

# Tea and biofertilizers: A below ground biodiversity sustainable approach

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## ABSTRACT

Tea is produced in the humid tropical and sub-tropical regions, which are home to rich biodiversity. The tea plantation environments have the potential to conserve a rich biodiversity which could serve as a source of biofertilizers. The natural abundance of microorganisms within the tea agro-ecosystems are expected to play a key role in sustainability of environment and increase tea performance and quality. However, agricultural and anthropogenic practices interfere with the tea agro-ecosystems and reduce the potential of the below ground biodiversity in sustainable tea production. The extent of this interference differs agro-ecologically and mapping of the biodiversities could provide a guide into the rich sampling sites which can be characterized, isolated, bulked and packaged into biofertilizers. Biofertilizers often double as efficient plant-aids in nutrient uptake and bio-pesticides, and offer an alternative to inorganic fertilizers and chemical pesticides which are a threat to humans and environments. Currently, the biotechnological manipulation of the tea rich biodiversities is very limited despite the opportunity it offers in biofertilizer production and utilization.

**Key words:** Biodiversity, Biofertilizers, Tea plantations, Sustainable agro-ecosystems.

## INTRODUCTION

Tea is produced in the humid tropical and sub-tropical regions which harbour rich biodiversity. The influence of plants on microbial population structure and function in the rhizosphere has important ecological implications for soil function, including biogeochemical cycles (Bloemberg and Lugtenberg, 2001). Soil microbes influence plant health and productivity. The rich biodiversity in tea plantations, if well managed is, capable of facilitating soil-plant-microbe interactions and enhancing tea productivity and quality. Application of inorganic fertilizers is an energetically costly process and considerable pollution is caused through both the production and use of mineral N-fertilizers. This is exacerbated by the relatively low efficiency of their uptake by the plants due to non-extensive root system and may also delete soil organic matter in the long term (Khan *et al.*, 2007; Ladha *et al.*, 1997; Ladha and Reddy, 1995). Inoculant biofertilizers are more environmentally sound and their introduction in agricultural production systems could be one of the means to mitigate the onset of global warming as well as the reduction in fertilizer input costs, prevent depletion of organic matter and increase crop yields (Mia and Shamsuddin, 2010; Kennedy *et al.*, 2004; Jeyabal and Kuppaswamy, 2001).

Organic and inorganic fertilizers are used primarily to increase nutrient availability and the type or amount of fertilizer added to soil could directly affect the function performed by the various microbial groups in the soil (Marschner *et al.*, 2003). Low organic matter content, predominant low activity clays, and strong acid reactions depress microbial activities which in turn disturb the

nutrient cycle in the soils (Marschner *et al.*, 2003). Under humid-tropic conditions, fertilizer loss through leaching, volatilization, and/or fixation represents an economic loss as well as a potential environmental contamination. Use of bio-fertilizers has the potential to improve functional relationship between above-and below-ground bio-diversity (Goenadi *et al.*, 2009). A biotechnological method of soil management provides a new approach in resolving the problems untackled by conventional practices. Biofertilizers are microorganisms which can improve the availability of nutrient to plants (Goenadi *et al.*, 2009). They are believed to be an important component of sustainable agriculture, as they can reduce significantly the use of chemical fertilizers (Goenadi *et al.*, 2009). Improving rhizospheric microbial activities through bio-fertilizer application, nutrient solubilization can be enhanced and consequently less conventional fertilizers will be needed (Goenadi *et al.*, 2005). This study aimed at unveiling the below-ground biological diversity (BGBD) across land use gradients and specifically at evaluating the biotechnological potential of the biodiversity rich tea plantations.

