# EFFECTS OF ORGANIC AND INORGANIC FERTILIZER ON THE POPULATION DYNAMICS OF SOIL MICROORGANISMS IN TEA RHIZOSPHERE AT KERICHO, KENYA


## ABSTRACT

The long-term cultivation of tea (*Camellia sinensis L.*) alters microorganism communities in the rhizosphere; it can increase saprotrophs, pathogenic microorganisms and reduce symbiotrophs. Fertilizers are sources of plant essential nutrients and can influence the activity and population of soil microorganisms. This study aimed to determine the effect of fertilization regimes on the population dynamics of soil microorganisms in the tea rhizosphere for its management. The study was carried out at the Tea Research Institute in Kericho, Kenya. Two main fertilizer types; organic (Phymix) and inorganic (Nitrogen, Phosphorus and Potassium- NPK) and foliar fertilizer (Tecamin Max, Tecnokel Amino Mix) as sub treatments application at the rate of 0, 75 and 150 kg N $ha^{-1}$) were applied in four replications. Sampling of soil was done before treatment application, during the dry season (February-March) and the wet season (June-July). The fungal and bacterial populations for both seasons were characterized. The data collected was analyzed using SAS (version 9) Statistical Software. Results showed that during the dry season the fungal colony units varied significantly ($P \leq 0.05$) between the types of fertilizer. Similarly in the rainy season, the fungal population varied significantly ($P \leq 0.05$) in where the organic fertilizer was applied in comparison with the fungal population where inorganic fertilizer was applied. Both interactions of fertilizer type and rate varied significantly ($P \leq 0.05$) for fungal populations during both seasons. No significant variation was noted for the bacterial population (cfu) for both seasons regardless of fertilizer type and rates. The fungi identified included;*Cylindrocarbon* spp., *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp. *Colletotrichum* spp., *Pestalotiopsis* spp. and *Fusarium* spp. The bacteria included; *Pseudomonas* spp., *Bacillus* spp., *Rhizobium* spp. and *Xanthomonas* spp. Organic fertilizer increased fungal populations significantly, an indication of enhanced soil health and may be recommended for use.



**Key Words**

Interaction, Nutrients, Pathogenic, Seasons and Variations.

**INTRODUCTION**

Soil microorganisms are vital in healthy plant development, protection, nutrient recycling and decomposition of organic matter. They also play a vital role in maintenance of the soil structure in agro-ecosystems (Prasad *etal.*, 2021). Soil physiochemical properties on fertilization can drive changes in microbial communities, structure and metabolic functions (Han, *et al*, 2021). Microbial diversity and biomass are responsive to changes in soil physiochemical properties and management and are useful in predicting changes in soil functions. Studies on the composition and diversity of microbial communities under fertilized soils would be beneficial for effective improvement of soil fertility and productivity in ensuring sustainable development of soil ecosystem and perhaps management of soil pathogenic microbes.

The perennial nature of tea crop growth requires continuous fertilization using both inorganic and organic fertilizer, which may alter the pH levelsof the soils. Inorganic chemical-based fertilizers have been applied over the years in an attempt to increase soil fertility and tea productivity (Adesmoyee and Kloepper, 2009). However, continuous application of inorganic fertilizer in tea farms acidifies the soils, inflict nutritional imbalance and deterioration of the rhizosphere micro-ecological environment.Similarly, the acidic soils suitable for tea cultivation are also suitable for the growth and development of fungi including pathogenic fungi. The usage organic fertilizers could possibly alleviate soil acidification, have a positive influence on reducing pollution, offer natural and safer sources of plant essential nutrients and can influence the activity and population of soil microorganisms including pathogens in the rhizosphere(Kamunya, *et al.*,2019).They have great potential in mitigating against problems associated with inorganic fertilizers and reduce the need for repeated application to maintain soil fertility.

