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LAND USE SYSTEMS AND DISTRIBUTION OF *Trichoderma* SPECIES IN EMBU REGION, KENYA

[RELACIÓN ENTRE USO DEL SUELO Y LA DISTRIBUCIÓN DE *Trichoderma* EN LA REGIÓN DE EMBU, KENIA]

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SUMMARY

The distribution of *Trichoderma* species in soils of Embu region in relation to land use practices was investigated. The study area was chosen because of its significant land use intensification. Soil washing and dilution plate techniques were used to recover *Trichoderma* spp from soil samples. The fungal isolates were identified and assigned to eight species. Greater populations as well as a wider range of species were obtained in soils collected from the natural forests while coffee farms were the poorest ones. Land use affected the distribution of *Trichoderma*. Napier farms had the highest abundance of this fungus. The species that showed the highest incidence in all cases was *T. harzianum*. Plant type was a major determinant of the occurrence of this fungus. *Trichoderma* favored plants with shallow and widespread rooting systems, to the deeply rooted perennial coffee and tea trees. The age of the plants also was a driving factor. Both inorganic and organic fertilizers are used in the region. There was a negative correlation between amount of chemical fertilizers and abundance of the fungus. Organic fertilizers were used exclusively in napier farms that had the highest fungal abundance. Soil pH and amount of phosphorus were limiting and influenced the occurrence and abundance of this fungus. However carbon and nitrogen were not limiting though they were high in the forests and napier farms where the fungus was also abundant. *Trichoderma* showed tolerance to soil acidity since it was abundant in the most acidic soils under napier. Land intensification affected *Trichoderma* distribution negatively.

Key words: Soil characteristics, *Trichoderma*, land management.

RESUMEN

Se estudió la distribución de *Trichoderma* en suelos de la región de Embu y su relación con las prácticas de manejo y uso del suelo. El área de estudio fue seleccionada debido a la intensificación en el uso del suelo. Se recuperó *Trichoderma* spp a partir de muestras de suelo. Las especies fungales aisladas fueron identificadas y asignadas a ocho especies. La mayor población y número de especies fue encontrada en el bosque natural, mientras que las fincas cafetaleras fueron las más pobres. El uso del suelo afecto la distribución de *Trichoderma*. Fincas con pasto Napier (*P. purpureum*) tuvieron la mayor abundancia de este hongo. La especie de mayor incidencia en todos los casos fue *T. harzianum*. El tipo de vegetación fue un determinante importante en la occurrencia de este hongo. *Trichoderma* favoreció plantas con raíces poco profundas y de amplia distribución, no así las plantas perenes y con raíces profundas como el café y té. La edad de las plantas también fue un factor importante. Fertilizantes orgánicos e inorgánicos son empleados en la región y se encontró una correlación negativa entre la cantidad de fertilizante empleada y la abundancia del hongo. Fertilizantes orgánicos fueron empleados exclusivamente en fincas con Napier. El pH del suelo y la cantidad de fósforo fueron limitantes e influenciaron la ocurrencia y abundancia de este hongo. Sin embargo, el carbono y nitrógeno no fueron limitantes. *Trichoderma* mostró tolerancia a la ácidez del suelo y fue abundante in la mayoría de los suelos ácidos con pasto Napier. La intensificación del uso del suelo afectó negativamente la distribución de *Trichoderma.*

Palabras clave: Características del suelo, *Trichoderma*, manejo del suelo.

INTRODUCTION

Microorganisms in soil are critical to the maintenance of soil function in both natural and managed agricultural soils because of their involvement in such key processes as soil structure formation, decomposition of organic matter, cycling of carbon, nitrogen, phosphorus and sulphur. Despite the importance of these organisms for ecosystem functioning, relatively little is known about the relationship between plant species composition and the diversity of soil microorganisms (Wardle *et al*., 1999; Broughton and Gross, 2000; Stephan *et al*., 2000; Niklaus *et al*., 2001; Knops *et al.*, 2002; Kowalchuck *et al.*, 2002). Soil microorganisms are mostly saprophytic, thus they use plant exudates or decomposing plant material for food. A reduction in food quantity and a change in food quality caused by a loss in plant diversity should modify the abundance, activity and diversity of soil microbial communities (Wardle and Lavelle, 1997; Hopper *et al*., 2000).

Land use type directly dictates the food quality and quantity of soil microorganisms and further determines soil management system. Several studies have documented that the treatment or management of soil affects microbial community structures. Application of pesticides, (Heilmann *et al.*, 1995) compost (Schionfeld *et al.*, 2002) and the introduction of genetically modified microorganisms (De Leiy *et al*., 1995; Mahaffee and Kloepper, 1997) have all been shown to affect soil microbial community structures.

Trichoderma species are cosmopolitan fungi in soils, decaying wood and vegetable matter. Their dominance in soil may be attributed to their diverse metabolic capability and aggressive competitive nature (Lewis and Papavizas, 1991; Eland, 2000; Haran *et al*., 1996a; Haran *et al.*, 1996b). These characteristics make them significant decomposers of woody and herbaceous material and are also necrotrophic against other decomposers. *Trichoderma* also play key roles in suppressing soil borne plant diseases and promoting plant growth (Garbeva *et al*, 2004). They colonize roots, attack, parasitize and gain nutrition from other fungi, thus enhancing root growth. *Trichoderma* species have developed rhizosphere competence through evolving numerous mechanisms for both attack of other fungi and for enhancing plant and root growth. These properties include mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of organic nutrients, induced resistance, inactivation of the pathogens enzymes and these properties have been demonstrated by several scientists including Chet (1987, 1993); Hjeljord and Tronsmo, 1998, Altomore *et al*., 1999; Eland and Kapat, 1999; Howell *et al*., 2000; Yedidia *et al*., 1999; These diverse activities of *Trichoderma* render them a beneficial component of soil ecosystem. This study was conducted to determine the influence of land use systems on the occurrence and distribution of *Trichoderma* species in Embu district, Kenya.

Embu district which is located in Mount Kenya region was chosen because of its land use intensity gradients. The main land use systems in the region range from indigenous forests through planted forests, monocropped coffee and tea farms to mixed croppings of maize-based farms. These different land use systems and the intensification that characterize monocropped cash crops like tea and coffee, provide a good baseline comparison with the occurrence and abundance of beneficial microorganisms like *Trichoderma*. Further the Mount Kenya region is one of the priority biodiversity hotspots in the country with approximately 31% plant species which have become extinct or in danger of extinction and 81 endemic plant species. There are also a number of rare and endangered animal species (Newmark, 1998). Being the most coherent and extended natural forest block of the country, Mount Kenya has been recommended as one of the four forests for biodiversity conservation in Kenya (Wass, 2000).