## MATERIALS AND METHODS

Inventory of micro-organisms across land use types was carried out at the central highlands of Kenya at Embu District. The soil in Embu is classified as Humic Nitisoils (FAO, 1989). The District lies approximately between latitudes 0° 8' and 0° 35' South and longitudes 37° 19' and 37° 40' East and is divided into five divisions. The central point of the study area (Mount Kenya Forest near Irangi market and bordering agricultural lands) was traversed by longitude 37° 28' East and latitude 0° 20'. Altitudes for the area range between 1,500 m and 4,500 m. Six land use types were identified as prevalent in the area. These were; maize-bean intercrop, tea; napier grass (*Pennisetum purpureum*); coffee (*Coffea arabica*); fallow or pasture, and natural undisturbed forest. Soil samples were taken from 60 pre-determined sampling points, distributed among the main land use types at each benchmark sites. The sampling points were marked using a grid and were 200 m apart. At each sampling point, two vertically crossing lines and two concentric circles of radius 3 m and 6 m were drawn. An auger was used to take four samples from the 0 – 20 cm depth. The 0 – 20 cm soil depth was chosen for mycological considerations since it contains the majority of soil microbiota (Skujins, 1984). The micro-organisms evaluated include beneficial microbes such as saprophytic (*Trichoderma*), symbiotic mycorrhiza (*Arbuscular mycorrhiza*), biological nitrogen fixing bacteria and antagonistic microbes (*Trichoderma* and Nematophagus).

### Assessment of mycorrhizal colonization and spore abundance

Roots and soil samples were assessed for presence of AMF. Assessment was done by evaluating the percentage root colonization, spore abundance and infective propagules. The roots were stained according to procedures by Koske and Gemma (1989). In to each bottle 2.5% KOH was added before heating in the oven at 70°C for one hour. The KOH was poured off and the roots rinsed to remove KOH. Alkaline hydrogen peroxide was then added and roots left for one hour to distain, to remove the phenolic substances. Alkaline hydrogen peroxide was then poured off, the roots thoroughly rinsed with tap water and 1% HCl added and left for one hour. After pouring off HCl 0.05% Trypan blue was added and the roots placed in the oven for one hour. Destaining solution (500 ml glycerol, 450 ml of distilled water and 50 ml of 1% HCl) was added. Analysis of AMF colonization was done according to McGonigle (1990). Slides were prepared with 30 pieces of roots each one cm long then examined under a compound microscope. The percentage of each piece covered by arbuscules, vesicles and hyphae was assessed to determine the intensity and frequency of AMF colonization.

Spores were extracted from soil using Jenkins (1964) procedure with modifications by Ingleby (2007). The soil (50 g) was pre-soaked in water and washed through 710 µm and 45 µm pore sieves with running water. The beaker content was decanted into 50 ml centrifuge tubes and centrifuged for five minutes at 1750 rpm. Water was carefully decanted from the tubes and

floating debris discarded and 48% of sucrose (227 g dissolved in 500 ml of water) was added and centrifuging for one minute at 1750 rpm. Immediately after centrifugation, sucrose solution was carefully decanted through a 45 µm sieve. The spores were rinsed thoroughly with water to wash out the sucrose. Spores were transferred from the 45 µm sieve into a small Petri dish for examination under a dissecting microscope. Under a dissecting microscope, spores were counted and grouped according to their morphotypes. Microscope slides were prepared with very small drops of PVLG-Polyvinyl lactoglycerin and Melzer's + PVLG and characterized under a compound microscope based on appearance, morphology, sub-cellular features and reaction to Melzer's.

