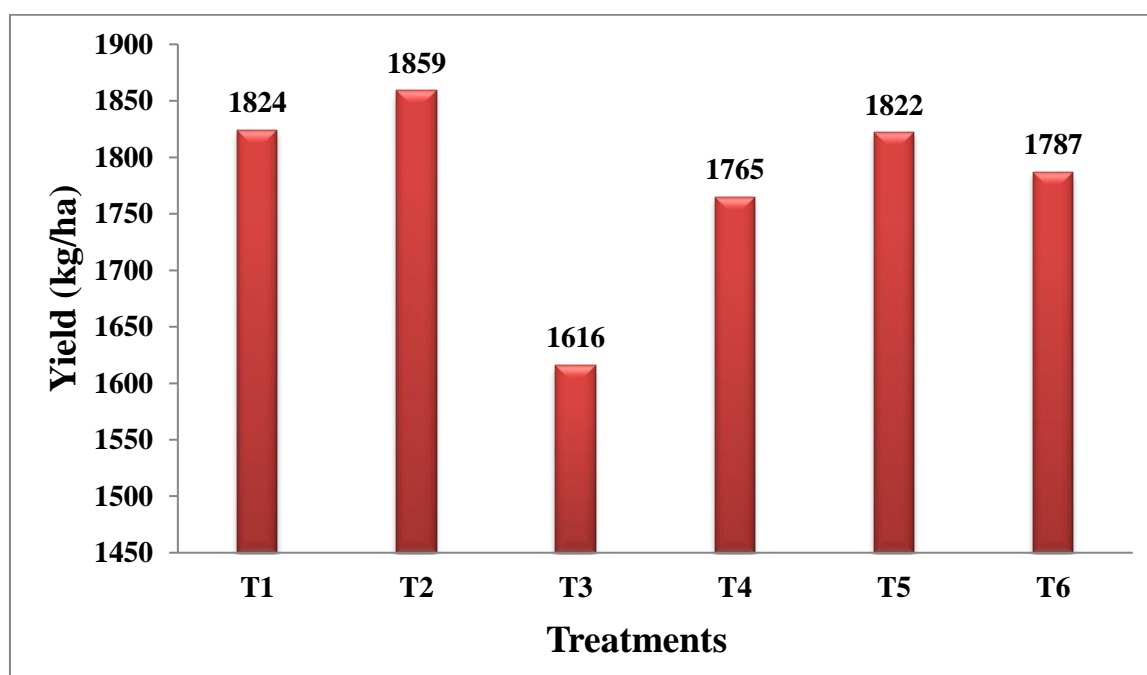
Yield of made tea (kg ha<sup>-1</sup>) after application of AGN soil amendment in various treatments are illustrated in Fig. 1. From the figure, it was indicated that AGN soil amendment @ 14.4 L/ha when applied with 75 per cent recommended dose of fertiliser (T<sub>2</sub>) recorded the maximum made tea yield (1859 kg ha<sup>-1</sup>) which was followed

by T<sub>1</sub> (1824 kg ha<sup>-1</sup>) and T<sub>5</sub> (1822 kg ha<sup>-1</sup>) though there was no significant variation among the treatments. Treatment 2 (T<sub>2</sub>) produced about 4 per cent increase in yield compared to 100 per cent of RDF alone (T<sub>6</sub>), indicating a possibility in reduction in fertiliser dose. A reduction in yield to the tune of 9.6 per cent was recorded when the dose of fertiliser was reduced to 50 per cent of RDF (T<sub>3</sub>). Moreover, doses of AGN soil amendment i.e. 14.4 L/ha (T<sub>1</sub>) and 24 L/ha (T<sub>5</sub>) produced similar yield of tea. Higher yield under T<sub>2</sub> may be due to higher number of plucking points per unit area and healthy plant growth. The lowest made tea yield recorded in treatment with 50% reduction in RDF (T<sub>3</sub>) which may be due to heavy reduction in fertiliser doses.

**Table 1. Effect of different treatments of AGN soil amendment on density of plucking points (No. m<sup>-2</sup>) and quality of harvested shoots (%)**

Treatments	Density of plucking points (No. m <sup>-2</sup> )	Fine shoot (%)
T <sub>1</sub> : 100% RDF + AGN soil amendment @ 14.4 L/ha	180.17	46.85
T <sub>2</sub> : 75% RDF + AGN soil amendment @ 14.4 L/ha	183.83	47.95
T <sub>3</sub> : 50% RDF + AGN soil amendment @ 14.4 L/ha	166.00	42.17
T <sub>4</sub> : 100% N & K + AGN soil amendment @ 14.4 L/ha	171.17	43.03
T <sub>5</sub> : 100% RDF + AGN soil amendment @ 24.0 L/ha	178.33	46.33
T <sub>6</sub> : 100% RDF only (Control)	173.33	44.73
SEm (±)	14.72	1.96
CD (0.05)	NS	NS

NS= Non significant



**Fig. 1. Yield of tea (kg made tea ha<sup>-1</sup>) as influenced by different treatments of AGN soil amendment**

### 3.4 Effect on Softness of Harvested Shoots

In general, softness in plucked shoots indicates easy plucking, help to maintain even plucking table thereby possibilities to increase fineness in harvested shoots, increase plucking efficiency, reduction in fibre content in made tea and better rolling in manufacturing process.

