Advanced Journal of Microbiology Research Vol. 2010
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Antagonism of *Trichoderma farzianum* isolates on soil borne plant pathogenic fungi from Embu District, Kenya

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Accepted 22 January, 2010

Species in the genus *Trichoderma* are important as commercial source of several enzymes and as biofungicides/growth promoters. The most common biological control agents of the genus are strains of *T. harzianum*, *T. viride* and *T. viriens.* In this study, sixteen selected isolates of *T. harzianum* from different land use types in Embu, Kenya were tested for anatognism against five soil borne phytopathogenic fungi (*Rhizoctonia solani*, *Pythium sp, Fusarium graminearum, F. oxysporum f. sp phaseoli* and *F. oxysporum f. sp Lycopersici*) using dual culture assay and through production of non-volatile inhibitors. Seven isolates were further characterized using RAPD-PCR procedure to determine genetic variability. All *T. harzianum* isolates had considerable antagonistic effect on mycelial growth of the pathogens in dual cultures compared to the controls. Maximum inhibitions occurred in *Pythium sp*-055E interactions (73%).The culture filtrates obtained from Czapek's liquid medium reduced the dry weight (mg) of the mycelia significantly while those from the potato dextrose broth showed minimum inhibition growth. *Pythium sp* was inhibited the most compared to other pathogens. Genetic similarities generated using Jacquard's coefficient of similarity ranged from 0.231 between isolates 055E and 011E to 0.857 between isolates 010E and 015E. The technique of RAPD was efficient in demonstrating the DNA polymorphism in the isolates of *T. harzianum* tested showing intraspecific genetic variability. Since all *T. harzianum* isolates evaluated were effective in controlling colony growth of the soil borne pathogens both in dual cultures and in culture filtrates they could be tried as a broad spectrum biological control agent in the green house and under field conditions.

Key words: *Trichoderma harzianum,* growth antagonism, genetic similarity, RAPDs.

INTRODUCTION

Modern agriculture, based on growing one or a few crop cultivars over large areas, is an ecologically unbalanced system, which invites disease epidemics. Prevention of such epidemics has traditionally been achieved through use of chemical fungicides such as methyl bromide (Hermosa et al., 2000). Toxicological environmental and sociological concerns have lead to drastic reduction in the availability of efficient commercial compounds and also the use of fungicides may lead to the appearance of new resistant strains of pathogens (Behzad et al., 2008). Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Shalini and Kotasthane, 2007; Eziashi et al., 2007). *Trichoderma* species have been known since the 1930s to show antifungal activity and there have been extensive efforts to use them for plant disease control since then (Hjeljord and Tronsmo, 1998). They have been used as biological control agents (BCAs) and their isolates have become commercially available of late (Freeman et al., 2004).

In one of the earliest studies Weindling (1932) observed that a strain that he believed to be *Trichoderma lignorum* was antagonistic to other fungi. *T. harzianum* is one efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi (Shalini et al., 2006). *T. harzianum* alone or in combination with other *Trichoderma* species or chemical adjuvant, has been used in control of several diseases

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such as *Rhizoctonia* damping-off in radish, corn and soybean (Lifshitz et al., 1985) and grey - mould on tomato (Migheli et al., 1994). Different mechanisms have been suggested as being responsible for their bio-control activity which include mycoparasitism, antibiosis, competition for nutrients and space and secretion of chitinolytic enzymes (Harman, 2000).

Since the distinguishing morphological characteristics of a fungus, the physiological and biochemical technique applied are frequently too limited to allow its identification, molecular methods have recently been introduced into *Trichoderma* taxonomy.

Random Amplified Polymorphic DNA (RAPD) is an electrophoretic method used for taxonomy at the species level to discriminate species (Hadrys et al., 1992). RAPD is a DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence (Williams et al., 1990; Welsh and McClelland, 1990).

Random markers as products of the PCR-RAPD technique (Williams et al., 1990) have been developed to differentiate numerous fungi, including *Trichoderma* species (Zimand et al., 1994). RAPD analysis has been used in many applications and various organisms, especially in the plant sciences (Kapteyn and Simion, 2002). RAPD fingerprinting was used by Arisan-Atac et al. (1995) to identify subgroups of *Trichoderma* capable of chestnut blight biocontrol; they found that the biocontrol activity was spread among several distinct groups and subgroups of *Trichoderma*. Using RAPD Dubey and Suresh (2006) analyzed strains of *Trichoderma*, verifying that with RAPD it was possible to distinguish among the isolates of *T. harzianum* and *T. viride* into three distinct groups.

