Fermentation and antimicrobial activities of extracts from different species of fungus belonging to Genus, *Trichoderma*

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SUMMARY

The present paper discusses the effects of the crude extracts of *Trichoderma* spp. on a number of fungal and bacterial organisms. These include *Paecilomyces variotii*, *Penicillium notatum*, *Nematospora corylii*, *Mucor miehei*, *Bacillus brevis*, *Bacillus subtilis*, *Enterobacter dissolvens* and *Sarcina lutea*. The culture broth extracts of different isolates of *Trichoderma harzianum*, *T. longibrachiatum* and *T. koningii* cultured in different media were investigated individually for *in-vitro* antifungal and antibacterial activities by agar diffusion technique. The culture broth extracts of *T. harzianum* produced definite antifungal and antibacterial activities against most of the test organisms. The results indicate that the extracts were fungicidal and antibacterial at a concentration of 100 µg per 6mm paper disks. *Trichoderma harzianum* showed the highest activity while *T. koningii* showed the least activity against most of the test organisms especially the yeast *Nematospora coryli*. The results of this study indicate that the *Trichoderma* species especially *T. harzianum* is a possible source of useful antimicrobial agents against pathogenic microorganisms which include gram-negative, gram-positive, fungi and yeasts.

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Introduction

Trichoderma is one of the commonest genera of fungi found in majority of the soils [1-3]. A number of *Trichoderma* species have been associated with mycoparasitism of a wide range of pathogens. Examples include *Rhizoctonia solani* Kuhn [4-7]. *Sclerotina sclerotorium* (Libert) de Bary [8] and *Fusarium oxysporum* Jarvis and Shoemaker [9]. Suppressiveness in these pathogens has been in many cases, attributed to some *Trichoderma* species [10-12].

Fungi in the genus *Trichoderma* Persoon are widespread and their taxonomical classification is difficult. This has led to the development of a species aggregate system of classification [1], which groups together several 'species'. It has been noted, for example that under the name *T. hamatum* (Bon.) Bain Aggr. there may be two, three or more different but morphologically very similar species aggregate and that isolates may behave quite differently under different conditions [1]. It is not surprising then that there is no clear pattern among the wide variety of secondary metabolites produced by *Trichoderma* strains [13,14]. Several non-volatile constituents, for example, epoxytrichothecenes and peptides have been described as biological active metabolites of *Trichoderma* strains [15]. Besides these, 6-pentyl-α-pyrone, a volatile lactone with an intense coconut-like odour showing high bioactivity has been identified [16, 17]. *T. harzianum* Rifai retards sporulation of *Cochliobolus salivus* (Ito Kurib) Dreschel ex. Duster and also parasitises it [18]. *T. harzianum* and *T. viride* reduced fruit rots of strawberries to the level achieved by a fungicide dichlorofluianid [19] showing that they produce antimicrobial substances.

Lately, considerable interest has been shown in the use of *Trichoderma* species as a source of bioactive substances [15]. In spite of the importance of the genus, there are no studies reported on the optimum fermentation conditions and antimicrobial activities of Kenyan isolates of *Trichoderma* strains.

Interest in the metabolites produced by *Trichoderma harzianum*, *T. longibrachiatum*, *T. koningii* was stimulated by their potential as antiinfective agents against human and plant pathogens [20]. Recently Onsando isolated and identified some strains of *Trichoderma* from soils in Eastern and Western Kenya and observed that *T. harzianum*, *T. longibrachiatum*, *T. koningii* are effective against *Armillaria mellea* fungus [21].

The present paper reports the bioassay results of the extracts obtained from fermentation of nine *Trichoderma* species under different fermentation conditions and media. The bioassays were carried out against some gram-positive, gram-negative bacteria, fungi and yeasts.

Materials and Methods

Strains of Trichoderma species

The nine isolates of *Trichoderma species* used in the study were isolated at Tea Research Foundation, Kericho in Kenya and identified at the International Mycological Institute, Kew, United Kingdom. These included four strains each of *T. koningii* (IMI 339493; IMI 342180; IMI 342182; IMI 342183); *T. harzianum*, (IMI 339496; IMI 339497; IMI 342184; IMI 342185) and one strain of *T. longibrachiatum* (IMI 339495).