In this study, the diversity, abundance and spatial distribution of *Trichoderma* species over the different land use systems in Embu was compared. The influence of chemical properties and farm management practices on the populations of this fungus was also studied. Morphological and cultural characters were used to identify *Trichoderma* isolates using taxonomic keys compiled by Samuels *et al*., (2004).

MATERIALS AND METHODS

Description of study site

The study site is divided into two main physiographic zones; namely the upper zones of open moorland above 3,350m and lower forest and cultivated area 1,400 to 2,000m above the sea level (Wokabi 1995). Three windows were identified within Nginda, Kibugu and Kaagati locations. Window 1 and 2 are 0.5km apart and both are 20km away from Window 3. Sixty sampling points, 200m apart were randomly chosen using GPS mappings. These points fell within eight land use systems; Tea farming (*Camellia sinensis*) 8 points, Coffee farming (*Coffea arabica*) 10 points, Maize based farming (*Zea mays*) 9 points, Fallow land (mainly *Digitaria abyssinica, Pennisetum cladestinum*) 8 points, Napier farms (*Pennisetum purpureum*) 8 points, Planted forests of Meru oak (*Vitex keniensis*) 6 points, Planted forests of mixed

eucalyptus (*Eucalyptus saligna, E. globulus*), 3 points and Indigenous Forests (8 points).

Soil sample collection

3m and 6m radius circles were drawn round each sampling point and 4 soil samples cored from the 3m radius and another 8 from the 6m radius at depth 0 - 20 cm. These samples were composed. The soil was collected and transported in paper bags. The samples were kept at 2- 5oC in the laboratory to reduce microbial activity.

Isolation of *Trichoderma* spp.

The 60 soil samples collected from the randomly chosen plots were processed using the soil dilution plate (Johnson *et al*., 1959) and soil washing methods (Gams *et al*., 1987; Bills & Polishook, 1994) in the laboratory.

Dilutions 1/10, 1/100, 1/1,000 of the samples were prepared as follows; to each flask containing 90ml sterile distilled water, 10g of soil was added from only one source and labeled accordingly. The flask was swirled to mix and suspend the soil thoroughly in the water. This is the 1/10 dilution. The dilution series were then prepared from this suspension. For 1/100 – 1ml of the 1/10 soil suspension was removed with a sterile pipette and added to a tube containing 9ml sterile water. The contents of the tube were mixed thoroughly. For 1/1,000 – 1ml of a 1/100 soil suspension was removed with a sterile pipette and added to a tube containing 9ml sterile water. The tubes were labeled accordingly. Before the setting of the organic matter and soil particles, 1 ml of the dilutions was applied to prepared plates of malt extract (MEA) and cornmeal agar (CMD) -with 2% dextrose both with streptomycin 50mg/L and cyclcosporin 10mg/L.

For isolation using the soil washing technique, 10g of soil was sieved in a set of 4.0 mm, 1.0 and 0.5 mm sieve. This was done by suspending 10g of the soil in 2L tap water and pouring through the nest of the sieves. The procedure was then repeated with 2L of sterile water. After this treatment, the contents of the first mesh which were bigger in size 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 50mg /L and Cyclosporine 10mg/L. 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. This 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^{o}C for two weeks (Gams *et al*., 1987).

The colonies were counted and identified using the soil dilution plate method (this is not an identification method). The identified colonies were transferred to Petri dishes containing PDA (potato dextrose agar) and incubated at 15, 25, 30 and 35^{o}C for further identification to species level. Colonies developed from the isolates using the soil washing technique were also identified.

Identification of *Trichoderma* species

Genus identification of green fungus was undertaken using the method of Domsch *et al*., (1980). *Trichoderma* isolates were identified at species level following the taxonomic key of the genus *Trichoderma* (Samuels *et al*., 2004). Colony characters, growth rates in culture and morphological characters were used in identification. Microscopic examination was carried out by mounting the culture in lactophenol cotton blue but for size measurements KOH and water was used as the mounting fluid. A small amount of material was placed in a drop of 3% KOH on a slide and then replaced with water.

Soil chemical characteristics

The remaining soil samples were used to measure the following characters of the soil. The soil pH was determined in 1:2.5 soil water suspensions. Nitrogen was determined by the catalytic oxidation of organic and chemically combined nitrogen and subsequent alteration to NH_4 by the micro Kjeldhal process.

Available P and K were determined using Mehlich method (Hinga *et al.*, 1980). Organic carbon was determined by oxidation using sulphuric acid and titrating the unused residue against ferrous sulphate (Nelson and Sommer, 1975).

Land Management Practices

Information on use of organic or inorganic fertilizers; application of pesticides and herbicides; age of cultivated plants; percentage crop cover; monocropping and mixed cropping; type of conservation strategy were gathered from farmers using a questionnaire.

Statistical Analysis

Comparisons of the distribution of *Trichoderma* and land use systems were done using SPSS Statistical Computer Software Version 10. Logistic regression

analysis was done because the data was of a presence absence nature and the proportions observed needed transformation logistically in order not to violate normality assumption. Descriptive statistical analysis of variance was used. Using the Genstat computer package version 8, Logistic regression modeling was carried out to study the relationship between variables. Analysis of deviance tables were used to assess the significance of effects. Multiway tables of predicted proportions were used to summarize the results for the categorical explanatory variables. Parameter estimates were also used to quantify linear relationships.

RESULTS

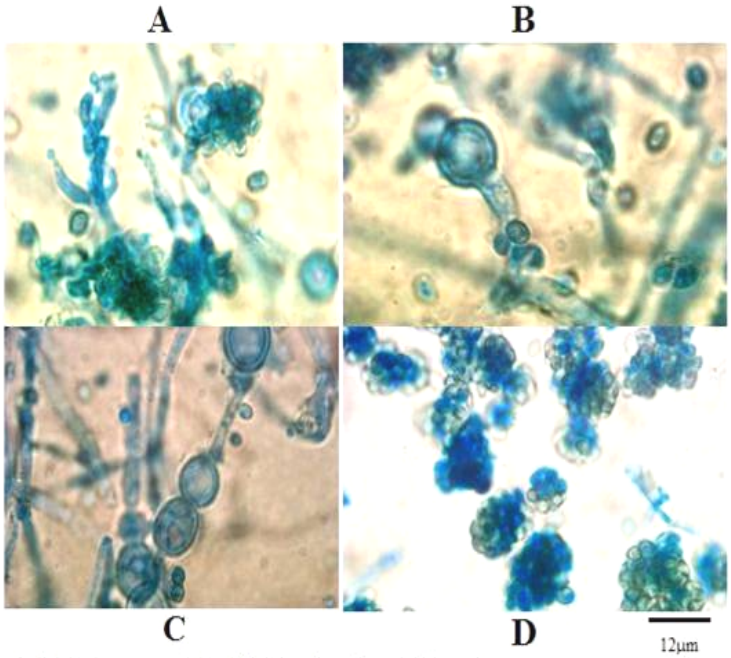
Distribution of *Trichoderma* in different land use systems

A total of 306 *Trichoderma* isolates were obtained from the analyses of the 60 soil samples collected from the sample site through soil dilution and soil washing methods. Identification of these isolates resulted in 8 species of *Trichoderma*, Plates 1, 2 and 3 and Table 1.