Data as noted in Table 2 indicated that, AGN soil amendment applied along with 100% and 75% RDF (T<sub>1</sub>, T<sub>4</sub> and T<sub>5</sub>) produced softer shoots in the month of November compared to 100% RDF alone (T<sub>6</sub>). Similar effect of AGN soil amendment was also noticed when the doses of fertiliser were curtailed to 75% of RDF (T<sub>2</sub>). However, shoots found harder as the dose of fertiliser further reduced to 50% RDF (T<sub>3</sub>).

### 3.5 Effect on Leaf Colour

It is evident from Table 3 that the leaf colour was remained unaffected under various treatments except in treatment T<sub>3</sub> (50% reduction in RDF + AGN soil amendment @14.4 L/ha) which may be due to heavy reduction in fertiliser doses.

### 3.6 Effect on Soil pH

A perusal of data presented in the Table 4 indicated that soil pH increased significantly after

application of treatments over control (T<sub>6</sub>) in both the soil depths and T<sub>4</sub> recorded the highest values (4.67). In top soil, T<sub>4</sub> was statistically similar to T<sub>3</sub>. Though, there was no significant difference observed among T<sub>6</sub>, T<sub>2</sub> and T<sub>1</sub>, yet T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> showed the significantly higher values as compared to T<sub>6</sub>. In sub-soil, significant increase in soil pH was observed in all the treatments over control (4.42) which were in the ranged from 4.56 – 4.67, however it was within favourable range.

### 3.7 Effect on Organic Carbon (%)

In case of organic carbon analysis, it was observed from the Table 4 that the treatment effect was not found to be significant.

### 3.8 Effect on Soil Moisture (%)

Available soil moisture is another factor in integrated nutrient management practices. The moisture of the soil recorded at the end of the season indicated that the effect of different AGN soil amendment treatments showed significant variations in respect of moisture content in both the soil depths (Table 4). In case of top soil, the highest value (20.87%) was recorded under T<sub>5</sub> which was statistically at par with that of T<sub>3</sub> (20.82%) and significantly higher than rest of the treatments. In sub-soil, the highest value (21.76%) was recorded under T<sub>3</sub> which

**Table 2. Effect of different treatments of AGN soil amendment on softness of harvested tea shoots**

Treatment	Softness of harvested shoots, months						
	June	July	August	September	October	November	December
T <sub>1</sub>	S	S	S	S	S	S	MH
T <sub>2</sub>	S	S	S	S	S	S	MH
T <sub>3</sub>	S	S	MH	S	MH	H	H
T <sub>4</sub>	S	S	S	S	S	S	MH
T <sub>5</sub>	S	S	S	S	S	S	MH
T <sub>6</sub>	S	S	S	S	S	MH	MH

*Index used: S = Soft; MH = Moderately hard; H = Hard*

**Table 3. Effect of different treatments of AGN soil amendment on colour of leaves**

Treatment	Colour of leaves, months						
	June	July	August	September	October	November	December
T <sub>1</sub>	G	G	G	G	G	G	YG
T <sub>2</sub>	G	G	G	G	G	G	YG
T <sub>3</sub>	G	GY	GY	G	GY	GY	YG
T <sub>4</sub>	G	G	G	G	G	G	YG
T <sub>5</sub>	G	G	G	G	G	G	YG
T <sub>6</sub>	G	G	G	G	G	G	YG

*Index used: G = Green; GY= Greenish yellow; YG= Yellowish green*

**Table 4. Effect of different treatments of AGN soil amendment on soil chemical properties and soil moisture content (%)**

Treatments	pH		Organic carbon (%)		Moisture (%)	
	Top soil	Sub soil	Top soil	Top soil	Sub soil	
T <sub>1</sub>	4.54	4.63	0.85	19.61	20.64	
T <sub>2</sub>	4.47	4.59	0.84	20.33	20.86	
T <sub>3</sub>	4.66	4.63	0.82	20.82	21.76	
T <sub>4</sub>	4.67	4.67	0.83	19.78	20.83	
T <sub>5</sub>	4.58	4.56	0.83	20.87	21.69	
T <sub>6</sub>	4.48	4.42	0.92	19.92	20.72	
SEm (±)	0.03	0.04	0.04	0.18	0.18	
CD (0.05)	0.06	0.09	NS	0.41	0.41	

NS= Non significant

was statistically at par with that of T<sub>5</sub> (21.69%) and significantly higher than those of rest of the treatments. Irrespective of treatments, soil moisture content was less at 0-15 cm depth than that of 15-30 cm depth due to quicker drying effect of surface soil.