However, this information about soil microbial diversity and composition in tea plantation with inorganic or organic fertilizer application is limited in Kericho, a major tea producing region in Kenya. The impact caused by application of both inorganic and organic fertilizer on the proliferation of soil pathogens need to be established to recommend on fertilizer regime as contribution to integrated disease management and improved tea productivity. The present study therefore, was performed in an already establishedfield trial to examine the soil pathogenic fungal and bacterial population dynamics and diversity in the soils under three fertilizer regimes of organic (Phymix) and inorganic (the standard NPK 26:5:5) fertilizer on rhizospheric soils at a depth of 0-15cm.



## MATERIALS AND METHODS

### Study site

The research was conducted in Tea Research Institute Kericho (TRI) at theTimbilil Estate; located at East of Kericho town, at Latitude 0°21'48.89"S; Longitude 35°21'39.61"E and altitude of 2178m above the sea level. The area has a mean annual rainfall of 1800 mm and minimum annual mean temperate of 18°C and of a maximum 23°C. Soil is red volcanic soil that is deep, well-drained with pHrange of 5.0-6.5.

**Experimental treatments and design**

Two fertilizer types; organic used is the Phymix (Phytomeia international; Nairobi, Kenya) and inorganic (NPK 26:5:5) were used as main factor A and randomized completely and two foliar fertilizer types namely Tecamin Max, Tecnokel Amino Mix (Agritechfertilizantes; Nairobi, Kenya) as sub-factor B applied in the three rates (equivalent to 0, 75kgN $ha^{-1}$ and 150kgN $ha^{-1}$) replicated four times (Total plots; (2 x 2 x 3 x 4 reps = 48plots). Tea cultivar used on the experimental plots is TRFK 11/4 with a spacing of 4mx2m.

**Soil sampling in the field**

Random sampling method was employed to collect soils using a sterilized soil auger from sites marked by Geographical Positioning Sites (GPS), before fertilizer application to establish initial microorganism population and the procedure was again repeated consequently after fertilization. Three tea bushes were randomly selected at each site and the rhizopheric soils (1-5cm around the root) was collected from each bush pooled to form a composite sample. The soil auger was properly sterilized using 5% sodium hypochlorite and rinsed with sterile water then dried using a sterile cloth before being used to sample soil from different plots. Sampling was done during the rainy season(June-July) and dry season (February-March). Sampling was done twice per season. The soil samples from each plot were mixed and then sieved through a 2 mm mesh sieve to remove plant debris and other coarse materials. The soil samples were placed in well-labelled polythene bags then taken to the laboratory and stored at 4 $^{o}$Cprior to analysis.

**Quantification of fungal populations and characterization**

Spread plate method in pre-sterilized Potato dextrose agar (PDA) was used to enumerate microbial populations of fungi in the sampled soil as described by Nur, (2013). Ten grams of the sample soil collected was suspended in 90 ml of sterile water blanks in 250 ml conical flasks. The soil was diluted serially to yield three dilutions. Using a sterile pipette, 1ml of the third dilution ($10^{-3}$) was plated onto three petri-dishes containing potato dextrose agar (PDA) and spread evenly using a sterile spreader. The PDA plates was then incubated at room temperature (24 ± 2$^{o}$C). After 72 hours of incubation the plates were observed and number of fungal colonies counted using a colony counter to estimate colony forming units per gram of the soil (CFU/g) in order to establish the initial fungal population using the formula;

$$\text{Number of fungi (cfu)/g soil} = \frac{\text{Number of colonies}}{\text{Amount plated}} \times \text{dilution factor}$$

Subculturing was then done to obtain pure colonies. Preliminary characterization of the isolates involved the examination of colony morphology and cultural features such as pigmentation, elevation, shape, size and growth form of the fungal and confirmed through microscopy from the Riddel slide cultures (Riddel 1950). Further, the subsequent isolations were done on selective media for the identified fungi to quantify the fungal population dynamics as a result of the applied fertilizer.

