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### **Distribution and Diversity of Indigenous** Trichoderma species in Machakos County, Kenya

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### Authors' contributions

This work was carried out in collaboration between all authors. Author PKM carried out the experiments, performed the statistical analysis, and wrote the first draft of the manuscript and managed literature searches. Authors PMW, SAO and JWK designed the study, managed the analyses of the study and literature searches and proofread the final draft. All authors read and approved the final manuscript.

#### Article Information

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### **ABSTRACT**

Aim: This study was undertaken in order to determine the effect of land-use intensification on occurrence, distribution, and diversity of *Trichoderma* fungus.

Study Design: Cross-sectional study.

Place and Distribution of Study: Mycology Laboratory, University of Nairobi between March and

September, 2014.

Methodology: Soil samples were collected from both Mwala and Kauti irrigation blocks in Kabaa irrigation scheme of Machakos County, in Kenya under three land use types (LUTs): intensively cultivated farmlands under irrigation, rainfed intensively cultivated farmlands and undisturbed lands. A total of 100 soil samples were obtained from the top 0-20 cm depth. Trichoderma species were isolated using the dilution plate technique using Trichoderma-selective media (TSM).

Results: A total of 369 Trichoderma isolates were recovered from the three LUTs. These were identified and classified into eleven species. The species identified were: T. harzianum, T. koningii,

T. viride, T. asperellum, T. atroviride, T. spirale, T. virens, T. tomentosum, T. brevicompactum, T. crassum and T. hamatum. The most abundant Trichoderma species was T. harzianum with a frequency of isolation of 38.87%, followed by T. koningii and T. viride at 18.03 and 15.49%, respectively. Trichoderma hamatum had the least isolation frequency at 0.41%. T. harzianum also had the widest distribution. The difference in abundance of Trichoderma in the three LUTs was significant (P=0.05). The undisturbed lands had a higher abundance of Trichoderma compared to the disturbed areas. Mwala irrigation block A had the least abundance while block D which is more recent in cultivation had highest mean abundance of Trichoderma. Difference in Trichoderma species mean richness between LUTs was not significant (P=0.203). Undisturbed lands had the highest richness. Undisturbed lands also had the highest diversity while irrigated lands were the least diverse

**Conclusion:** Enhanced land-use intensification lowers the abundance and diversity of *Trichoderma* in the soil.

Keywords: Trichoderma; biodiversity; biocontrol; fungi; pathogen; land-use.

### 1. INTRODUCTION

A rapid increase in human population in Kenya, coupled with shortage of arable land has posed a great challenge on food security [1]. The situation is made even worse by unpredictable weather patterns, declining soil fertility, upsurge in prevalence of crop pest and pathogens [2,3]. Since the arable land remains inelastic, there is enhanced intensification agricultural production in order to mitigate the food insecurity situation. Agricultural intensification has many negative implications. Farmers result in increased use of chemical inputs that not only increases the cost of production but also leads to environmental contamination.

Soil is a dynamic, living, natural body that is vital to the function of terrestrial ecosystems [4]. The current increased intensification affects the soil as it leads to loss of soil biodiversity and this affects the status of soil health which is critical to agriculture [5]. sustainable According Nannipieri et al. [6], higher soil biodiversity is an indication of the status of its health. Therefore, soil microbial populations may be used as indicators of soil health since an ecological balance between soil borne pathogens and organisms with biocontrol potential naturally suppresses the incidence of diseases [7]. One of the cost effective and eco-friendly way for higher agricultural productivity is maintenance of a higher soil biodiversity. Trichoderma, a soil fungus, is one of the components of soil biodiversity that has capacity of maintaining soil health. Members of this genus like T. harzianum, T. atroviride, T. atroviridis, T. asperellum and T. virens are antagonists against important soil borne crop pathogens. This fungus is cosmopolitan, colonising a wide range of soil niches from cool temperate to tropical climates [8].