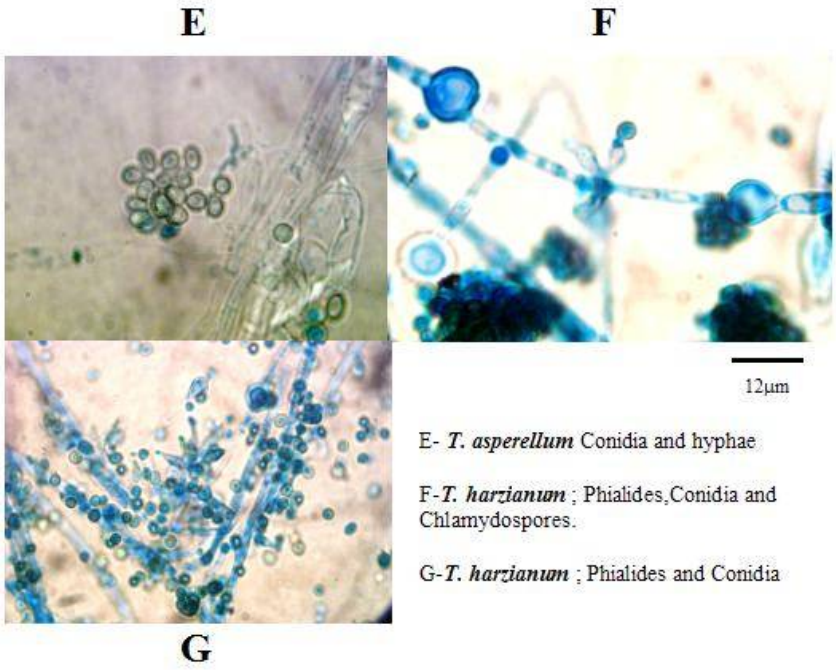
The most frequently isolated species was *T. harzianum*. Most of the isolates of this species were recovered from napier farms and the least from land left fallow for pasture. *T. harzianum* was the most frequently isolated species from the tea and maize farms, the natural forest, fallow land and planted forests. In the coffee farms *T. viride* was the most frequently isolated species. *T. citrinoviride* was the most common in napier farms and *T. viride* in the coffee farms. Of the eight species listed in Table 1, six were isolated with the soil dilution plate technique, and two from both methods (*T. harzianum* and *T. citrinoviride*). The number of isolates obtained by the soil dilution plate method was 274 and 32 were isolated using the soil washing technique.

There was a significant difference in abundance of the different species across the different land use systems (P value = 0.00 < 0.05). *T. harzianum* was the most abundant species, Fig 1. Different species were significantly abundant in different soils as summarized in Table 2. Species abundance under various land uses was also compared. The results show that there was a significant difference between the various land uses at 5% level of significance with P value of 0.00. Land use under napier had the highest abundance and coffee, the least (Figure 2).

Plates 1 (A,B,C,D), 2 (E,F,G) and 3 (A,B,C,D) : *Trichoderma* isolates showing conidia, phialides and chlamydospores.



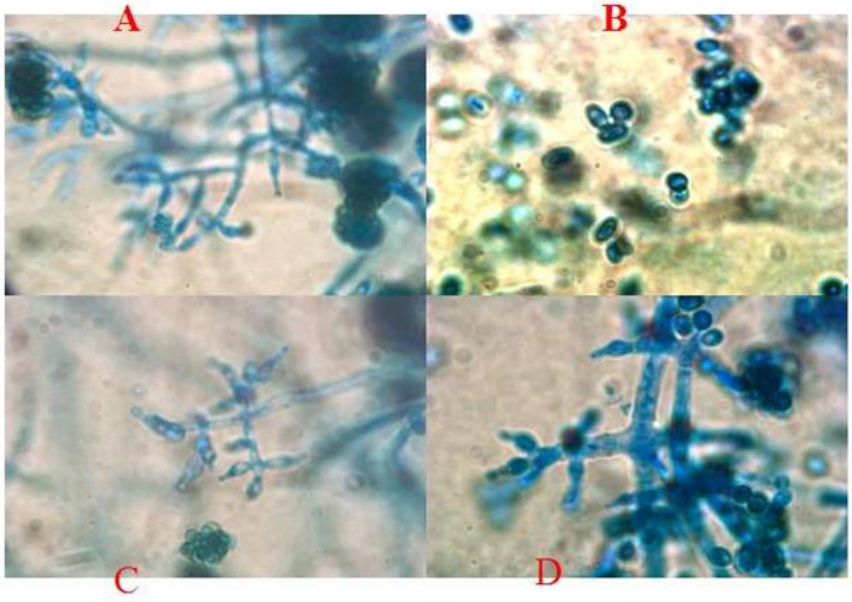
A-***Trichoderma atroviride***; Phialides, Conidia and Chlamydospores.
B- ***T. atroviride***; Conidia and Chlamydospores.
C- ***T. atroviride*** ; Conidia and Chlamydospores
D- ***T. asperellum***; Conidia whorls



E- ***T. asperellum*** Conidia and hyphae

F-***T. harzianum*** ; Phialides,Conidia and Chlamydospores.

G-***T. harzianum*** ; Phialides and Conidia



A- *T. citrinoviride* [Phialides], **B**- *T. citrinoviride*[Spores], **C**- *T. surrotunda* [[Phialides] and **D**- *T. surrotunda* [Phialides],

Table 1. Frequency of Isolation of *Trichoderma* species from the different land use systems.