MATERIAL AND METHODS

Isolation and identification of Trichoderma species

Trich*oderma* sp were isolated using dilution plate technique (Johnson et al., 1959) and soil washing methods (Gams et al., 1987) on malt extract agar (MEA) and cornmeal agar (CMA) with 2% dextrose) both with streptomycin 50 mg/L and cyclcosporin 10 mg/L. The colonies were counted and identified and transferred to Petri dishes containing potato dextrose agar (PDA) and incubated at 15, 25, 30 and 35°C for further identification to species level. Genus identification of green fungus was undertaken using the method of Domsch et al. (1980). *Trichoderma* isolates were identified to species level following the taxonomic key of Samuels et al. (2004). 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. The isolates were preserved in sterile soil and stored at 4°C.

Isolation of phytopathogenic fungi

Diseased plant tissues were obtained from the University farm at Kabete campus 10 Km north-west of Nairobi. *Rhizoctonia solani* was isolated from Spinach (Spinacea oleracea), *Fusarium oxysporum f.* sp *Lycopersici from* tomato (*Lycopersicum esculetum*), *F. oxysporum f.* sp *phaseoli* from beans (*Phaseolus vulgaris*) and *F. graminearum from* maize cob (*Zea mays*). Diseased plant tissues were washed under running tap water to remove surface soil, dust and other contaminants. Tissue pieces were cut out from the leading edge of lesion, and placed in 1% sodium hypochlorite for five minutes, washed briefly in sterile distilled water and dried on sterile filter paper. The dried pieces were cut into smaller pieces, plated onto PDA and incubated at 25°C. *Pythium* sp *was* obtained by baiting using beet root seedlings.

Dual culture tests

Plates of PDA were inoculated with a 5 mm disc from five-day-old cultures of the phytopathogens 10 mm from the edge of the plate. After two days a 5 mm disc of the *Trichoderma harzianum* isolate was placed 55 mm from the phytopathogens disc. *Pythium sp* and the biocontrol strains were inoculated at the same time. Paired cultures were incubated at room temperature for six days. The growth of the fungi was recorded by measuring the radial growth of the pathogens.

The percentage growths of the pathogens were calculated as follows: % Growth = Radius of the growth in the direction of the test strain/radius of the growth in the absence of the test strain x100 (Behzad et al, 2008).

Slide culture method

For *Rhizoctonia solani* and *Pythium sp -Trichoderma* interaction, a clean sterile glass slide was placed in 9 cm diameter plates. A small amount of autoclaved melted PDA was spread over the slide to make a thin film on the slide. 5 mm discs of one week old growing colonies cut from the margin of each pathogen and *Trichoderma* isolates were placed on the opposite sides of the slide 3 cm apart on the PDA surface.

A few ml of double distilled water was added to the plate to prevent drying and incubated 25 ± 1°C for a week. At the end of incubation period, meeting area of *Trichoderma* - Pathogen hyphae was stained with lactophenol in cotton blue and observed under a light microscope for the presence of mycelial penetration and for cell wall disintegration.

Determination of antifungal properties of *T. harzianum* culture filtrates

To harvest non-volatile antibiotics produced by *Trichoderma* isolates, the fungus was grown on potato dextrose broth (PDB) and Czapek's liquid medium (CLM) as described below.

Isolates cultured in potato dextrose broth

Each *T. harzianum* isolate was separately inoculated into 100ml PDB incubated at 20°C and Cultures filtered through 0.22 mm Millipore filters after 10 days. Two milliliter aliquots of these filtrates were placed in sterile Petri dishes and 25ml of PDA added. 5mm wide mycelial discs of the pathogen were placed at the center of the solidified agar plates and incubated at room temperature for 6 days. Colony diameters were measured daily and inhibition percentage obtained using the formula: I% = [(C2-C1/C2) X100] (Behzad et al, 2008).Where: C1 means growth of the pathogens in the presence of antagonist and C2 means the growth of control. Each experiment had three replicates and complete randomized design was adopted. PDA without the culture filtrates served as the control.

Isolates cultured in Czapek's liquid medium

To test for non-volatile antibiotics, the method of Kexiang et al. (2002) was applied. Flasks containing 50 ml of CLM were inoculated with a 5 mm disc of *T. harzianum* isolates and incubated at 25°C in the dark for two weeks. Culture liquids were filtered through filter paper and sterilized by bacterial filtration (Acrodisc nitrocellophane membranes, pore size 0.22 ∝m). The filtrates were diluted with PDB to a concentration of 70%. PDB lacking the filtrate of *Trichoderma* served as a control. Two agar discs containing the pathogen were inoculated in each flask, incubated at 25°C and mycelia filtered through filter paper after 8 days, dried and weighed. Each treatment was replicated three times.