Test cultures

The test cultures employed for assaying antimicrobial activity were gram-positive bacteria, *Bacillus brevis* (ATCC 9999), *B. subtilis* (ATCC 6633), *Sarcina lutea* (ATCC 381); gram-negative bacteria, *Enterobacter dissolvens* (LMG 2683); fungi, *Paecilomyces variotii* (ETH 114646), *Penicillium notatum* (isolated in H. Anke's laboratory , University of Kaiserslautern , Germany); mucor, *Mucor miehei* (TÜ 284) and yeast, *Nematospora corylii* (ATCC 10647).

The test cultures were standard and obtained from Laboratorium Voor Microbiologie, Ghent, Belgium (LMG); Mycological Institutes for Biology and Microbiology (ETH), Tubingen University, Germany (TÜ) or from American Tissue Culture Centre, USA (ATCC). These were cultured and maintained at the Department of Biotechnology, University of Kaiserslautern, Kaiserslautern, Germany.

Test Organism Culture Media

The culture media were Yeast Glucose Malt (modified) Agar medium from H. Carroux, Hamburg, Germany and Nutrient Broth Difco medium from Difco, U.S.A., prepared according to the method described by Schneider [22] and poured into petri dishes to give a uniform depth of approximately 4 mm.

Chemicals

Analar chemicals and solvents were obtained from Merck, Darmstadt, Germany and were of analytical grade. Ingredients for media were commercial products from various suppliers. The antifoam used was silicon M 30 (Roth, Karlsruhe, Germany).

Fermentation media

The media M1 to M7 were used in the fermentation of different strains of the fungus, *Trichoderma.* The composition of the different media were as follows:

Medium 1 (M1): Double malt extract medium, which consisted of: 40 g malt extract (Dr. Fränkle and Eck, Fellbach, Germany) and 1000 ml tap water.

Medium 2 (M2): Yeast malt glucose (modified) medium consisted of: 4 g malt extract (Dr. Fränkle and Eck, Fellbach, Germany), 10 g glucose (Deutche Maizenwerke, Hamburg, Germany), 4 g yeast extract (Hans Hartge GmbH, Hamburg, Germany) in 1000 ml tap water.

Medium 3 (M3): Yeast malt glucose medium which consisted of: 10 g malt extract, 10 g glucose, 4 g yeast extract and 1000 ml tap water.

Medium 4 (M4): Maltose- Glucose Peptone medium which consisted of: 20 g Maltose (Merck, Darmstadt, Germany), 10 g glucose, 2 g peptone (Difco laboratories, USA), 1 g yeast extract, 0.5 g KH_2PO_4 (Merck, Darmstadt, Germany), 10 mg $MgSO_4.7H_2O$ (Merck, Darmstadt, Germany), 10 mg $FeCl_3$ (Merck, Darmstadt, Germany), 1.78 mg $ZnSO_4.7H_2O$ (Merck, Darmstadt, Germany), 73.5 mg $CaCl_2$ (Merck, Darmstadt, Germany) dissolved in 1000 ml distilled water.

Medium 5 (M5): Richard's nutrient (modified) medium which consisted of: 10 g KNO_3
Merck, Darmstadt, Germany), 5 g $KH_2PO_4.3H_2O$ (Merck, Darmstadt, Germany), 2.5 g $MgSO_4.7H_2O$ (Merck, Darmstadt, Germany), 0.02 g $FeCl_3$ (Merck, Darmstadt, Germany), 50 g sucrose (Merck, Darmstadt, Germany) in 1000 ml distilled water.

Medium 6 (M6): Maize meal medium which consisted of: 40 g Glucose, 1.5 g $KH_2PO_4.3H_2O$,
0.5 g KCl (Merck, Darmstadt, Germany), 0.5 g $NaNO_3$ (Merck, Darmstadt, Germany), 0.5 g $MgSO_4.7H_2O$, 10 g maize meal in 1000 ml distilled water.

Medium 7 (M7): Potato-Glucose medium which was made up of: 4 g Potato puree (Pfanni, Munchen, Germany), 20 g glucose in 1000 ml distilled water.

Fermentation Conditions

All strains of the fungus *Trichoderma* were separately fermented in media **M1** to **M7**.

The seed cultures were prepared by placing an average of 4 agar blocks (1 cm x 1 cm) containing spores of the different strains of *T. harzianum, T. longibrachiatum* and *T. koningii* in 500 ml baffled Erlenmeyer shake flasks containing 200 ml of the various media autoclaved at 121°C and 15 psi for 20 minutes. The flasks were placed on a shaker set at 120 rpm and a temperature of 27°C. After 24 hours, the 200 ml of seed culture (pre-culture) was inoculated into a 20 litre Biolafite C-6 fermenter containing 20 litre of the fermentation medium sterilized at a temperature of 121°C at a pressure of 15 psi for 40 minutes. The fermenter was agitated at 120 rpm with aeration set at 1.5 litres of air per minute and maintained at 24°C. The fermentation was monitored and terminated when glucose was exhausted in the medium or when the bioactivity against *N. corylii* had reached a maximum.