Land Use / *SPECIES*	Tea Farms	Coffee Farms	Maize-Based Farms	Napier	Indigenous Forest	Planted Forests	Fallow	Overall Frequency of Isolation
Trichoderma citrinoviride	4	6	0	35	2	0	0	47
T. surrotunda	0	4	4	0	0	0	0	8
T. harzianum	23	8	24	28	32	34	16	165
T. asperellum	11	0	0	0	0	0	7	18
T. viride	0	12	12	0	16	4	0	44
T. atroviride	0	0	0	0	4	2	4	10
T. agrovissum	0	0	0	0	4	0	0	4
T. stromaticum	0	0	4	0	0	0	6	10
Total	**38**	**30**	**44**	**63**	**58**	**40**	**33**	306

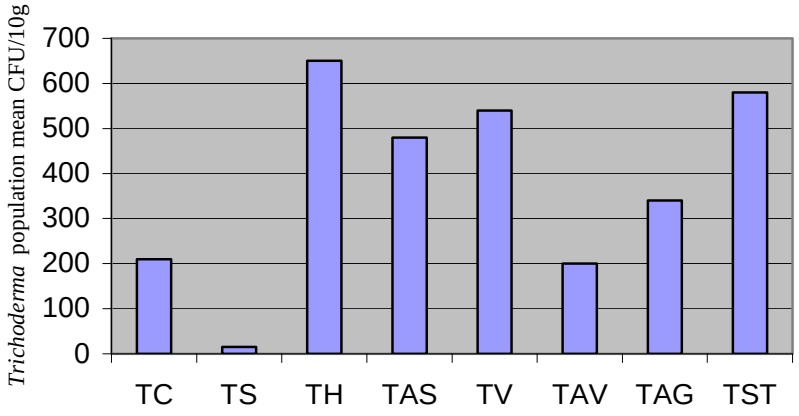


Figure 1. The abundance of *Trichoderma* spp. In Embu region, Kenya.
Key: TC-*Trichoderma citrinoviride*; TS-*T. surrotunda*; TH-*T. harzianum*; TAS-*T. asperellum*; TV-*T. viride*; TAV-*T. atroviride* TAG-*T. agrovissum* TST-*T. stromaticum.*

The abundance of the species obtained by the dilution plate technique was compared with the land use systems. Table 3 shows that there was significant difference in the abundance of *T. surrotunda, T. harzianum, T. viride* and *T. citrinoviride* across the land use systems. *T. surrotunda, T. harziarum* and *T. viride* were the most abundant in land under maize while *T. citrinoviride* was most abundant in land under napier.

There were no significant differences among the three windows with respect to the frequency of occurrence and abundance of the *Trichoderma* species ($P > 0.05$). Natural forest recorded the highest diversity of the fungus.

Table 3. Comparison of *Trichoderma* abundance with land use systems. ANOVA results**.**

***Trichoderma* species**	**P-value**	**Most abundant in this land use system**
T. surrotunda	0.001	Maize
T. harzianum	0.000	Maize
T. asperellum	0.406	
T. viride	0.056	
T. stromaticum	0.967	
T. citrinoviride	0.000	Napier

Table 2. Variation of *Trichoderma* with land use: anova results.

Land Use System	Species Recovered	P-Value	Most Abundant Species
Tea farm	*Trichoderma citrinoviride*	0.00	*T. asperellum*
	T. surrotunda		
	T. harzianum		
	T. asperellum		
Coffee farm	*T. citrinoviride*	0.29	
	T. surrotunda		
	T. harzianum		
	T. viride		
Maize based farm	*T. citrinoviride*	0.00	*T. harzianum*
	T. surrotunda		
	T. harzianum		
	T. viride		
	T. stromaticum		
Napier farm	*T. citrinoviride*	0.00	*T. citrinoviride*
	T. surrotunda		
	T. harzianum		
	T. viride		
Indigenous forest	*T. citrinoviride*	0.00	*T. harzianum*
	T. surrotunda		
	T. harzianum		
	T. viride		
	T. atroviride		
	T. agrovissum		
Planted forest	*T. citrinoviride*	0.00	*T. harzianum*
	T. surrotunda		
	T. harzianum		
	T. viride		
Fallow	*T. citrinoviride*	0.00	*T. stromaticum*
	T. surrotunda		
	T. harzianum		
	T. asperellum		
	T. atroviride		
	T. stromaticum		

Effect of soil chemical characteristics on occurrence of *Trichoderma* species

The pH, acidity, Carbon (C), Nitrogen (N), Phosphorus (P), and Potassium (K) varied significantly across the land use systems ($p = 0.00 < 0.05$), Table 4. The chemical characteristics of soil showed positive significant influence on the occurrence of all *Trichoderma* species though at varying levels. Table 5 shows that the distribution of *Trichoderma* in the study site could be explained in terms of soil chemical characters by up to 34.7%, (the highest value obtained (0.347)). Therefore 56.3% of the occurrence of this fungus has to be explained by some other factors. Of the soil chemical attributes tested C, P and K strongly showed significant positive correlation with the most common species of *Trichoderma*, *T. harzianum* and *T viride*.

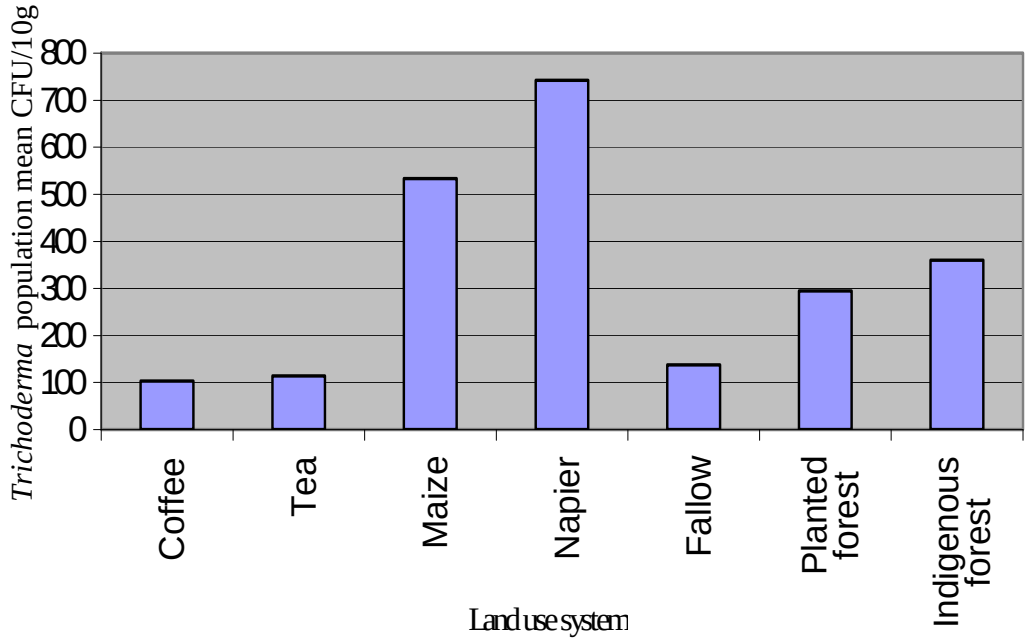


Figure 2. Comparison of *Trichoderma* abundance with land use.