Isolation of DNA from filamentous fungi

Mycelia for DNA extraction were cultured in 50 ml of yeast potato dextrose broth (YPDB) at 20^{O}C with rotary shaking at 120 rpm, harvested by filtration through a filter paper and washed with distilled water after two days. The mycelia were freeze - dried and grounded in the presence of sand and stored at -20°C. One hundred milligrams of the mycelial powder was transferred into eppendorf tubes. 500∝l of 2xCTAB buffer (equilibrated to 65°C) and 1.0% of β-mercaptoethanol were added and the tubes heated at 65°C for 30 min. 500 ∝l of chloroform-isoamyl alcohol (24:1) was added to the tube and vortexed for 30s and centrifuged at 12,000 rpm for 15 minutes. The upper portion of the aqueous phase was recovered and 1/5 volume of 5% CTAB and mix. Another chloroform-isoamyl alcohol (24:1) extraction was performed. Equal volume of CTAB precipitation buffer was mixed with the recovered supernatant and left to stand on ice for 20 minutes. The mixture was centrifuged for 15 min at 12,000 rpm and the supernatant discarded. The pellet was rehydrated with high salt TE buffer (heat at 65°C for 5 -10 min). DNA was precipitated by the addition of an equal volume of cold absolute ethanol with incubation at -20°C for 10 min. DNA was collected by centrifugation at 12,000rpm for 10 min, washed with 70% ethanol, dried, and resuspended in 100 µl of 1XTE buffer and stored at -20°C. The quality of the DNA was checked by use of 0.7% agarose gel and quantified spectrophotometrically.

RAPD analysis and PCR conditions

Amplification reactions were performed in 0.6 ml micro centrifuge tubes in a 25 µl reaction volumes containing 5 ng of template DNA, *Taq* buffer, 2.5 mM $MgCl_2$, 0.8 mM each dNTP, 1.15 ng/µl Primer and 1unit *Taq* DNA polymerase. Amplification reactions were performed in a Perkin-Elmer, Gene amp PCR system 2400 thermal Cycler programmed for 35 cycles of denaturation at 94°C for 30 seconds, low stringency annealing temperature at 31^{O}C for 1.0 minute and polymerization at 72°C for 1.0 min with a final extension step at 72°C for 10 minutes. PCR products were separated on 2.5% agarose/ 1X TBE gels. A 50 bp DNA molecular size marker was loaded on the first well and used for comparison. The banding pattern was visualized on Ultraviolet transilluminator and documented by MultiDoc digital imaging system.

Data analysis

Bands were manually scored 1 for presence and 0 for absence and the binary data used for statistical analysis using the software R-command version 2.1.1. The size of the fragments (molecular weight in base pairs) was estimated by using 50 bp ladder marker, which was run along with the amplified products. A genetic dissimilarity matrix was calculated according to Squared Euclidean Distance which estimated all pair-wise differences in the amplification product and Cluster analysis was done by Wards method using a minimum variance algorithm (Ward, 1963).

Genetic Similarity (GS) was analyzed using the equation: $GS = (N_{ab})/ (N_a+N_b-N_{ab})$, where N_{ab} is the number of shared fragments between isolates a and b, N_a is the number of scored fragments of isolate a, and N_b the number of scored fragments of isolate b. Genetic distance (GD) was then calculated as GD = 1-GS (Yang et al., 1998). The data for growth inhibition measurement were arcsin transformed, subjected to analysis using the Statistica version 6 software.

RESULTS

Inhibition of growth of pathogenic fungi by *T. harzianum* on culture media

As single cultures *Phythium* sp grew actively and colonized the 9mm entire agar surface within two days, whereas *F. Oxysporum* took two weeks, and *F. graminearum* and *Rhizoctonia* sp filled the plate in six days. Confrontation experiments (dual culture assays) provided evidence that *T. harzianum* isolates substantially reduced the growth of the pathogens. Nine out of the sixteen isolates tested were able to inhibit the growth of three pathogens each by more than 50%.Three of the *T. harzianum* isolates inhibited four pathogens. Isolates 015E and 051E were more superior to others since they inhibited the growth of the five pathogens tested. Isolates 030E and 011E were inferior since they only controlled at least one pathogen. *T. harzianum* showed parasitic behavior against *Pythium* (Figure 1) by coiling round the host hyphae and degrading it. *Pythium* sp grew faster than *T. harzianum* its growth stopped immediately upon physical conduct with *T. harzianum.* The parasitic *T. harzianum* over grew the host resulting into complete degradation of the latter and sporulation of the former over the entire plate. The isolate 055E gave the lowest percentage inhibition of 31.76% against *T. harzianum* while 015E gave the highest inhibition of 73.33%.