### ***Trichoderma* assessment**

*Trichoderma* spp. were isolated from soil using serial dilution plating (Jonson *et al.*, 1959). 1/10, 1/100, 1/1000 dilutions of the samples were prepared. Before the setting of the organic matter and soil particles, 1 ml from the second and third dilutions was applied to prepared plates of malt extract (MEA) incubated at 25°C. *Trichoderma* spp. were isolated from the 120 soil samples using the soil dilution plate (Johnson *et al.*, 1959) and soil washing methods (Gams *et al.*, 1987; Bills and Polishook, 1994). The dilution plate method was used for the estimation of the fungus. 1/10, 1/100, 1/1000 dilutions of the samples were prepared. Before the setting of the organic matter and soil particles, one millilitre of the dilutions were applied to plates containing malt extract (MEA) and cornmeal agar (CMD) with 2% dextrose both with streptomycin 50 mg/L and cyclosporin 10 mg/L antibiotics. For isolation using the soil washing technique, 10 g of soil was sieved in a nest of 4.0 mm, 1.0 mm and 0.5 mm sieve. This was done by suspending 10 g of the soil in two litres tap water and pouring through the nest of the sieves. The procedure was then repeated with 2 litre of sterile water. After this treatment, the contents of the first mesh which were large organic particles were surface sterilized by transferring the contents into a sterile Petri dish with sterile water containing streptomycin. Organic particles floating on the surface of the water and the washed soil particles were picked up with a loop and forceps and transferred onto plates of MEA and CMD (Cornmeal agar with 2% dextrose) both with streptomycin 50 mg/l and Cyclosporine. 10mg/l antibiotics. Two replicates per media were used. The small pieces of debris retained on the other two sieves could not be surface sterilized because they were too small and porous. The debris was damp-dried on sterile paper towels and then dried over silica gel for 24 hours before plating on the isolation media. The plates were incubated at 25°C for two weeks (Gams *et al.*, 1987). The colonies were counted and identified using the soil dilution plate method. The identified colonies were transferred to Petri-dishes containing PDA (potato dextrose agar) and incubated at 15, 25, 30 and 35°C for further identification to species level. Colonies developed from the isolates using the soil washing technique were also identified.

### **Nematode assessment**

The soil sub-samples were mixed homogeneously to constitute a composite sample from which 500 g of soil were taken, placed in a plastic bag, sealed and then kept under shade. The samples were then transported to the laboratory in a cool box and stored at 4°C. Nematodes were extracted from the soil using the sieving and centrifugation techniques (Jenkins, 1964).

## **RESULTS AND DISCUSSION**

### **Effect of land use types and mycorrhiza abundance**

Spore abundance was highest in the intensively cultivated/disturbed and least in forest systems (Figure 1). Active propagules were highest in less disturbed forest, Tea and nappier, and least in intensively cultivated/disturbed land use types. Fungi in symbiosis are able to live symbiotically with over 80% of living plants. Beneficial mycorrhizal associations are found in many of our

edible and flowering crops. Mycorrhizal symbiosis increases plant access to water and nutrients from the soil (McGonigle and Miller, 1996), play an important role in the formation of soil aggregates (Hamel *et al.*, 1997), influence plant biodiversity (van der Heijden *et al.*, 1998), help protect against pests and diseases (Azcon-Aguilar and Barea, 1997) and improve fitness of plants in polluted environments (Hildebrandt *et al.*, 1999).

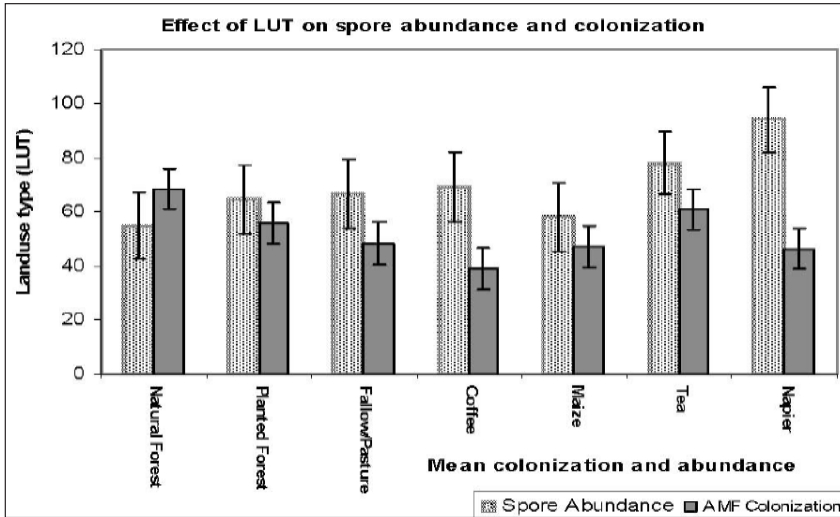


Figure 1: AMF spore abundance and colonization across land use types in Embu District.