### 3.9 Effect on Soil Microbial Population (CFU g<sup>-1</sup> of Soil)

The CFU count was calculated as total fungal count including the yeast and *mold*, actinomycetes count and bacteria count. Molds play important role in breakdown of organic matter in soil. They also help in binding soil particles and can help in reducing soil borne diseases. Data presented in Table 5 indicated that the population of yeast was either in traces or absent as it was not detected in all the treatments. Population of mold was reported to be 5.0 x 10<sup>4</sup> CFU g<sup>-1</sup> of soil before application of treatments. After application of treatments, it increased from 6.2 x 10<sup>4</sup> to 9.2 x 10<sup>4</sup> CFU g<sup>-1</sup> among the treatments when CFU was counted at the end of the flushing period. Application of

AGN soil amendment along with RDF had no negative impact on mold population in soil. Increase in fungal population after application of biostimulant was also reported by Hellequin et al. (2020). The increase in mold population in soil may be due to quicker multiplication and growth of microbes as the availability of higher amount of growth promoting substances, vitamins as well as enzymes and microenvironment provided by organic amendment. Similar observations were reported by Arslan et al. (2008), Kumari et al. (2016) and Devi et al. (2012). However, population of actinomycetes was slightly reduced in T<sub>1</sub> and T<sub>5</sub> after application of AGN soil amendment along with RDF when compared with their initial population (20.0 x 10<sup>5</sup> CFU g<sup>-1</sup>). More or similar population of actinomycetes was observed in T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. In case of bacterial population in soil, the initial population was reported to be 177.0 x 10<sup>5</sup> CFU g<sup>-1</sup> of soil which was reduced in the range of 113.0 x 10<sup>5</sup> – 173.0 x 10<sup>5</sup> at the end of the cropping period. This finding was in accordance to the observations of Cong et al. (2025).

**Table 5. Effect of different treatments of AGN soil amendment on soil microbiological population (CFU g<sup>-1</sup> of soil) in pre and post treatment application**

Treatments	Microbial population (CFU g <sup>-1</sup> of soil)			
	Total fungi		Actinomycetes	Bacteria
	Yeast	Mold		
T <sub>1</sub>	-	7.5 x 10 <sup>4</sup>	19 x 10 <sup>5</sup>	121 x 10 <sup>5</sup>
T <sub>2</sub>	-	6.2 x 10 <sup>4</sup>	22 x 10 <sup>5</sup>	132 x 10 <sup>5</sup>
T <sub>3</sub>	-	6.4 x 10 <sup>4</sup>	20 x 10 <sup>5</sup>	113 x 10 <sup>5</sup>
T <sub>4</sub>	-	6.2 x 10 <sup>4</sup>	21 x 10 <sup>5</sup>	152 x 10 <sup>5</sup>
T <sub>5</sub>	-	9.2 x 10 <sup>4</sup>	17 x 10 <sup>5</sup>	173 x 10 <sup>5</sup>
T <sub>6</sub>	-	9.78 x 10 <sup>4</sup>	22 x 10 <sup>5</sup>	179 x 10 <sup>5</sup>
Initial	-	5.0 x 10 <sup>4</sup>	20 x 10 <sup>5</sup>	177 x 10 <sup>5</sup>

#### 4. CONCLUSION

From the present experimental findings, it can be concluded that the integrated use of 75% RDF + AGN soil amendment @ 14.4 L/ha/year produced 4.03 per cent more yield compared to 100% RDF (Control). However, the effect of treatment was found to be not significant. The same treatment also recorded 6.06 per cent more plucking points/m<sup>2</sup> over control. Though organic carbon in soil was not affected significantly after application of AGN soil amendment, there was slight increase in soil pH in both the depth of soil. Among the microbial populations, population of mold was increased from 5.0 to 9.2 x 10<sup>4</sup> CFU g<sup>-1</sup>. However, population of actinomycetes was slightly reduced in T<sub>1</sub> and T<sub>5</sub> after application of AGN soil amendment when compared with their initial population though higher or similar population was observed in T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. In case of bacterial population in soil, the population was not increased after application of AGN soil amendment. Thus, the application of AGN soil amendment can be used as a sustainable way to increase crop yield and to maintain soil health in tea ecosystem. The result as discussed is only an indication in first year of experimentation and requires for further validation.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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