All isolates of *T. harzianum* tested inhibited the growth of *R. solani* with isolate 029E giving the highest percentage inhibition (61.55%) while 063E the lowest (25.88%). The inhibition process was parasitic (Figure 2), the parasite penetrating the host cell wall directly without formation of appressorium-like structures suggesting mechanical activity.

The host hyphae eventually disintegrated (Figure 3). *T. harzianum* isolates grew considerably faster on PDA than did the *Fusarium* pathogens in the same conditions of culture. In dual cultures the isolates had antagonist activity towards the pathogens since most isolates allowed a percentage radial growth inhibition above 50%. However for *F. oxysporum* f. sp *lycopersici* a junction was always formed between it and the isolates. (Figure 4A).The isolates also sporulated on the pathogen colony (Figure 4B).

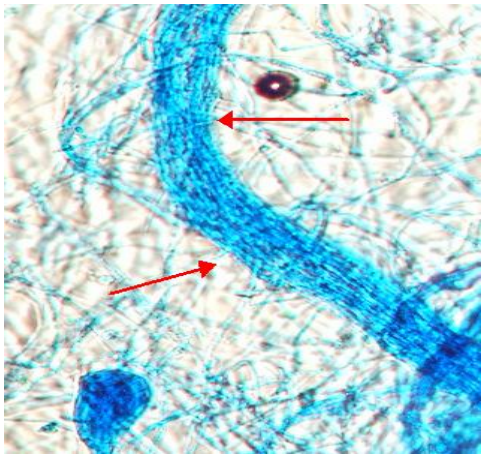

Figure 1. Light microscope micrograph showing *Trichoderma harzianum* encircling mycelia of *Phythium sp* as shown by the arrows.

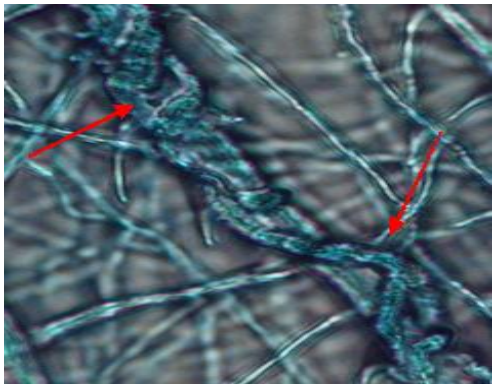

Figure 2. Micrograph of light microscope showing coiling of *T. harzianum* over *R. solani* Arrows point to interaction zones between .*T harzianum* and *R. solani*.

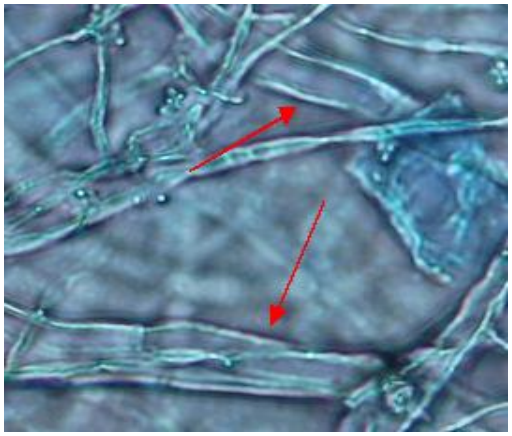

Figure 3. Light microscope micrograph showing disintegrated hyphae of *R solani*. The arrows indicate the disintegrated hyphae.

Inhibition of growth of pathogenic fungi by *T. harzianum* through production of non-volatile compounds

Effect of culture filtrates produced from Potato dextrose broth

Slight colony inhibition of the pathogens *(F. oxysporum f.* sp *Lycopersici, F. oxysporum f.* spp. *phaseoli, Pythium* spp., *Fusarium graminearum* and *Rhizoctonia solani)* was observed when exposed to the culture filtrates of *T. harzianum* cultured in PDB (Table 1). There was no inhibition of radial growth by all tested isolates on *Pythium* spp*.* Isolate 014E inhibited F. oxysporum growth by 80%. Isolates 057E, 021E, 055E, 015E, 0051E, and 010E had no effect on *F. graminearum.* The culture filtrates from isolates 042E, 063E, 030E, 029E, 046E, 044E, 011E, 049E, and 014E inhibited the growth of R. solani.