**Quantification of bacterial populations and morphological characterization**

For colony growth, 1ml of $10^{-5}$ serial diluted soil was plated onto three replicate petri-dishes containing pre-sterilized nutrient agar (NA) using sterile pipette and incubated at 35ºC± 2ºC for 24 hours. After incubation, the number of colonies formed was counted using a colony counter to obtain bacteria colony forming units per gram of the soil. Only plates containing greater than 30 bacterial colonies and less than 300 bacterial colonies in every sample was considered for estimating the number of CFU/g of the soil. Plates containing less than 30 colonies are considered to be too few to count (TFTC) while those containing greater than 300 colonies are considered to be too many to count (TMTC) (Sieuwerts*et al.*, 2008).

The Characterization of the isolates involved the examination of colony morphology and culture features such as pigmentation, elevation, shape, size and growth formof safranin-stained bacterial isolates under the dissecting and compound microscope, further microscopy in oil immersion eye piece magnification was used and gram staining. The subsequent isolates were done using selective media of the identified bacterialspecies

**RESULTS**

**Effect of fertilizer on the diversity of fungi and bacteria on tea rhizosphere**

The composition of the rhizospheric microbial community in tea plants varied though was not significantly different(p≤0.05) depending on the season and the fertilization regime. Results obtained from the first season (February-March)indicated that fungal colony units in the tea rhizosphere varied significantly (P≤0.05) between organic and inorganic fertilizer form applied. Only the organic amendment showed significant (P≤0.05) variation of fungal cfuamong the different foliar fertilizers. In addition, both interactions of fertilizer and rate plus foliar and rate also varied significantly (P≤0.05) for fungal populations. There was no significant (P≤0.05) variation in bacterial cfu population for organicamendment and foliar fertilizerbut a variation was noted in inorganic (Table 1).

**Table 1: The effect of different types and rates of fertilizer on fungal and bacterial population during the dry season (February- March).**

| Fertilizer type | Foliar type | Fungi (cfug-1 soil) x$10^3$ | | Bacteria (cfug-1 soil) x$10^5$ | |
|---|---|---|---|---|---|
| | | 75kg N ha-1 | 150kg N ha-1 | 75kg N ha-1 | 150kg N ha-1 |
| Organic (Phymix) | Tecamin Mix | 19.5±2.56 | 26.8±3.35 | 31.3±6.6 | 32.3±3.19 |
| | Tecnokel Amino Mix | 12.5±1.04 | 26±2.16 | 36.5±3.3 | 33.8±11.15 |
| | Control (0) | 10±1.08 | 11±1.08 | 29.5±3.97 | 25.8±1.99 |
| P=0.05 | | 0.01 | 0.10 | 0.68 | 0.68 |
| CV (%) | | 21.4 | 28.4 | 44 | 44 |
| Inorganic (NPK) | Tecamin Mix | 29.5±5.55 | 24±3.16 | 31.5±3.85 | 23.8±3.46 |
| | Tecnokel Amino Mix | 24±1.78 | 15±2.8 | 37±6.82 | 45.8±5.44 |
| | Control (0) | 14±1.58 | 25±2.08 | 39.8±6.66 | 34±4.66 |
| P=0.05 | | 0.05 | 0.10 | 0.68 | 0.04 |
| CV (%) | | 22.1 | 28.5 | 35 | 27.3 |

Results during the rainy season showed that, the fungal population varied significantly (P≤0.05) in the organic fertilizer applied into tea rhizosphere than with the fungal population in the inorganic fertilizer applied to rhizosphere. The organic amendment showed significant (P≤0.05) variation of fungal population between the different foliar fertilizers regimes but there was no significant (P≤0.05) variation in inorganic amendment. In addition, both interactions of fertilizer type and rate of foliar also varied significantly (P≤0.05) for fungal populations (Table 2).

There was significant (P≤0.05) variation in bacterial cfu for both organic and inorganic amendment however, a significant variation (P≤0.05) between different foliar fertilizers and rate was recorded as shown in Table 2.