Members are commonly present in the soil's top horizons [9]. Other *Trichoderma* species have been recovered in extreme environments like mangrove swamps, salt marshes and estuarine. Okoth et al. [10] carried out a study in Kenya in two bench mark sites with significant land use intensification and being biodiversity hot spots. They reported that the most frequently isolated species was *T. harzianum* while other species had a restricted distribution due to difference in ecological parameters and type of crop grown. Previous studies have revealed that *Trichoderma* species can be influenced by soil temperature [11,12,13] soil moisture [14], soil pH range [15], salinity [13,16], among other soil factors.

Trichoderma species have been gaining importance as a potential and effective biocontrol agent and also as a plant health promoter due to their fascinating mechanisms like the production of antifungal metabolites, competition for space and nutrients, mycoparasitism, induction of defense responses and promotion of salinity and drought tolerance [17,18]. Several reports indicate that Trichoderma species can effectively suppress crop pathogens [19]. Several studies in Kenya have demonstrated ability of *Trichoderma* isolates to manage some crop diseases [20,21,22]. Harman et al. [23] have shown that the genus Trichoderma can induce localised or systematic resistance to diseases through the release of metabolites that tends to promote plant production of ethylene or terpenoid phytoalexins.

Studies have confirmed that agricultural intensification has effects on soil *Trichoderma* biodiversity [24,25,26]. As intensification

becomes a real phenomenon in order to feed the growing population, its impact on soil biodiversity need to be assessed. According to Okoth et al., [10], land-use types have an effect on abundance. richness and diversity Trichoderma. Information on the occurrence and diversity of indigenous Trichoderma species in Kenya under intensively cultivated lands is scanty. There is need to assess the effect of land-use intensification on the occurrence and diversity of this soil fungus. Quantification of Trichoderma in the soil would assist in estimating the capacity of soil in controlling soil-borne fungal pathogens. This study therefore aims at evaluating the effect of land-use intensification on Trichoderma occurrence and diversity. This will be achieved by comparing Trichoderma biodiversity in intensively cultivated lands with those from undisturbed lands.

### 2. MATERIALS AND METHODS

# 2.1 The Study Area and Soil Sample Collection

Soils were obtained from Kabaa irrigation scheme, in Mwala Sub-county of Machakos County, Kenya, This county experiences arid and semi-arid type of climate. The temperatures vary from 6 to 29°C. The study area was selected due to variation of land use which included high intensively cultivated land and also availability of rainfed land use and adjacent undisturbed land. The main crops grown under irrigation include French bean, maize, tomatoes, common bean and kales. Under rain-fed agriculture, diverse crops are grown including maize, common beans, sorghum, cassava, kales, millet among other crops. The undisturbed lands had grass as the main vegetation type. The study was replicated in two regions within the county; which were Mwala and Kauti irrigation schemes. Mwala irrigation scheme is located in Lower Midland zone 4, majorly occupied with cambisol soils. Mwala irrigation scheme is divided in to four blocks; A to D based on their history of cultivation; with block A having a longest period of cultivation as it was opened in 2006, followed by block B opened in 2009, block C opened in 2010 and block D opened in 2011. Kauti irrigation scheme lies in the Upper midland zone 4 and is characterized with Alfisol and acrisol soils. The scheme is divided into two main blocks based on elevation, the upper and the lower blocks.

In each of the two regions, soils were sampled from the three land use types (LUTs): intensive agricultural lands under irrigation, rainfed intensive agricultural lands and undisturbed lands. From each sampling point, 12 soil subsamples were obtained as described by Okoth et al. [10]. Two circles measuring 3 m and 6 m radii were drawn and 4 and 8 soil samples were augured at 0-20 cm depths, respectively (Fig. 1).

The soil samples were mixed thoroughly into a single composite sample and a 100 g subsample taken for *Trichoderma* isolation. The soil auger was sterilised for each sampling point to avoid cross contamination. A total of 100 samples were collected from the two regions, in the three LUTs. These samples were transported to in paper bags to mycology laboratory in University of Nairobi (Chiromo campus) where they were kept at 5°C awaiting further analysis.