This could explain the abundance of this fungus in the forests and Napier and maize farms. Seventy five percent of the leverage points from regression analysis, that is the points that influenced the analysis, fell within the forests, napier and maize farms, and they were also points with higher abundance of the fungus and of C, P, K. This suggests that the influence of C, P, K could be observed clearly at point level suggesting further that individual management of the farms influenced the soil content of C, P, K. Maize and napier farms were the only farms with exclusive usage of organic fertilizers. Again the results above suggests that organic fertilizers had an influence on the occurrence of this fungus.

Both organic and inorganic fertilizers are used in the site, though in the napier and maize farms only organic fertilizers are used. There was a significant relationship between addition of organic fertilizers to farms and amount of C, N, P, and K ($p=0.000$). In all the cases the regression was positive. However the relation between organic fertilizers and occurrence of fungi was positive but weak ($p=0.05$) suggesting that it was not only organic fertilizers that explained the occurrence of the fungus. The significance of organic fertilizers was reduced by the forests that also recorded high populations of the fungus and the fallow land that had high C, P, K but recorded low fungal populations. Addition of organic fertilizer influenced the occurrence of *T. surrotunda* highly and *T. harzianum* and *T. koningii* marginally. The influence was positive. Organic fertilizers were added to both annuals (maize based plants) and perennials (coffee, tea and napier farms). There was a marginal relationship between addition of organic fertilizers to annuals or perennials. *T. surrotunda* responded more to addition of fertilizers to annual plants than to perennial ($p = 0.025$; parameter estimate 2.64). Though coffee and tea farms recorded the lowest abundance of fungal frequency of isolation and abundance (Table 1, Fig. 2), at point level all the coffee (points 8, 14, 15, 18, 29) and tea (points 1 and 9) farms with organic fertilizers recorded fungal occurrence. This indicates a relationship between the fungus and organic fertilizers.

Simple regression analysis of the *Trichoderma* sp. abundance on quantity of inorganic fertilizer applied to the farms was significant ($p = 0.04$). There was a negative regression coefficient (-0.195) meaning that with the increase in quantity of chemical fertilizer application, the fungal abundance decreased. This is represented in the scatter graph, Fig. 3. Addition of inorganic fertilizers to both annual and perennial plants influenced the occurrence of *T. harzianum* negatively ($p = 0.02$, $p = 0.01$ respectively) *T. surrotunda* ($p = 0.04$, $p = 0.11$), Table 6. The prediction values estimated that the probability of isolating *T. harzianum* from an annual or perennial farm without inorganic fertilizers is 83.8% and this decreases to 33.0% with addition of inorganic fertilizers.

Table 4. Variation of soil chemical properties with land use system.

Soil Attributes	P value	F- value	Land use	Mean	SD	Range
pH	0.000	25.621	Coffee	3.9778	0.1852	3.66 – 4.32
			Tea	3.4640	0.2503	3.08 – 4.06
			Maize	4.1725	0.3027	3.62 – 4.83
			Napier	4.2115	0.2417	3.59 – 4.70
			Fallow	4.3658	0.2205	3.93 –4.60
			Planted forest	3.8789	0.6953	2.98 – 5.00
			Indigenous forest	3.5017	0.1582	3.20 – 3.80
Acidity (M.e%)	0.011	6.626	Coffee	3.9037	0.4776	1.80 – 4.57
			Tea	2.9467	0.8651	1.40 – 4.20
			Maize	1.1379	0.8829	0.20 – 3.01
			Napier	0.8739	0.6432	0.20 – 2.54
			Fallow	1.6667	0.6020	0.70 – 2.80
			Planted forest	2.0944	1.2358	0.20 – 4.70
			Indigenous forest	2.3438	0.9092	0.70 – 3.40
Nitrogen (%)	0.041	4.247	Coffee	0.5611	0.9285	0.17 – 4.34
			Tea	0.3810	0.1665	0.13 – 0.70
			Maize	0.3712	9.140	0.22 – 0.54
			Napier	0.3217	7.413	0.23 – 0.45
			Fallow	0.6650	0.442	0.27 – 1.76
			Planted forest	0.8056	0.3737	0.37 – 1.65
			Indigenous forest	0.6256	0.1843	0.35 – 0.90
Carbon (%)	0.004	8.546	Coffee	3.7811	0.7283	2.73 – 5.23
			Tea	3.8590	1.4909	2.00 – 6.77
			Maize	3.6475	1.3904	0.3 – 5.82
			Napier	3.7608	0.5941	2.98 – 4.96
			Fallow	5.5762	1.6964	2.25 – 8.60
			Planted Forest	6.3917	0.8554	5.01 – 7.94
			Indigenous Forest	5.5469	0.2768	5.10 – 5.97
Phosphorus (ppm)	0.000	16.819	Coffee	11.5185	5.0791	2.00 – 26.00
			Tea	16.6000	10.8812	7.00 – 40.00
			Maize	15.1667	6.5652	5.00 –30.00
			Napier	13.3030	4.6936	7.00 – 24.00
			Fallow	15.5833	7.0641	8.00 – 30.00
			Planted Forest	13.3333	7.2324	5.00 – 36.00
			Indigenous Forest	20.7500	11.4141	2.00 – 45.00
Potassium (%)	0.000	48.197	Coffee	7.2211	7.2679	0.33 – 16
			Tea	0.26	0.1420	0.1 – 0.45
			Maize	0.37	0.2031	0.18 – 0.56
			Napier	0.242	0.1410	0.1 – 0.48
			Fallow	0.3838	0.2246	0.17 – 0.79
			Planted Forest	0.2144	0.1117	0.1 – 0.32
			Indigenous Forest	0.3614	0.1359	0.2 – 0.47

Table 5. Logistic Regression Analysis: Relationship between Soil Chemical Characteristics and Occurrence of *Trichoderma* Species

Trichoderma Species	R^2	P value	pH		Exchangeable Acid		Carbon		Nitrogen		Phosphorous		Potassium	
			Coef	P value	Coef	P value	Coef	P value	Coef	P value	Coef	P value	Coef	P value
T. surrotunda	0.347	0.000	N/S		N/S		N/S		-227	0.000	1.961E-03	0.094	2.772E-02	0.000
T. harzianum	0.254	0.000	N/S		N/S		0.243	0.000	N/S		-1.6515E-02	0.031	3.82E-02	0.057
T. koningii	0.158	0.000	N/S		N/S		N/S		N/S		N/S		1.051E-02	0.012
T. viride	0.087	0.025	N/S		N/S		N/S		N/S		N/S		1.0050E-02	0.001
T. asperellum	0.120	0.001	N/S		0.187	0.000	N/S		N/S		-8.887E-03	0.035	N/S	
T. stromaticum	0.068	0.1	N/S		N/S		N/S	0.1	N/S		N/S		N/S	
T. citrinovivide	0.073	0.086	N/S		N/S								-0.02670	0.034
T. atrovivide	0.331	0.000	N/S		N/S		N/S		0.479	0.000	0.000		1.004E-02	0.001
T. aggrovissum	0.152	0.000												

Coef: Coefficient; NS : Not significant

Table 6. Influence of farm management on occurrence of *Trichoderma* species: Regression analysis table.