**Table 2: The effect of different types and rates of fertilizer on fungal and bacterial population during the wet season (June-July)**

| | | Fungi (cfug-1 soil) x$10^3$ | | Bacteria (cfug-1 soil) x$10^5$ | |
|---|---|---|---|---|---|
| Fertilizer type | Foliar type | 75kg N ha-1 | 150kg N ha-1 | 75kg N ha-1 | 150kg N ha-1 |
| Organic (Phymix) | Tecamin Mix | 42.5±2.75 | 31.2±3.50 | 47.8±6.08 | 28.8±3.52 |
| | Tecnokel Amino Mix | 34.8±4.07 | 34.0±3.29 | 74.5±3.3 | 38.3±2.60 |
| | Control (0) | 21.2±3.12 | 33.7±1.65 | 30.7±1.49 | 33.7±2.10 |
| P=0.05 | | 0.02 | 0.74 | 0.002 | 0.05 |
| CV (%) | | 22.1 | 16.4 | 19 | 12.5 |
| Inorganic (NPK) | Tecamin Mix | 17.5±4.29 | 23.5±3.23 | 25.8±2.66 | 60.3±1.55 |
| | Tecnokel Amino Mix | 16.8±1.79 | 17.5±1.71 | 24.0±1.87 | 66.0±2.16 |
| | Control (0) | 12.3±0.85 | 27.5±2.63 | 27.8±4.01 | 28.8±3.12 |
| P=0.05 | | 0.49 | 0.05 | 0.74 | 0.0001 |
| CV (%) | | 40.7 | 20.1 | 26 | 8 |

The soil pH in all the sampled plots were within the ideal requirement for tea production (4.5 and 5.6). Organic fertilizer (Phymix) however, increased the soil pH with Tecnokel Amino mix foliar to 5.17 than with the inorganic NPK fertilizer combined withTecnokelAmino mix foliar (4.41). However, there was no significant differences (P≤0.05) in pH between rates and foliar (Table 3).

It was noted that there was a decrease in bacterial population with a lower pH at the rate of 75kg N ha-1 in organic fertilizer(Tecnokel amino mix) with a mean of 24cfu/g while inorganic (Tecnokel amino mix) with a mean of 74cfu/g, while the population of fungi increased in the organic (Tecamin foliar) with a mean of 42.5cfu/g.

**Table 3: The effect of different types and rates of fertilizer treatments on the soil pH**

| | | Fertilizer rates | |
|---|---|---|---|
| Fertilizer type | Foliar type | 75kg N ha-1 | 150kg N ha-1 |
| Organic | Tecamin Mix | 4.16±0.3 | 3.86±0.04 |
| (Phymix) | Tecnokel Amino Mix | 5.17±0.2 | 4.37±0.2 |
| | Control(0) | 4.52±0.5 | 4.65±0.4 |
| P=0.05 | | 0.08 | 0.05 |
| CV (%) | | 11.3 | 8 |
| Inorganic | Tecamin Mix | 4.56±0.1 | 4.66±0.3 |
| (NPK) | Tecnokel Amino Mix | 4.415±0.1 | 4.728±0.3 |
| | Control(0) | 4.85±0.2 | 4.67±0.3 |
| P=0.05 | | 0.04 | 0.96 |
| CV (%) | | 3.93 | 7.65 |

**Morphological characterization of fungi**

Among the fungal genera identified in the rhizosphere of tea plants were *Trichoderma* sp. (Plate 1), *Pestalotia*spp. (Plate 2), *Cylindrocarbon* spp. (Plate 3), *Penicillium* spp., *Aspergillus* spp. *Colletotrichum* spp., *Fusarium* spp. (Plate 3), and*Beuverriabassiana*