Czapek's liquid medium culture filtrates

The different culture filtrate from the *T. harzianum* isolates caused significant (P = 0.05) effect on the dry weight of the phytopathogens: *F. oxysporum f.* sp *Lycopersici, F. oxysporum f.* sp *phaseoli Pythium* sp*, Fusarium graminearum* and *Rhizoctonia solani*. The culture filtrates of the isolates suppressed the growth of the pathogens and reduced the dry weight (mg) mycelia (Figure 5). *Pythium* sp was the most influenced as compared to the control. However some isolates' filtrates seems to have enhanced the accumulation of mycelia since the percentage weight obtained of the mycelia was more than 100%.the pathogen *Pythium* for example was enhanced by the isolates 042E, 049E, 046E, 045E, 021E and 051E while the rest (014E, 029E, 010E, 044E, 055E, 011E, 015E, 057E, and 031E) greatly suppressed the accumulation of the mycelia of *Pythium* by more than 50%. The filtrate from the isolate 011E was superior to others since the dry weight of the mycelia of four (*F. oxysporum f. sp Lycopersici, F. oxysporum f. sp phaseoli Pythium sp* and *F. graminearum)* phytopathogens was less than 50%. The *Fusarium* pathogens were always susceptible to the culture filtrates from all the *T. harzianum* isolates.

DNA polymorphism analysis of the *T. harzianum* isolates

In the study four random primers namely 203, 230, 220, 0p13 were used which gave bands ranging from 350bp to 2000bp as shown in Table 2. All the primers produced intense bands with some being faint. A total of 81 bands were produced. The 7 samples used for DNA poly-morphism were antagonistic to the phytopathogens used. In dual cultures the isolates 015E and 051E were superior

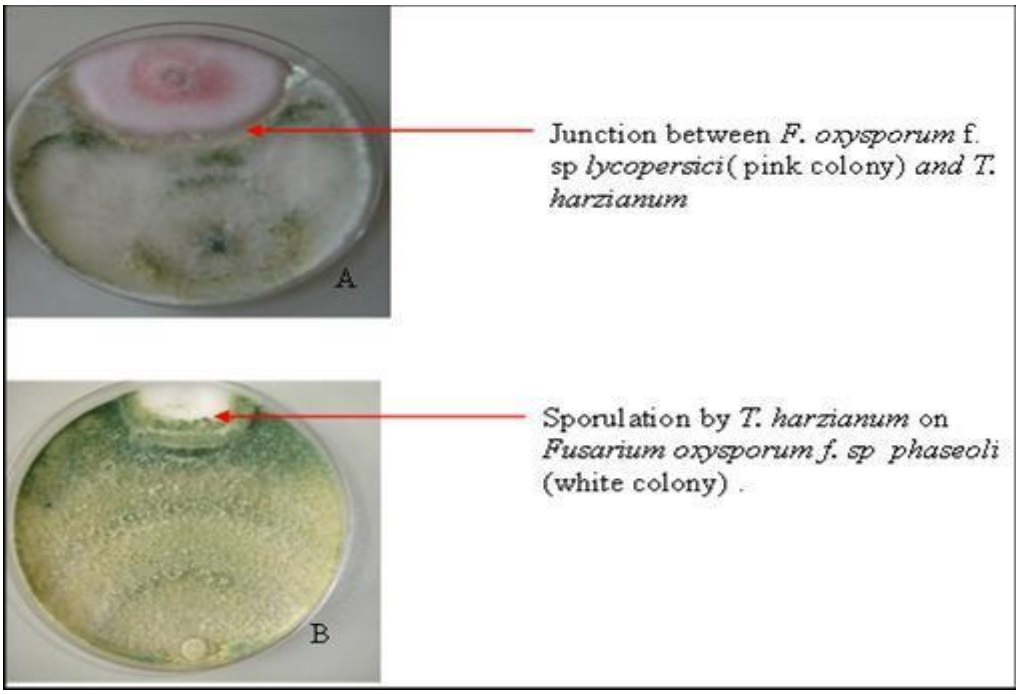

Figure 4. Plate confrontation test of *T. harzianum* against; A. *Fusarium oxysporum* f.sp *lycopersici* and B. *Fusarium oxysporum f.sp phaseoli* Performed on PDA medium.

Table 1. Effect of different isolates of *T. harzianum* on radial growth inhibition (%) of phytopathogens by the production of non-volatile compounds from Potato Dextrose Broth after 6 days of incubation.