# 2.2 Trichoderma Isolation and Identification

Soil dilution plate technique was used to isolate Trichoderma strains from the soil using Trichoderma Selective Medium (TSM) [27]. Plating was done from the second and third dilution in three replicates for each dilution. The plates were then incubated in the dark at 20°C for 10 d to allow fungal growth [11]. Trichoderma colonies were then sub-cultured onto a half strength Potato Dextrose Agar (PDA) plates amended with chloramphenicol (½ PDA+c). Single spore cultures were plated on ½ PDA+c and incubated at 20°C for 14 day at 15, 25, 30 and 35°C. For the first 7 days, the Trichoderma colonies were incubated in the dark then under 24 h blue light to encourage sporulation [27]. The isolates were identified to species level following the taxonomic keys of Samuels et al., [28] and Gams and Bissett, [29]. Colony characters, growth rates in culture and morphological characters were used 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.

#### 2.3 Statistical Analysis

The data obtained was subjected to analysis of variance (ANOVA) using statistical software GenStatver. 9.0 (VSN International Ltd). Trichoderma mean abundance, mean richness and Shannon index will be calculated as described by Tuomisto [30]. Significance was evaluated at P < 0.05 for all analyses and means separation accomplished by Fisher's protected least significant difference (LSD) test.

### 3. RESULTS AND DISCUSSION

# 3.1 Isolation of *Trichoderma* Species across the Various Land Uses

From the 100 soil sampling points across the different land use types, a total of 369 Trichoderma isolates were recovered. isolates were grouped into 11 Trichoderma species. The species include; T. harzianum, T. koningii, T. viride, T. asperellum, T. atroviride, T. spirale. T. virens. T. tomentosum. brevicompactum, T. crassum and T. hamatum. The most abundant Trichoderma species was T. harzianum with a frequency of isolation of 38.87% (Table 1). This species was followed by T. koningii and T. viride at 18.03 and 15.49%, respectively. Trichoderma hamatum had the least isolation frequency at 0.41% and was only isolated form Mwala rainfed farmlands (Table 1). Among the Trichoderma species, T. harzianum had the widest distribution with representatives from all the LUTs under study. Some species were isolated from only one land use type; T. virens (Kauti undisturbed), T. brevicompactum (Kauti irrigated), T. crassum (Mwala undisturbed) and T. hamatum (Mwala rainfed).

Trichoderma asperellum, T. virens and T. crassum were only recovered from undisturbed

farmlands while *T. tomentosum* was isolated from rainfed farmlands only. *Trichoderma tomentosum* was isolated from rainfed lands only while *T. brevicompactum* was only isolated from irrigated areas of Kauti. Isolates of *T. crassum* and *T. hamatum* were restricted to Mwala region, being isolated from undisturbed and rainfed lands, respectively.

The occurrence of *Trichoderma* varied negatively with age of cultivation. The lands with longer history of cultivation had lower abundance of this fungus. This was reflected in Mwala irrigation blocks, where block D which has a shorter history of cultivation having the highest isolation frequency at 54.17% while block A having the least isolation frequency at 4.16% (Fig. 2). From this block, only T. harzianum could be isolated while from block D, three species were isolated. namely T. harzianum, T. viride and T. spirale (Table 2). The isolation of individual species also varied with age of cultivation. Although T. harzianum was the most isolated species within these blocks at 58.34%, it was more abundant in block D (Table 2). Trichoderma viride and T. spirale which were the second and third most abundant species, at 33.33 and 8.33%, respectively, were only isolated from block D (Table 2) which had the shortest history of cultivation.