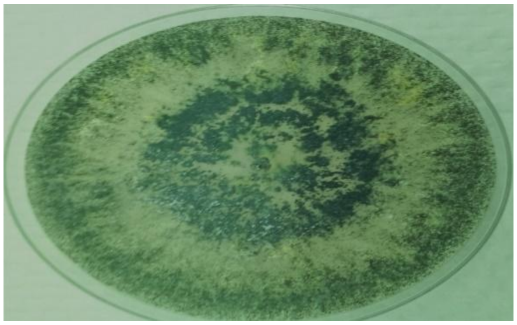


Plate 1: Trichoderma culture on PDA

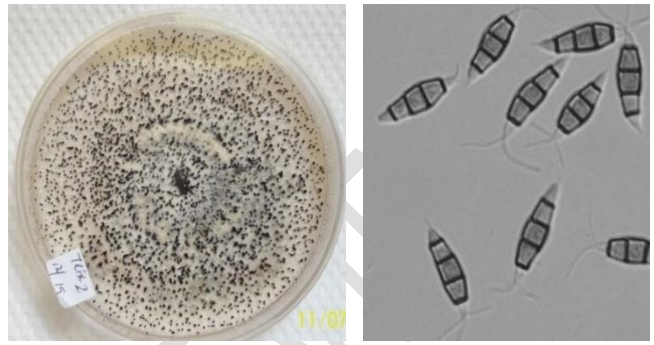


Plate 2: Pestalotia culture and conidia

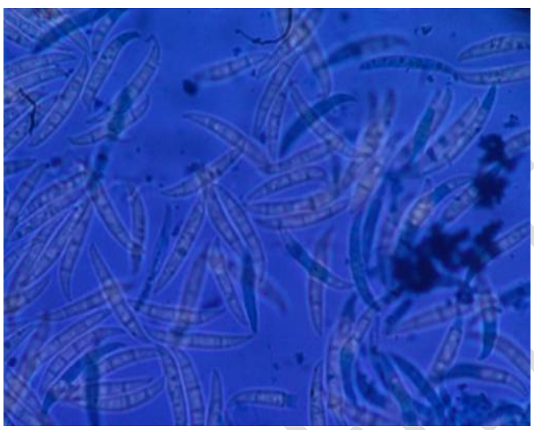


Plate 3: Macroconidia of *Fusarium* spp.

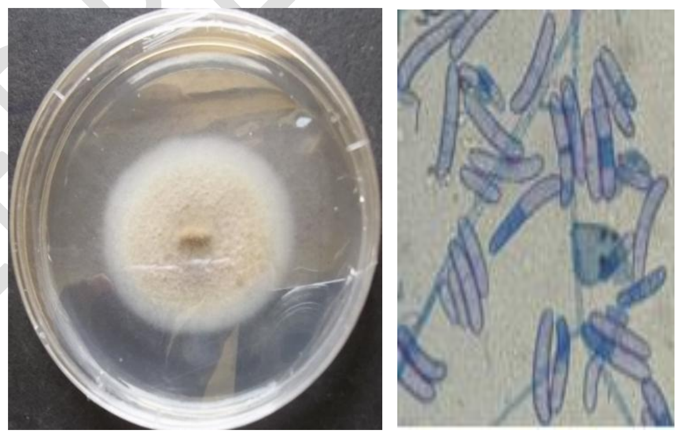
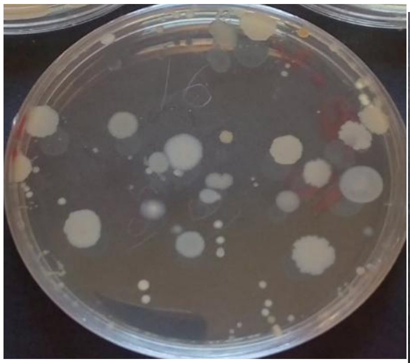


Plate 4: Cylindrocarbon culture and spores

**Morphological characterization of bacteria**

Some common bacteria that were found in the tea rhizosphere include; *Pseudomonas* spp., *Bacillus* spp.(Plate 5 and 6), *Rhizobium* spp. and*Xanthomonas* spp.