***T. harzianum* Isolates**	**Radial growth inhibition (%)**				
	F. oxysporum f.sp Lycopersici	***F.oxysporum f. sp phaseoli***	***Pythium sp***	***F. graminearum***	***R. solani***
042E	12.74	9.61	0	66.27	4.31
063E	2.27	13.46	0	44.70	17.64
030E	20.29	23.71	0	40.39	28.627
029E	32.51	1.92	0	85.88	11.76
046E	31.94	23.07	0	43.92	31.37
044E	40.08	20.51	0	36.47	30.98
057E	11.02	17.30	0	0	0
045E	19.72	27.56	0	6.66	0
011E	16.80	28.20	0	7.05	8.23
049E	4.02	10.89	0	5.88	5.88
021E	15.07	14.74	0	0	0
055E	14.50	26.28	0	0	0
015E	10.33	16.66	0	0	0
051E	11.55	15.38	0	0	0
010E	11.57	17.30	0	0	0
014E	80.22	82.05	0	8.62	71.37

to others since they inhibited the growth of five pathogens by more than 50% and 029E inhibited the growth of four pathogens. In the culture filtrate activity the isolates 011E and 055E had a wide range of activity by suppressing the growth of five pathogens while isolates 0440E and 010E suppressed the growth of four pathogens each. The DNA profiles obtained for *Trichoderma harzianum* isolates were scored (Figure 6) and a dendrogram or dissimilarity matrix developed using Squared Euclidean Distance and Clustering based on Wards method (Figure 7).