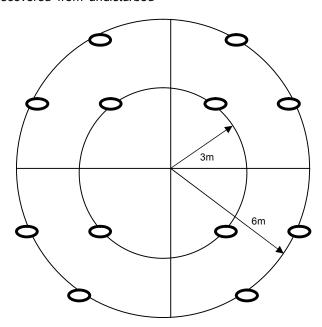


Fig. 1. Schematic representation of the twelve soil sampling points which comprised one main sampling point of the 100 sampling points

Table 1. Frequency of isolation of *Trichoderma* species from the three land-use types in the two regions

Trichoderma spp	Proportion (%) per land use type							
	Mwala irrigated	Mwala rainfed	Mwala undisturbed	Kauti irrigated	Kauti rainfed	Kauti undisturbed	overall%	
T. harzianum	58.34	28.57	40.78	33.33	60.61	37	38.87	
T. koningii	0	0	24.8	39.4	0	15.25	18.03	
T. viride	33.33	0	18.65	0	0	15.01	15.49	
T. asperellum	0	0	5.53	0	0	11.35	8.43	
T. atroviride	0	34.29	1.64	0	21.21	9.93	7.61	
T. spirale	8.33	0	6.97	0	0	5.08	5.41	
T. virens	0	0	0	0	0	6.38	3.7	
T. tomentosum	0	7	0	0	18.18	0	0.89	
T. brevicompactum	0	0	0	27.27	0	0	0.61	
T. crassum	0	0	1.64	0	0	0	0.55	
T. hamatum	0	17.14	0	0	0	0	0.41	
Frequency of isolation (%)	1.65	2.4	33.45	2.26	2.26	57.98	100%	

Table 2. Frequency of isolation of *Trichoderma* species in Mwala irrigation blocks A to D

Trichoderma spp	% of isolation in the irrigation blocks						
	A	В	С	D	Overall %		
T. harzianum	100	33.33	50	69.24	58.34		
T. koningii	0	0	0	0	0		
T. viride	0	66.37	50	15.38	33.33		
T. asperellum	0	0	0	0	0		
T. atroviride	0	0	0	0	0		
T. spirale	0	0	0	15.38	8.33		
T. virens	0	0	0	0	0		
T .tomentosum	0	0	0	0	0		
T. brevicompactum	0	0	0	0	0		
T. crassum	0	0	0	0	0		
T. hamatum	0	0	0	0	0		

The results revealed that the abundance of *Trichoderma* species was affected by the land use type. The abundance of this fungus for different LUT was significantly different (*P*<0.01). The undisturbed lands had a higher abundance of *Trichoderma* species than the intensively cultivated lands (Fig. 3). These results were true for the two regions under study (Figs. 4 and 5). However, in both regions, the rainfed farmlands had a higher mean abundance than the irrigated lands.

The difference in Trichoderma species abundance between irrigation blocks in Mwala was not significant (P=0.418). However, block D had highest mean abundance of Trichoderma followed by block B (Fig. 3). Block D has a recent history of cultivation compared to the others as it was opened in 2011. Block A, which has the longest history of cultivation since 2006, had the least mean abundance of Trichoderma species (Fig. 6). Difference in Trichoderma species richness between LUTs was not significant (P=0.203). However, undisturbed lands had the highest mean richness. The irrigated farmlands had the least mean richness of Trichoderma (Fig. 7). This trend was replicated in the two regions under study.

The difference in *Trichoderma* species richness among Mwala blocks was not significant (P=0.285). However block D had the highest mean richness while A had the least (Fig. 8). This study further revealed that undisturbed

lands had the highest *Trichoderma* species diversity (Fig. 9). The irrigated farmlands had the least mean Shannon index. However within Kauti region, rainfed lands were the least diverse. Within Mwala irrigation blocks, block D had the highest diversity, followed by C. Block A was the least diverse (Fig. 9).

Among the Mwala irrigation blocks, block D had the highest mean diversity followed by block C while block A had the least diversity (Fig. 10).