Farm management	***Trichoderma* species that presented significant trend**	**Estimates of parameters**		**Chi probability**
		Estimate of farm management practice	**Standard error**	
Pesticides	*T. harzianum*	-2.34	1.12	0.011
Herbicides	*T. harzianum*	-8.0	15.4	0.020
	T. stromaticum	-5.7	25.4	0.571
Organic fertilizer	*T.surrotunda*	10.6	50.1	0.023
input	*T. koningii*	9.8	50.1	0.112
	T. harzianum	9.4	58.8	0.105
Inorganic fertilizer	*T. harzianum*	-1.584	0.573	0.004
input	*T. surrotunda*	-5.7	32.8	0.049
Crop density	*T. harzianum*	-1.491	0.563	0.006
	T. citrinoviride	-1.576	0.850	0.044
	T. atroviride	-8.9	37.2	0.104
Perennial crop	*T.harzianum*	-0.714	0.870	0.025
cover	*T. viride*	-0.046	0.920	0.040
	T. citrinoviride	-0.8	0.956	0.020

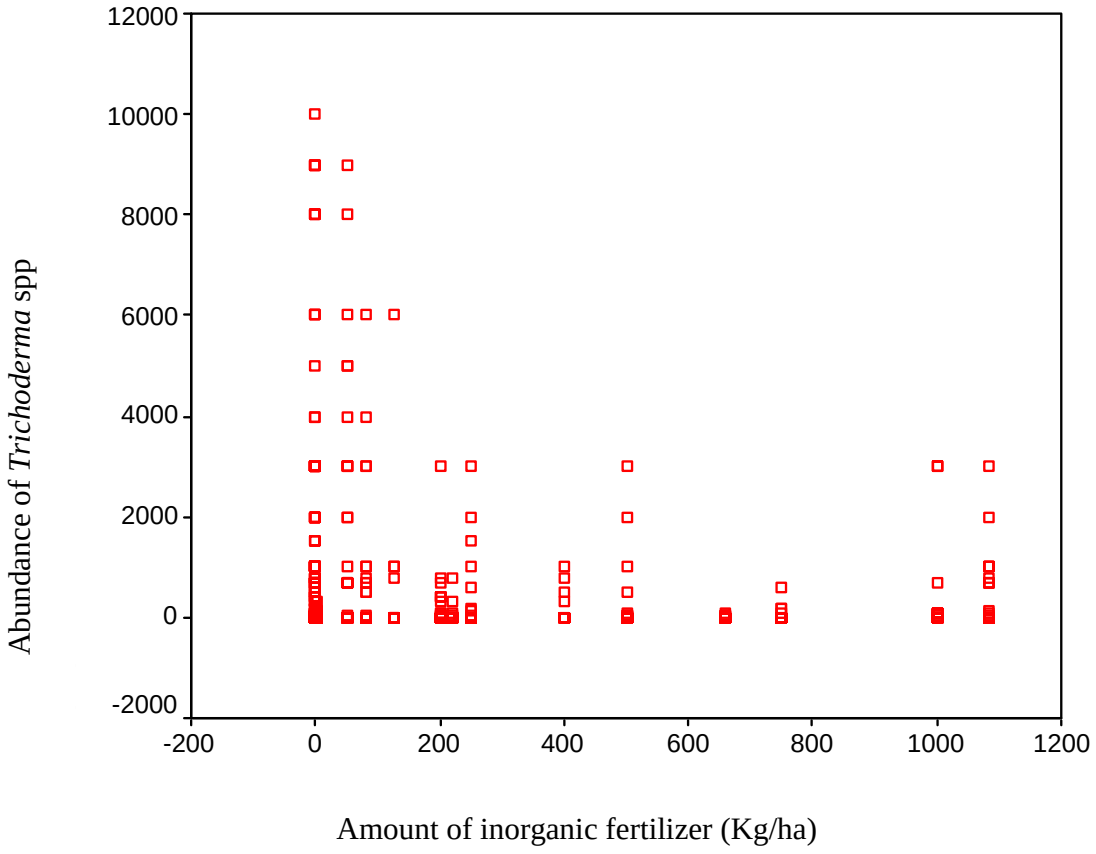


Figure. 3. Effect of inorganic fertilizer application on abundance of *Trichoderma* spp.

The soils in the study site were acidic (pH 3-5). This varied significantly across the land use types but did not influence the distribution of the fungus Fig. 4. The same applied to acidity.

The cultivated crop formed different densities of above ground cover. For example, tea farms formed higher densities compared to coffee farms. This was measured in percentages and compared with distribution of the fungus. Crops that had thick above ground cover had less occurrence of *T. atroviride, T. harzianum* and *T. citrinoviride* Table 6. However *T. harzianum and T. citrinoviride* species was high in forests and napier farms that also had high cover and less in coffee farms that had thin above ground cover). This means that the crop density alone cannot be used to explain the occurrence of the fungus. Other factors such as the type of above ground cover, in other words the plant species could also be contributory factors. Farms with high crop densities tended to be the ones that had addition of fertilizers. The distribution of the most common species of *Trichoderma, T. harzianum* was highly influenced positively with additions of organic fertilizers (p = 0.037; deviance ratio 2.56) which increased with increase with crop density. Inorganic fertilizers and crop density, on the other hand, highly influenced the distribution of *T. harzianum* negatively (chi pro = 0.026; deviance ratio 2.77), Table 7.

Again as observed above the factor influencing the fungus negatively is the fertilizer, and not crop density. Soil organic carbon increased with crop density (p = 0.04; deviance ratio 2.20) and the probability of isolating *T. harzianum* increased with increase in C as shown by the predictions from regression model, Table 8.