### Land use gradients and *Trichoderma* abundance

*Trichoderma* populations were highest in nappier > maize > indigenous forests and planted forests in that order (Figure 2). The populations were significantly lower ( $P < 0.005$ ) in fallow, coffee and tea land use systems. *Trichoderma* are significant decomposers of woody and herbaceous materials, parasitic against other decomposers, mycoparasitic hence regulates populations of other fungi. *Trichoderma* also possess have an antibiosis nature and are bio-enhancers hence enable plants to tolerate stress and compete for nutrients. *Trichoderma* spp., have been studied as biological control agents against soil-borne pathogenic fungi (Küçük, 2000; Henis, 1984; Sivan and Chet, 1993). Knowledge concerning these fungi as antagonists is essential for their effective use because they can act against pathogens especially against parasitic nematodes that affect tea (Chet, 1987; Küçük, 2000).

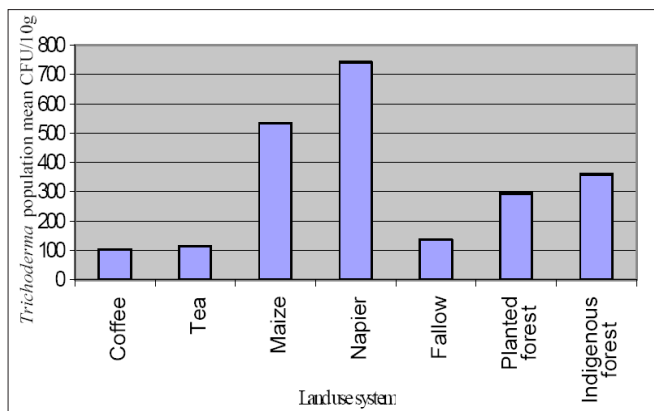


Figure 2: *Trichoderma* populations across land use types in Embu District.

## Nematophagus populations

Nematophagus are natural enemies of plant parasitic nematodes. They parasitize nematode eggs and other life stages. They are widespread in distribution and have a high potential as biological control agents of plant and animal parasitic nematodes. The highest percent frequency of isolation (33.7%) was recorded in the vegetable cropping system followed by the maize (27.9%), nappier (20.9%), shrub (11.6%) and forest (5.8%) (Table 1). This study reveals that nematophagus populations increase with intensity of land cultivation as evidenced by the high numbers in the vegetable cultivation. This is consistent with the conclusion of Freckman and Ertema (1993) that nematode abundance was higher in high input organic systems than in perennial cropping systems while species diversity was greatest under minimum tillage treatments. According to Yeates (1999), nematode diversity tends to be greatest in ecosystems experiencing long-term human interference and changes in nematode community may be a reflection of changes in soil and ecological processes.

**Table 1: Nematophagus populations across land use types in Embu District.**

Land use	n	Frequency of isolation %	Mean evenness	Mean richness	Mean Shannon
Forest	8	5.8	0.375	0.625	0.17.
Maize/bean	8	27.9	1.000	3.000	1.07
Napier	8	20.9	1.000	2.250	0.76
Shrub	8	11.6	0.625	1.250	0.36
Vegetables	8	33.7	1.000	3.625	1.26
P-value		$3.81 \times 10^{-07}$	$7.14 \times 10^{-9}$	$3.81 \times 10^{-07}$	$1.062 \times 10^{-06}$

## CONCLUSION

The study reveals the existence of a rich biodiversity across land use types and specifically in tea plantations. The abundance of *Mycorrhiza*, *Trichoderma* and *Nematophagus*, all of which are beneficial microorganisms unravels a biotechnological potential that can be harnessed in the development of biofertilizers for direct inoculation and restoration of environmental health through integrated management practices that enhance beneficial microbes' growth and interactions. This could greatly enhance plant health and productivity.

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