This study has revealed that Trichoderma is widely distributed in the study area. In a previous study on occurrence of *Trichoderma* from different land-use types by Okoth et al. [10], 9 and 10 species were isolated from Embu and Taita regions of Kenya, respectively. It has also been reported that members of this genus are cosmopolitan and occur in diverse ecosystems and climatic regions [31]. The frequent occurrence of Trichoderma species in the soils under different crop species is in accordance with the fact that species of Trichoderma are an abundant and biologically important component of soil micro-flora, though differences in their abundance can occur [28]. The occurrence of Trichoderma species is modulated by several factors which include microclimates, substrate availability, as well as complex ecological interactions [32]. Survival ability of Trichoderma in diverse geographical areas can be attributed to their high reproductive capacity, competitive capability and diverse metabolism [33].

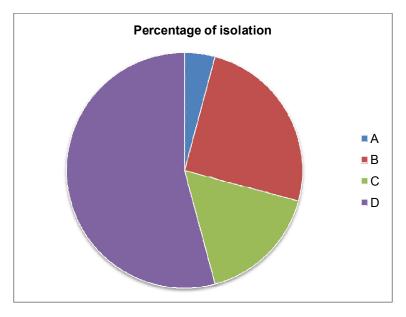


Fig. 2. Frequency of isolation of Trichoderma species in Mwala irrigation blocks A to D

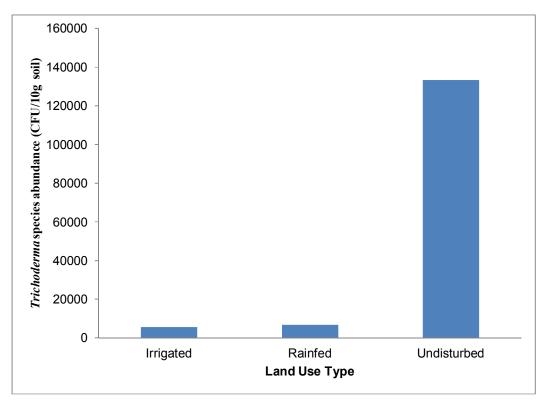


Fig. 3. Trichoderma species mean abundance for the three land-use types

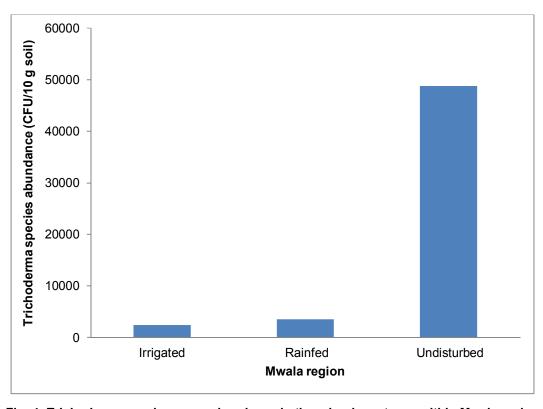


Fig. 4. Trichoderma species mean abundance in three land-use types within Mwala region

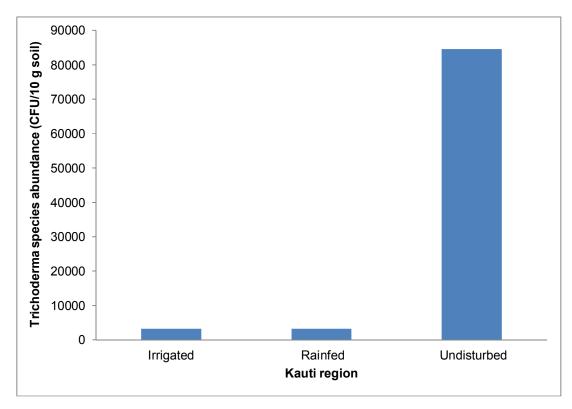


Fig. 5. Trichoderma species mean abundance in the three land-use types within Kauti region