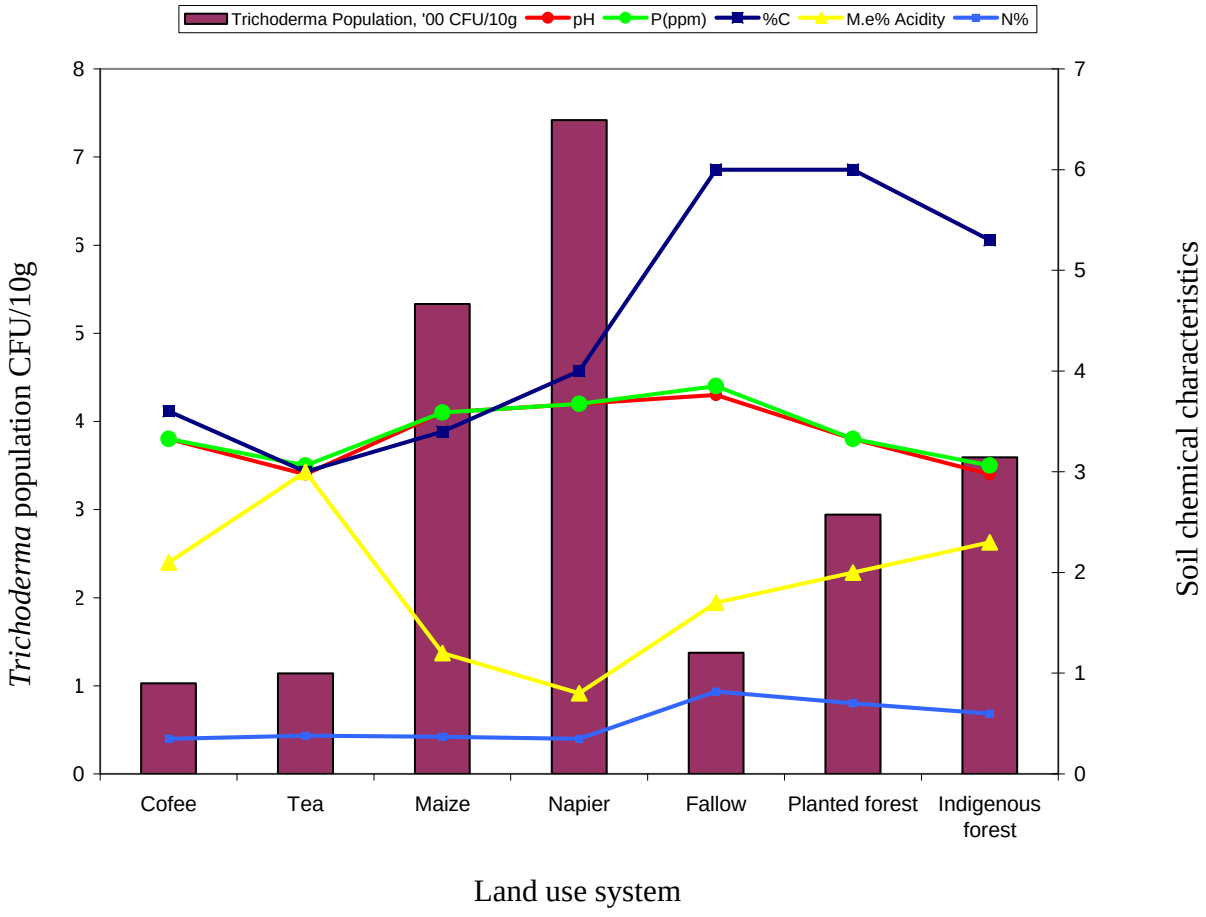


Figure 4. Effect of soil chemical characteristics on the occurrence of *Trichoderma* spp.

Table 7. Influence of inorganic fertilizer and crop density on the distribution of *Trichoderma harzianum.*

Farm Management practice: crop density	Species	Estimates of parameters		Chi probability
		Estimates of parameter	Standard error	
Crop diversity	*Trichoderma harzianum*	Inorganic Fert 1= -1.25	1.03	0.026
		Crop density 8= -7.7	16.2	
		Crop density 16= -0.67	1.22	
		Crop density 24= -0.262	0.946	
Crop density	*Trichoderma harzianum*	Organic Fert.1= -0.85	1.06	0.037
		Crop density 8= -7.6	16.2	
		Crop density 16= -2.66	1.24	
		Crop density 24= -1.355	0.774	

Table 8. Influence of crop density on amount of carbon in soil and occurrence of *T. harzianum.*

Crop density grouping	Forest Prediction	s.e	8 Prediction	s.e	16 Prediction	s.e	24 Prediction	s.e
C group								
< 4	0.6624	0.2123	0.0014	0.0228	0.1813	0.1510	0.4590	0.1530
4 - 5	0.6522	0.1897	0.0013	0.0218	0.1747	0.1267	0.4477	0.2157
5 - 6	0.8592	0.1026	0.0044	0.0706	0.4079	0.2909	0.7252	0.2426
> = 6	0.7194	0.1424	0.0018	0.0298	0.2244	0.2120	0.5257	0.2721

Effect of other farm management practices on soil chemical characteristics and fungal occurrence

Farmlands within the study site were cleared at different times to start the tea and coffee cultivation. The farms were as old as 18 to 55 years. The age of the farms showed a relationship with occurrence of *Trichoderma* (Table 9) and the amount of carbon (C) in the soil. The more the years of cultivation the less the probability of occurrence of the fungus. The relationship with C was significant at 0.05 level of probability. Carbon decreased with age of the farms. This relationship influenced the occurrence of *T. citrinoviride* (p=0.10). Predictions from regression model estimated that the probability of isolating *T. citrinoviride* from farms decreased with the age of the farm as carbon decreased. The probability of isolating *T. citrinoviride* from farms that have been under cultivation for 6.5 years or less increased from 52.1% to 66.6% with increase in carbon as shown in Table 10. The predictions estimate that the probability of isolating *T. citrinoviride* decreased with the age of the farm but increased with increase in carbon in farms of all ages. The same results were obtained with N, P and K.

64% of the farms were managed using soil conservation strategies. The types of soil conservation strategy in the region are bench terracing, fanya juu, cultivation on contours and strip cropping. All tea and coffee farms had conservation strategy while some maize based farms did not have. There was no influence of the type of soil conservation strategy on the occurrence of the fungus.

Pesticides and herbicides highly influenced occurrence of *T.. harzianum* and *T. stromaticum* negatively as shown in Table 6. The parameter estimate for pesticide is negative and therefore tells us that the probability of having *T. harzianum* on a particular farm decreases with pesticide application.

Trichoderma species abundance was influenced by land use intensity index (P value = 0.00< 0.05) at 5% level of significance. The intensity index for the coffee farms ranged between 0-0.3063; tea farms 0.1433-0.4083; maize based farms 0.2916-0.4942; napier farms 0.0200 and forests 0.000. Simple regression analysis showed that land intensity index measured by the cultivation intensity and frequency of application of farm inputs affected the abundance of *Trichoderma* species negatively.