The parameters that were monitored during the fungal growth include pH, glucose content, growth (mycelial dry weight), and the bioactivity against *N. Corylii*. The parameters that were being monitored were determined in 100 ml aliquots of culture broth withdrawn aseptically from the Biolafite fermenter. The aliquots were taken every 12 hours but this was changed to 24 hours depending on the strain of *Trichoderma* being fermented. When *T. longibrachiatum* was cultured in medium M5 to M7 the fermentation period was between 16 days to 52 days, this made it necessary to extend the time intervals the aliquots were taken to once in one week or to every fortnight.

Fermentation Monitoring

Glucose analysis. The glucose concentration in the culture broth was determined using urine sugar test strips (Boehringer, Mannheim, Germany).

pH. The pH of the culture broth was measured with a pH metre (CG825, Schott, Hofheim).

Mycelial dry weight measurement. The mycelial dry weight was measured by withdrawing aseptically 100 ml of the culture broth from the fermenter and the mycelia was separated from the broth culture by filtration using a pre-weighed filter paper which had been dried at 80°C. The mycelia and filter paper were thereafter dried in an oven at 80°C until there was no further weight changes, then cooled in a desicator to room temperature and re-weighed, the difference gave the mycelial dry weight [22].

Diffusion assay of some known antibiotics against a few selected target organisms

The standard antibiotics, Clotrimazole (CL) (Sigma, USA), Ampicillin as sodium salt (A1) (Boehringer Mannheim,Germany), Ampicillin Trihydrate (A2) (Roth Chemicals, USA) and tetracylin HCl Pharm. (T) (Serva, Feinbiochemica Heildelberg, Switzerland) were tested each at 5 and 10 µg per 6mm disc against some selected test organisms. The diameter of inhibition zone was measured in mm with a standard laboratory line ruler.

Bioassay tests

Gram-positive bacteria: The bioassay of the ethyl acetate culture broth extract against the gram-positive bacteria, *B. brevis, B. Subtilis, S. lutea* (*Micrococcus leuteus*) was done using the agar diffusion technique. 50 and 100 µg of the extract was dissolved in methanol and loaded onto a 6 mm filter paper (Schleicher and Schüll, Felbach, Switzerland), dried and placed on petri dishes containing the nutrient broth medium and seeded with appropriate test organisms. The dishes were then incubated at 37°C for 24 hours and the zones of inhibition measured from the end of the growth on one side of the disk to the beginning of growth on the other side including the diameter of the disk [23].

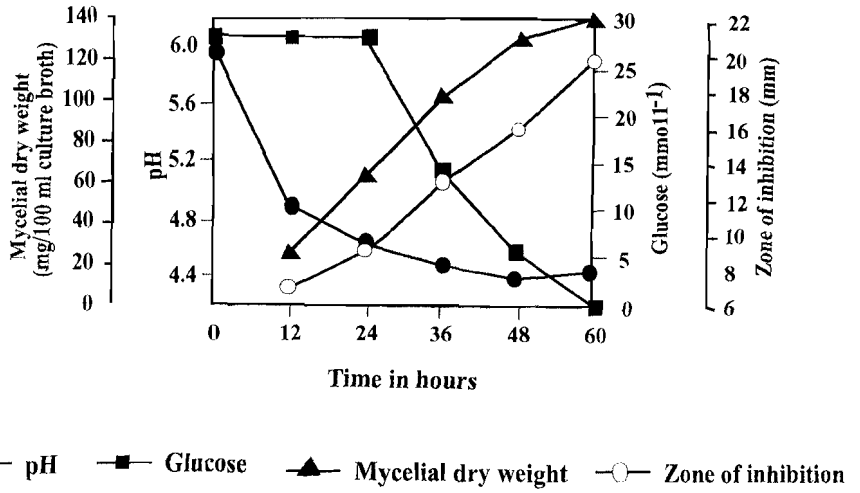
Gram-negative bacteria: The bioassay of the ethyl acetate culture broth extract against *E. dissolvens* was carried out as outlined in the above section of the gram-positive bacteria. The only difference was in the incubation temperature of *E. dissolvens* being 27°C. The recording of the inhibition zones was measured as described above.