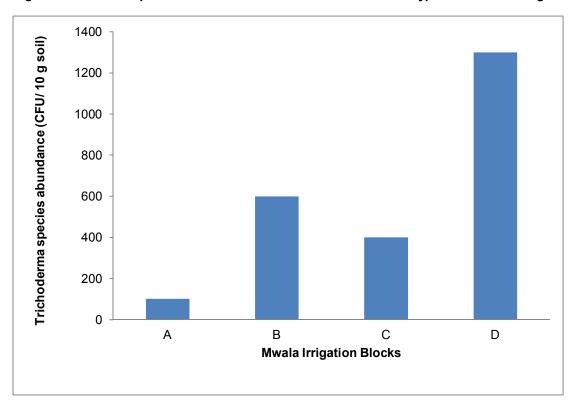


Fig. 6. Trichoderma species mean abundance in the four irrigation blocks in Mwala region

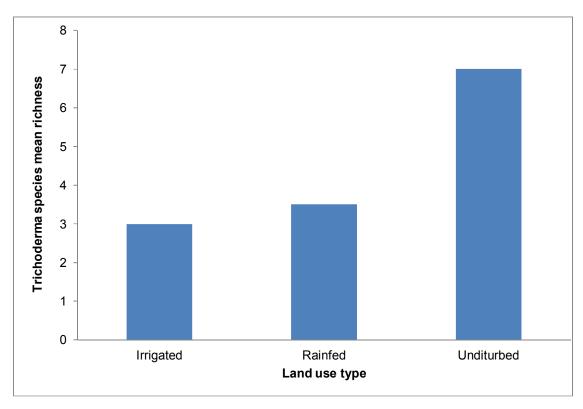


Fig. 7. Trichoderma species richness for the three land use types.

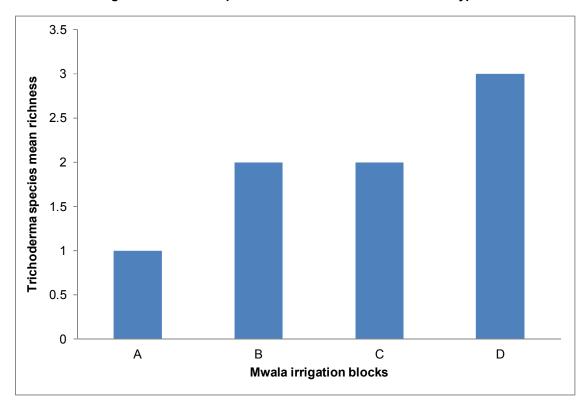


Fig. 8. Trichoderma species richness within Mwala irrigation blocks

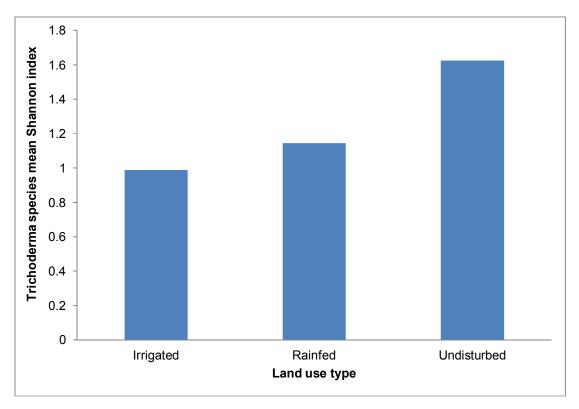


Fig. 9. The diversity of Trichoderma species in the three land-use types

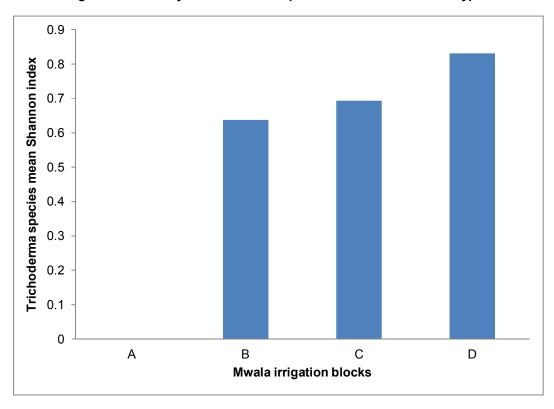


Fig. 10. Trichoderma species mean Shannon indices for the four Mwala irrigation blocks