Table 9. Influence on landscape management on the occurrence of *Trichoderma* species: Regression analysis table.

Landscape management	*Trichoderma species*	Estimate of parameters		Chi probability
		Estimate of landscape management	Standard Error	
Duration of cultivation	*T. koningii*	-8.4	40.3	0.018
	T. asperellum	-0.0279	0.0470	0.543
	T. citrinoviride	-0.1123	0.0566	0.015

Table 10. Influence of age of farm on amount of carbon in soil and occurrence of *Trichoderma* spp.

Cultivation group C group	18 years Prediction	s.e	20 years prediction	s.e	30 years Prediction	s.e	45 years Prediction	s.e
< 4	0.521782	0.266021	0.144885	0.169976	0.000017	0.000917	0.000014	0.000958
4 – 5	0.717327	0.271048	0.282673	0.271056	0.000041	0.002132	0.000033	0.002227
5 – 6	0.666223	30.565894	0.236615	24.828380	0.000032	0.004704	0.000026	0.003079
> = 6	0.666223	30.565894	0.000032	0.004704	0.236615	24.828380	0.000026	0.003079

DISCUSSION

More isolates of *Trichoderma* were recovered using the soil dilution technique compared with the soil washing technique. This implies that most of the *Trichoderma* propagules were spores which were washed out in the soil washing technique (Klein and Eveleigh, 1998). This method aims at sieving out the spores of the many fungi in the soil and to end up with actively growing mycelium. Hence for *Trichoderma* isolation, the dilution plating technique remains adequate, agreeing with the findings of Klein and Eveleigh, 1998.

Land use type and soil management practices influenced the distribution and abundance of *Trichoderma* species. Though *Trichoderma* species was isolated throughout the study site, the diversity and abundance varied. The plant type played a major role in determining the occurrence of the fungus. This agrees with the findings of Ibekwe *et al.,* (2002) and Grayston *et al.* (1998). Plants contribute to litter formation and therefore affect soil nutrients, Loranger – Maerciris *et al.,* 2006. Further the plant type determines the rooting system and *Trichoderma* species are known to be favored by the presence of high levels of plant roots which they colonize readily. Some strains of *T harzianum* and *T. viride* are known to be strongly rhizosphere competent. They grow and proliferate best when there are abundant healthy roots (Heljord and Tronsmo, 1998; Yedida, 1999) as they are also attracted by root exudates. This could explain why the fungus was abundant in napier farms and not in coffee and tea farms whose trees are deeply rooted. The age of the plants also affected the distribution and abundance of the fungus. Coffee and tea farms that were maintained for up to fifty years and above showed low populations of the fungus compared to annuals or intercropped (with annuals) perennials. Garland (1996) and Picard *et al.,* (2000) also reported that the composition of root exudates is strongly affected by plant development stage which in turn affect rhizosphere communities over time. He showed that young roots and root tips represented excellent niches suitable for colonization. It is evident from the results above that annuals encouraged the growth of *Trichoderma* as well as intercropped perennials compared to monocropping system.

The use of herbicides and pesticides had a negative influence on the occurrence and diversity of the fungus. This was in line with the findings of Heilmann, *et al.* (1995). Amendment of soils with manure, however, had a positive effect on the fungus.

The soil key nutrients, C, N, P, and K varied across the land types and influenced the distribution and abundance of *Trichoderma*. These were determined by the land cover and management practices. The forests and napier farms favored the population of *Trichoderma* probably due to the higher pH, N, C and P in these sites. The most limiting soil factors were P and the low pH. Nitrogen, C and K, were within the acceptable range for soils (Wokabi, 1995). Phosphorus limitation may also explain the variation in the occurrence of the fungus. Coffee farms recorded the poorest population of the fungus and also the lowest level of P (average of 12ppm, but most farms recorded less than 7ppm). Low level of P could be attributed to low level of soil pH (< 4.5 for all land uses), resulting into conversion of phosphate ions into insoluble forms. The natural forest had the highest level of P (average

of 21ppm). Napier farms recorded an average of 13ppm. The coffee farms also recorded the lowest level of N and C (second last to tea). The exclusive use of organic fertilizers in the Napier farms could have had a positive effect of improving soil quality by increasing soil concentration of the nutrients P, N, C, and increasing soil pH and in effect favouring the population of *Trichoderma.* Napier and tea soils were notable extremely acid though the former recorded high population of the fungus and the later, very low. This suggests that *Trichoderma* is tolerant to acidity.

Land intensification had a negative effect on the fungal occurrence. This was a measure of the cultivation intensify and frequency of application of farm inputs. Land in intersification was lowest in napier farms followed by the forests yet the abundance of *Trichoderma* was great in napier farms followed by the maize based farms. These results indicate that apart from land intensification, other factors also contributed to the abundance of the fungus confirming the discussion above that plant type influence the distribution and abundance of the fungus. Napier is a perennial plant with low tillage activity compared with maize which is an annual crop. The high abundance of *Trichoderma* in these two farming systems suggests the fungus can survive in tilled soils as reported by Kundsen *et al.,* (1995) and Luque *et al.,* (2005). Gomez *et al.,* 2006 observed high proportions of *Trichoderma* in both conventionally cropped and no-till cropping systems.

The frequency of application of farm inputs, which was the other factor used in calculation of land intensification, was low in napier and maize based farms and zero in the forests. This furather confirms that application of inorganic fertilizers, which was high in tea and coffee farms did not improve abundance of the fungus. The napier and maize based farms exclusively had inputs of organic manure which from the results can be attributed to the abundance of the fungus. These findings agree with those of Gomez *et al.,* who stated that agricultural intensification may result in deterioration of soil quality, affecting soil productivity. Roper and Gupta (1995) and Toresani *et al.,* (1998) also reported that management practices can have a significant implication on the composition of soil biota.

CONCLUSION

Long term monocropping and land intensification in terms of frequent application of inorganic fertilizers and herbicides greatly affects the population of *Trichoderma* spp. The use of organic soil amendments results in a soil that has higher amounts of the fungus. Land use system and type of fertilizer used influenced the distribution and abundance of *Trichoderma*. Populations of this fungus in soil with a history of organic production practices were higher than in soils under conventional production practices. Less disturbed soils of the forests also recorded high carbon levels which together favored the occurrence of the fungus. *Trichoderma* also seemed to favor certain plant types and not others. Above ground plant type influenced the occurrence of the fungus with annual plants being favored to monocropped old plants, indicating that the age of the plant cover also determined the belowground populations of *Trichoderma* spp.

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