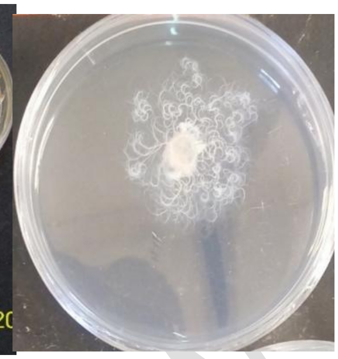


Plate 5: Bacterial culture on Nutrient Agar media: Plate 6: Colonies of *Bacillus mycoides*

**DISCUSSION**

The application of fertilizer in the tea fields is a common practice to maximize yields and to replenish the nutrients lost through harvesting. Excessive chemical fertilizer though has resulted to numerous effects such as nitrogen leaching, soil degradation, soil compaction, reduction in organic matter, loss of soil carbon and micro-organisms activity. Though both organic fertilizers provide similar nutrient elements as inorganic fertilizer, the effect on rhizospheric micro-oganisms vary significantly, results have indicated that there is a rise in soil acidity with frequent use of nitrogenous fertilizer (Owuor *et al.,* 2014). There are many different types of fungi and bacteria that can be found in the rhizosphere of tea plants, and the specific diversity may depend on factors such as soil type, climate, and management practices. Both fungi and bacteria are important components of the rhizospheric microbial community in tea plants, and their interactions with the tea plant can have significant impacts on plant growth, yield and quality(Kamunya, *et al.*,2019). Additionally, results indicated that organic fertilizer treatment improved soil pH.The currentstudyestablished that the use of organic fertilizer significantly increased the bacterial population during the wet season. This data is in agreement with previous findings that NPK chemical fertilizer caused a significant decrease in bacterial populations (Sun *et al.,* 2015).

There were significant differences in the soil fungi and bacteria composition observed in the tea rhizosphere with organic fertilizer and inorganic fertilizer. Comparable results were observed by Lin *et al*., (2019), with long-term continuous cropping of tea fungalpopulation increased significantly in soils where inorganic fertilizer was applied, because fungi are acidophilic and are capable of flourishing in an acidic environment (Yang *et al*., 2021). The study has further shown that the application of chemical fertilizers decreased the soil pH, stimulated the increase of fungi. However, application of organic fertilizer was able to increase this effect. Organic fertilizers act as an effective source of energy for soil microbes that improve soil structure and plants growth and development (Shaji *et. al*,2021), this could explain the observation in this study. Zhang *etal*., (2012) have documented the progressive soil-plant response potential benefits from organic fertilizer showing a rise in microbial activities enhancing crop growth and restraining pest and diseases.

The number of fungi was found to increase in the soils applied with inorganic however, Lin *et al*., (2019) described tea as a unique crop because the soil becomes strongly acidified following its planting The tea plant itself acidifies soil and also the fertilizers being applied during management (Le *et al*., 2022), which favours fungal growth and unsuitable for bacteria. Further with the application of organic fertilizer increased the soil pH which was favourable for bacteria and their ecological functions as observed in the current study agreeing with Aziz et al., (2021).

There were significant differences in the soil fungi and bacteria composition observed in the tea rhizosphere with organic fertilizer and inorganic fertilizer. Among the microorganisms identified in the rhizosphere of tea plants included *Cylindrocarbon* spp., *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp. *Colletotrichum* spp., *Pestalotiopsis* spp. and *Fusarium* spp. The bacteria that were found in the tea rhizosphere included*Pseudomonas* spp., *Bacillus* spp., *Rhizobium* spp. and *Xanthomonas* spp. which was comparable results to Lin *et al*., (2019); Mandic-Mulec & Prosser (2011) and reports of Chen *et al*., (2023).

**Conclusion**

Organic fertilizer enhanced fungal and bacterial populations in rhizospheric soil during wet season. It was noted thatsoil pH was related to fertilizer treatment in that organic treatments increased the soil pH compared to inorganic treated plots. These findings therefore, suggest that fertilizer can shape microbial composition and population into the tea rhizosphere. Thisstudy provides a promising strategy to soil health by application of organic fertilizers as opposed to inorganic fertilizer, though was found to favour fungi.

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