Fungus: The test organisms, *M. miehei, P. variotii, P. notatum, N. corylii* were seeded onto petri dishes containing Yeast-Malt-Glucose agar medium. The ethyl acetate culture broth extract was dissolved in methanol and loaded onto a 6 mm filter paper, dried and placed on the petri dish. The petri dishes seeded with *M. miehei* and *P. variotii* were incubated at 37°C while those seeded with *P. notatum* and *N. corylii* were incubated at 27°C. The measurements of the zones of inhibition were carried out as described for the gram-positive bacteria.

The Culture Broth Extraction: The culture broth was extracted with a resin, Diaion HP 21 (Mitsubishi).

The Diaion HP 21 resin was maintained in 10% methanol and 90% water. The resin was degassed before being packed into a column. The culture filtrate (culture broth) was passed through the resin in the column and the water fraction discarded. The column was then eluted with 50% acetone: 50% water and the column was thereafter washed with 5 litres of distilled water. The acetone in the 50% acetone fraction in water was evaporated under reduced pressure and the resultant aqueous layer was extracted six times with ethyl acetate (EtOAc). The ethyl acetate extract was dried with anhydrous sodium sulphate (Na_2SO_4) and the EtOAc evaporated under reduced pressure at 45°C. Any water remaining in the extract was removed by the addition of a small amount of iso-propanol (2-propanol), which aided in the removal of water at 45°C under reduced pressure. The water free extract was dissolved in the least amount (1 ml) of analar grade methanol and an ultrasonic bath was used to aid in dissolving the extract into the solvent. Special pipettes were used to remove the extract into a pre-weighed sample bottle. The solvent was then removed by putting the sample in a speed vacuum (centrifuge), which was maintained at a temperature of 45°C and at a high vacuum for a period of upto 3 hours. The crude extracts obtained were mostly dark brown oils with the exception of the extracts from *T longibrachiatum*, which were intensely yellow coloured gummy solids. The masses of the crude extracts were recorded to be between 0.8000 g and 1.3000 g for all the strains of *Trichoderma* except for *T. longibrachiatum* that had a high yield of 4-5 g of extract per 20-litre fermentation.

Figure1. Fermentation of *Trichoderma harzianum* (T4 IMI 339496) in 20 Litres double malt (M1) medium.



Results

Fermentation monitoring

Most of the fermentation profiles for all the *Trichoderma* species studied followed the expected trends of fungal growth. This entailed a decrease of glucose levels until it is exhausted. pH on the other hand reduces initially but rises slightly towards the end of the fermentation. The mycelial dry mass and the bioactivity of the culture broth both increased steadily at first and then eased off slightly at the end of the fermentation. The fermentation profiles of the *Trichoderma* species varied depending on the media used and the isolate. Some isolates used all the glucose in 12 hours while others could take twenty to fifty two days. *T. harzianum* (IMI 339496) on the other hand took between 60 and 120 hours in its fermentation in most of the media to exhaust the glucose, thus, necessitating the termination of the fermentation (Figure 1).

Figure 2. Fermentation of *Trichoderma longibrachiatum* (T3, IMI 339495) in Potato-Glucose medium (M6)