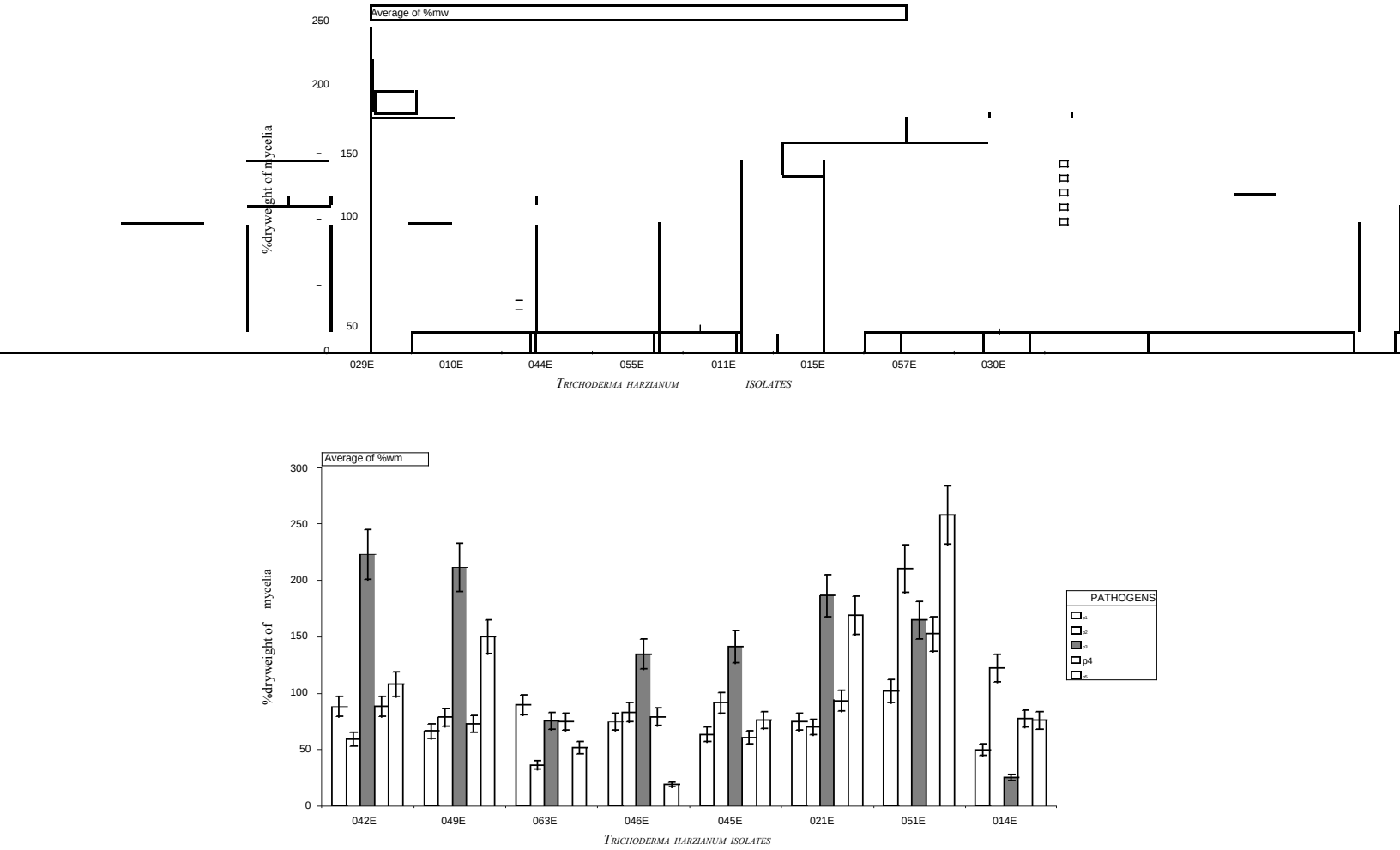

Figure 5. Percentage dry weight (mg) of mycelia of fungal phytopathogens p1: *F. oxysporum f .sp Lycopersici,* p2: *F. oxysporum f. sp phaseoli* p3: *Pythium sp, p4: Fusarium graminearum* and p5: *Rhizoctonia solani* as influenced by non-volatile antibiotics of *T harzianum* isolates.

Table 2. Synthetic deoxyribonucleotides used as primers for amplification of *Trichoderma harzianum* DNA.

Primer code	Primer sequence 5'- 3'	Total no. of bands	Size range of bands(bp)
203	CACGGCGAGT	7	600-2000
220	TCGATGTCG	7	600-2000
0P13	CAGCACCCAC	7	300-650
230	CGTCGCCCAT	7	350-1800

DISCUSSION

T. harzianum inhibited the growth of the target organisms through its ability to grow much faster than the pathogenic fungi thus competing efficiently for space and nutrients. Starvation is the most common cause of death for microorganisms, so that competition for limiting nutrients results in biological control of fungal phyto-pathogens. A second mechanism of pathogen control that *Trichoderma* displayed was mycoparasitism. Micro-scopic observation of the interaction region between *R. solani* and *Pythium* spp. with *T. harzianum* showed that the mycelia of *T. harzianum* grew on the surface of the pathogens always coiling round their mycelia and later penetrating their cell walls directly without formation of appresorium structures. The pathogen mycelia then disin-tegrate suggesting enzyme action. Lorito et al. (1998), Metcalf and Wilson (2001) and Sharon et al. (2001)

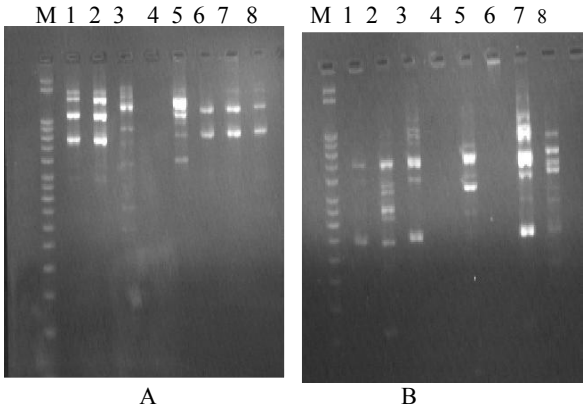

Figure 6. A RAPD profiles obtained with primer203. Positions 2-9 corresponds to isolates T010, T015, T011, 021E, 051E, 029E, 055E, and 044E.**B** RAPD profiles obtained with primer230. Positions 2-9 corresponds t0 isolates 044E, T011, T015, T014, 051E, 055E, T010, and 029E.

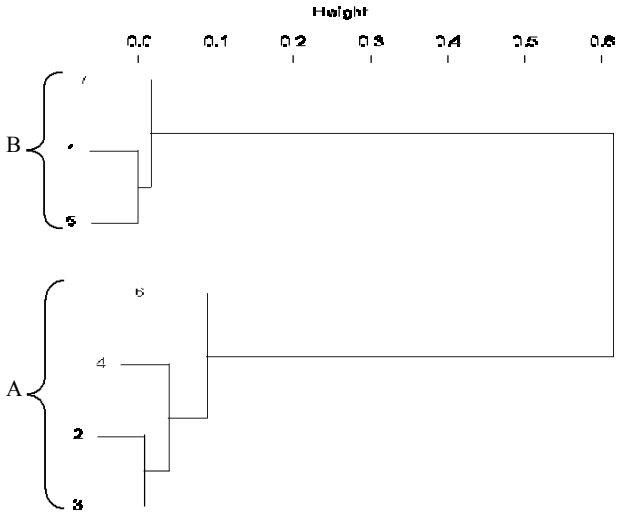

Figure 7. Dendrogram for 7 *Trichoderma harzianum* isolates as revealed by RAPD markers.Where : 1:044E, 2:011E, 3:015E, 4: 051E, 5: 055E, 6:010E, 7:029E