Results of this study revealed that T. harzianum was the mostly frequently isolated species with a frequency of 38.87% (Table 1). This showed that it was the most dominant in the region and was therefore isolated from all the land use types. Trichoderma harzianum species is known to be strongly rhizosphere competent and proliferates best in soils rich in organic matter and plant root exudates. This may explain why this fungus is abundant in soils in this study. In agreement to this study, Okoth et al. [10], reported that T. harzianum was the most abundant species in two ecologically different regions in Kenya. In a similar study covering Russia, Siberia and the Himalayas, Kullnig, et al. [34] isolated T. harzianum, T. asperellum, T. atroviride, T. ghanense, T. hamatum, T. koningii, oblongispirum, and T. virens, with T. harzianum. being the most prevalent species. Sun et al. [26] also reported that T. harzianum was the most prevalent species in China soils. A study by Jaklitsch [35] covering 14 European countries in temperate climate detected 75 species with T. harzianum being the most abundant.

However, these results do not agree with those reported by Devi et al. [36] who isolated Trichoderma strains from various points in India subcontinent. Out of the 70 isolates they obtained, the most abundant species was T. Longibrachiatum. However, T. harzianum, T. asperellum and T. virens were also isolated similar to this study. Similarly, Attitalla et al. [37] isolated T. harzianum from only five of the 23 soil samples examined from Al-Jabal AL-Akhdar region of Libya. This could be attributed to geographical and climatic differences. Sun et al. [26] reported the effect of temperature on Trichoderma distribution with some species only occurring in cool regions. For example, T. polysporum and T. viridescens were restricted to cooler regions while T. brevicompactum, T. erinaceum and T. ghanense were prevalent in hot areas. Similarly, in this study, T. virens, T. brevicompactum T. crassum and T. hamatum had a more restricted distribution and were only isolated from only one land-use type. This could be attributed, probably to their low rhizosphere competence or other unfavourable soil factors. Okoth et al. [10] reported that although T. harzianum had the widest distribution, T. reesei, T. asperellum, and T. polysporum were only isolated from Taita, a region of relatively elevated temperatures. Trichoderma citrinoviride and T. aggressivum were only isolated from Embu, a region of lower temperature.

This study revealed that disturbance has significant impact on abundance of Trichoderma species. The undisturbed lands had the highest abundance, richness and diversity Trichoderma. These results were evident in the regions of Mwala and Kauti. This could be attributed to the fact that less disturbed soils are naturally balanced to support a higher Trichoderma diversity. Undisturbed lands are characterised by a high floristic diversity, which could have influenced the greater Trichoderma diversity. In this study, it seems that some agricultural practices carried out by farmers could have had a negative impact on *Trichoderma* in the soil. Most farmers in the study area grow annual crops. This could have contributed to reduced organic matter in the soil as they harvest and take away the crop plants from the fields. These results are similar to those of Okoth et al. [10] who reported a higher occurrence of Trichoderma in the less disturbed soils. In contrast, however, Sun et al. [26] reported a higher diversity in vegetable soils in China than the pasture soils probably due to cropping system adopted that would favour a higher abundance and diversity of Trichoderma.

Bourguignon, [38] demonstrated that different crops and cropping regimes affect Trichoderma populations. Some crops grown by farmers could also be producing root exudates with antifungal activity and hence contributing to low agricultural Trichoderma populations in farmlands. Celar, [39] demonstrated that onions could cause decrease in T. harzianum, T. viride and T. longibrachiatum. Irrigaon may also reduce Trichoderma populations in the soils. According to Eastern and Butler, [40] extreme soil water content have a detrimental effect on the hyphal growth, spore production and germination of Trichoderma. The effect of use of inorganic fertilizers could have contributed probably to rise in pH and hence negatively affecting Trichoderma populations. Similarly, tillage is known to affect the soil physical and chemical environment in which the soil organisms live, thereby affecting soil Trichoderma in different Janusauskaite et al. [41] demonstrated that tillage intensity results in lower organic carbon and nitrogen and lowers soil quality, factors that would lower Trichoderma occurrence and diversity. According to Torensan et al. [42], soil management practices like tillage, fertilizer application, addition of organic manure, application of pesticides and irrigation may greatly negatively affect soil populations. Muniappan and Muthukumar [43]

also reported that soil pH negatively influenced relative abundance of *T. koningii* while soil phosphorous affected it positively.