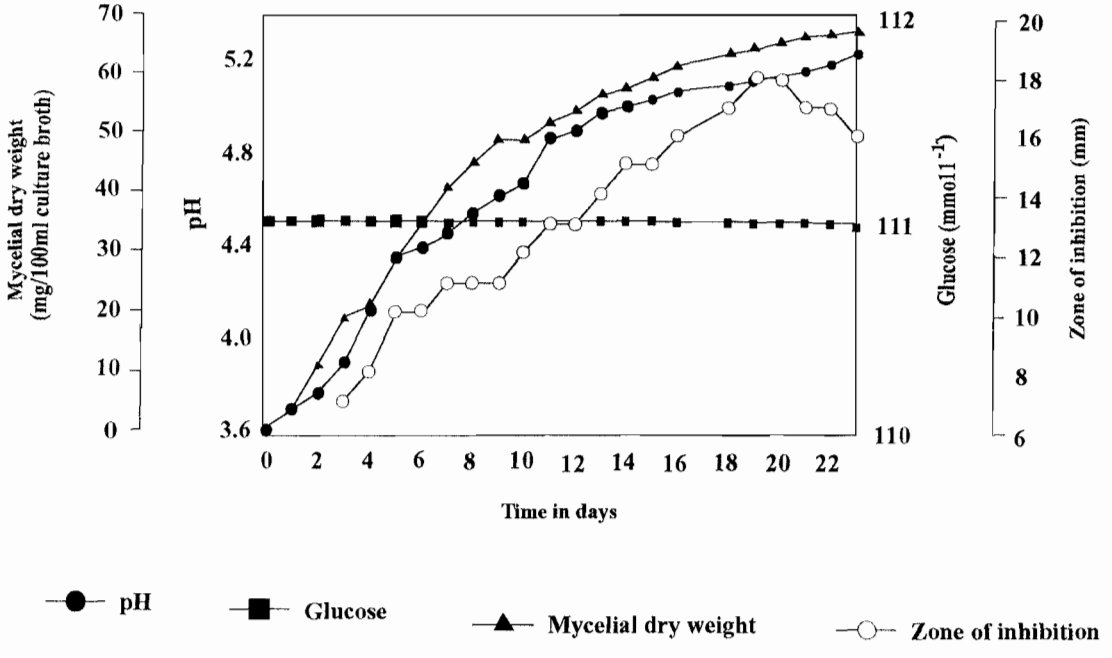
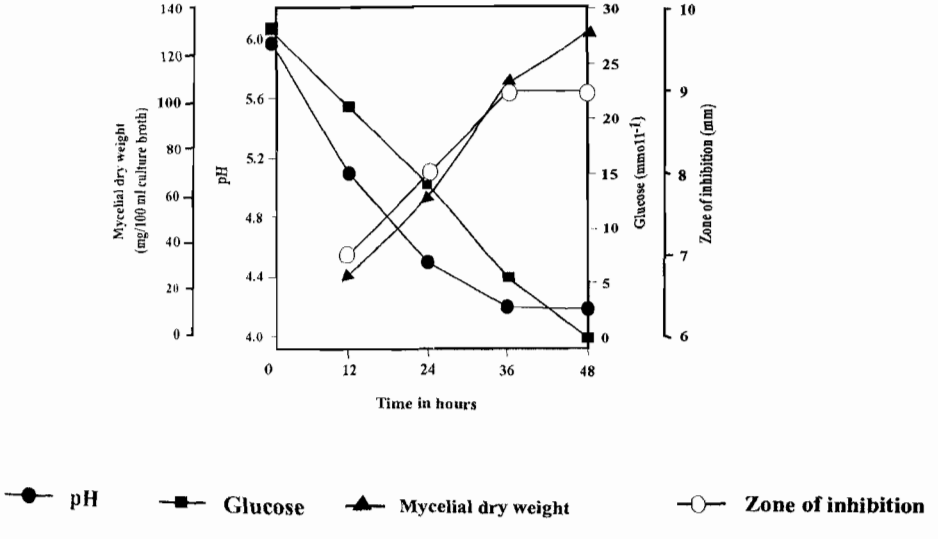


Figure 3. Fermentation of Trichoderma koningii (T9, IMI 342183) in 20 Litres medium (M1) .



T. longibrachiatum could not consume all the glucose when cultured in medium M5, M6 and M7 as illustrated in Figure 2. The fermentation was stopped after 22 days when the activity of the extracts had reached an optimum value. Glucose levels remained fairly constant throughout this fermentation.

T. koningii (IMI 342183) showed a fairly typical fungal fermentation profiles **(Figure 3)** when cultured in M5 but with the other media the fermentation durations were much shorter, less than 60 hours.

The other strains of fungi *T. harzianum*, (IMI 339497, IMI342184, IMI 342185) and *T. koningii*, (IMI 339493, IMI 342180, IMI 342182) had very short fermentation times of 12 hours or less using M1, M2 and M3 and showing very low activity against the test organisms, *N. corylii* (Figure 4). This low activity is also observed against the other test organisms.