In the Dendrogram, all the 7 isolates were distinctly divided into two major clusters 'A' and 'B' at 20 units. Isolate 051E and 029E spanned the extremes of the entire Dendrogram. Genetic dissimilarity ranged from a lowest of 0.143 (between T010 and T015) to a highest of 0.857 (between 055E and 051E).Isolate 051E, T011, T015, and T010 were assigned to cluster 'A'. Genetic dissimilarity among the entries in this cluster ranged from a lowest of 14.3 percent (between T015and T010) to a highest of 35.7 percent (between T010and 051E). The other cluster 'B' comprised of three accessions. In this cluster isolate 044E 055E and 029E were grouped together. The genetic dissimilarity in this group was ranging from 33.3 percent between 055E and 029E to a high of 75 percent between 044E and 029E.

demonstrated possible role of chitinolytic and/or glucanases enzymes in bio-contro l by *Trichoderma.* These enzymes function by breaking down the polysaccharides, chitin, and glucans that are responsible for the rigidity of fungal cell walls, thereby destroying cell wall integrity limiting the growth of the pathogen. A mixture of several enzymes might be necessary for efficient cell wall lysis. *T. harziunum* has been reported to apply high chitinase and β-1,3-glucanase activities (Sivan et al., 1984).

A third mechanism of pathogen control by *Trichoderma* was through antibiosis. Culture filtrates of the isolates had an inhibitory effect on the radial growth of the patho-gens and mycelial accumulation suggesting action of non-volatile antibiotics in the filtrates. This agrees with the findings of Sivan et al. (1984) who noted that culture filtrates of *T. harzianum* strongly inhibited the growth of *Pythium aphanidermatum* whereas *T*. *hamatum* filtrates caused only minor inhibition of growth. Antibiotic inhibittions have been documented by Claydon et al. (1987), Dubey and Suresh (2006), Kucuk and Kivanc (2003) and Lynch (1990). Claydon et al. (1987) reported inhibition due to antibiotics trichodermin, harzianum A and harzianolide. Dubey and Suresh (2006) found that non-volatile substances produced by *T. harzianum* are inhibittory to *F. oxysporum f.* sp*. ciceris* causing chickpea wilt. Culture filtrate from PDB inhibited *Fusarium* spp. and *R. solani* but not *Pythium* sp. The difference in activity of the culture filtrates displayed by PDB and Czapek's liquid media on the pathogens indicates the importance of substrate in fungal production of secondary metabolites. The presence of inhibition zones in dual cultures between *F. oxysporum f.* sp *Lycopersici* and *T. harzianum* suggested secretion of diffusible non-volatile inhibitory substance by the *T. harzianum* isolates. This has also been documented by Grodona et al. (1997) and Behzad et al. (2008).

The DNA analysis of *T. harzianum* isolates from the same region (Embu) showed existence of intraspecific variation. Based on RAPD data isolates from the same land use appeared in different groups due to intraspecific variation. Isolate 044E and isolate 011E collected from camphor plantation were clustered in different groups and were 54.4% dissimilar. This agrees with Moller et al. (1995) whom detected intraspecific diversity not only between isolates of *Chaunopycnis alba* from different geographic regions or hosts, but also between isolates from a single location. Goes et al (2002) also found intraspecific genetic variation among *Trichoderma* isolates. In his work the isolate Tm isolated from corn seed was grouped in the Dendrogram with the isolate 2820 isolated from sugar cane. Although belonging to the same species, the isolates presented low similarity (55.5%).

Conclusion

T. harzianum is a good candidate for biological control due to the different modes of action the fungus employs in inhibiting the growth of other fungi. Through the technique of RAPD, it was possible to identify intraspecific

genetic variation between *T. harzianum* isolates. The results presented here support the existence of cryptic species with similar morphology.

ACKNOWLEDGEMENTS

We would like to express our special thanks to the University of Nairobi for laboratory equipment and enabling environment to conduct research and the Con-servation and sustainable management of below ground biodiversity (CSM-BGBD) project. A project executed by TSBF/CIAT with co-financing from the Global Environment Facility (GEF) and implementation support from the United Nations Environment Programme (UNEP) for financial support of the research.

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