In spite of their coexistence in many soils, populations of some of the Trichoderma species were negatively related to each other. This suggests that these soil fungi compete in the soil environment or the fungi may be reacting variedly to the given environmental conditions. The first view is supported by the fact that strains of T. viride are known to be highly competitive and are known to proliferate best in the presence of healthy plant roots, whereas, T. koningii tends to occur in diverse soil conditions [28,44]. The second view is supported by the fact that soil pH has been thought to be of great importance to the activity and occurrence of Trichoderma species [23,45,46]. Kredics et al. [45] showed that species of Trichoderma grow optimally around pH 4.0 to 5.0, and exhibit little or no growth below pH 2.0 or above pH 6.0. This clearly shows that even the taxa within a genus respond differently to the environmental factors. Eastburn and Butler [47] also reported that soil pH had significant influence on the distribution of T. harzianum in a wide pH range of 6.2 to 7.9. Simon et al. [48] reported that application of ammonia to the soil can increase the populations of Trichoderma species.

The intensity of land use was found to have an effect on Trichoderma occurrence, richness and diversity. Wu et al. [49] reported that agricultural practices alter mycofloral diversity. Further, according to Lehman et al. [4], human disturbances, land management, cultivation, climatic factors, elevation, agriculture and environmental variability modify the diversity and ecology of microfungi. Though there may be important abiotic or biotic factors, which were not accounted for in this study, the establishment and wide-spread occurrence of Trichoderma species, appears to be more directly determined by the environment and anthropogenic activities. A similar conclusion was reached by Geisseler and Scow, [50], Attitalla et al. [37]; Okoth et al. [10]; and Sariah et al. [51]. They found that the environment had the greatest effect on the densities of several species of Trichoderma. Other factors of primary importance are those involving competition, antagonism and synergism among soil microorganisms.

The results further revealed that the history of cultivation had an effect on *Trichoderma* abundance, richness and diversity. Within Mwala

irrigation blocks, block A have been in cultivation since 2006 had lower abundance, richness and diversity while a more recent block D (opened in 2011) had the highest abundance, richness and diversity of Trichoderma (Fig. 2). Long history of cultivation may have led to changes in soil organic matter levels and addition of synthetic chemicals. Studies have shown that changes in Trichoderma populations tend to occur in response to temporary changes in soil moisture, soil organic matter, soil temperature and nutrient availability [30,46]. Tillage represents disturbance of the soil habitat and can mechanically disrupt filamentous organisms such as Trichoderma, decrease soil structure, and alter water and nutrient content and distribution [52]. Plant species also exerts a selective pressure on soil fungal communities [43,53,54]. This could account for the difference in abundance. richness and diversity Trichoderma between the farmlands with different histories of cultivation. The results indicate that, as period of cultivation increases, there is likelihood of losing important soil fungi such as Trichoderma. This has subsequent negative consequences as loss of inherent capacity of soil to maintain soil health is lost. According to Lehman et al. [4], activities associated with intensive agriculture such as aggressive soil tillage, annual cultivation, application of broad spectrum agrochemicals and excessive crop residue removal may continue to decrease soils inherent capacity to maintain health.

### 4. CONCLUSION

The results of this study demonstrate presence of *Trichoderma* within different land-use types with varying abundance, richness and diversity. The undisturbed lands, which represent low level of intensification, had the highest abundance, richness and diversity while irrigated intensively cultivated farmlands had the least of these parameters. This study clearly showed that soil disturbance and crop species influences the population structure of the soil *Trichoderma*.

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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