Antimicrobial bioassay of the extracts from the fermentation of T. harzianum (IMI 339496)

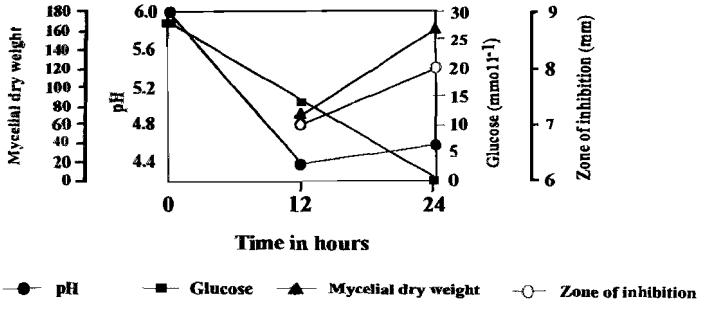
The results indicated in Table 1 show that the extracts from *T. harzianum* (IMI 339696) showed the highest activity among the nine *Trichoderma* strains of fungi studied in this work when cultured in different medium. The highest activity was recorded when this strain was cultured in medium, M1, M5, M6 and M7. The bioactivity was highest against *N. corylii* with an inhibition ranging from 20-30 mm at concentrations of 50 µg of the extract. Fermentations in the media M2, M3 and M4 showed the lowest activity when T4 was cultured in this media, with an inhibition zone of less than 10 mm at concentrations of 50 µg per 6 mm filter paper disc against *N. corylii*. T4 showed no activity against the gram-negative bacteria, *E. dissolvens* (LMG 2683) when cultured in medium M1 to M4 and showed very small inhibition (≤ 9 mm) in culture medium M5 to M7.

T. harzianum (IMI 339496) extracts showed both antifungal and antibacterial inhibition though the fungal test organisms were inhibited more. The extracts from fermentations in media M1 and M5 showed a slightly higher inhibition of *B. brevis*, *B. subtilis*, while the extracts obtained using medium M5, M6 and M7 as culture media showed a higher activity against the mucor, *M. miehei* (TU 284). The activity of *T. harzianum* (IMI 339496) extracts against the fungi, *P. variotii* (ETH 44646) and *P. notatum* was highest when cultured in medium M1, M5, M6 and M7. The results show that these four media were the best to use as the culture media to obtain antifungal extracts.

Antimicrobial bioassay of the extracts from the fermentation of T. longibrachiatum (IMI 339495)

When *Trichoderma longibrachiatum* was cultured in different media, the extracts showed higher antifungal and antibacterial activity when grown in medium M5, M6 and M7 (Table 2).

Figure 4. Fermentation of *Trichoderma harzianum* (T10, IMI 342184) in 20 Litres Double malt medium (M1) .



Antimicrobial bioassay of the extracts from the fermentation of T. koningii (IMI 342183)

Trichoderma koningii showed much lower activity against all the test organisms (Table 3) than of *T. harzianum* and *T. longibrachiatum* cultured in medium M1 to M5 and tested at a concentration of 50 µg per disc. There was no growth when *T. koningii* (IMI 342183) was cultured in Medium M6 and M7. This was due to the inability of *T. koningii* to utilize or

metabolise the two starch media.

From the above results *T. harzianum* (IMI 339496) gave the most active extracts. Further, the most suitable media were medium M1, M5, M6 and M7. *Trichoderma longibrachiatum* extracts also showed substantial antibacterial and antifungal properties while *T. koningii* (IMI 342183) showed much lower activity against all the test organisms. The remaining strains of fungi had little or no activity. Changing the medium had no effect on the bioactivity of the extracts towards the test organisms.

Antimicrobial bioassay of the extracts from the fermentation of T. harzianum (IMI 339497, 342184, 342185) and T. koningii (IMI 339493, IMI 342180 and IMI 342182) strains of fungi

The six *T. harzianum* and *T. koningii* strains listed above showed very low activity in the preliminary studies when cultured in all the media (Table 4).

The only medium that showed slight activity was M1. This, therefore, led to the use of this medium in the fermentation of the six strains of fungi. The bioactivity of most of the extracts were very low with *T. harzianum* (IMI 339497) being the most active with an activity of 12 mm against *N. corylii* while *T. koningii* (IMI 342182) extract had no activity against all the eight test organisms. Otherwise these strains of fungi showed very little or no activity against the test organisms and were therefore not investigated further.

Table 1. Zones of inhibition in millimeters for the culture broth extracts from *Trichoderma harzianum* (IMI 339496) in different fermentation media.

Media Test organism	M1	M2	M3	M4	M5	M6	M7
Paecilomyces variotii	17	-	9	-	15	24	25
Penicillium notatum	15	+	9	-	16	22	23
Nematospora corylii	20	10	10	10	25	25	30
Mucor miehei	9	-	-	-	14	17	18
Bacillus brevis	16	+	9	9	24	11	10
Bacillus subtilis	17	+	9	+	25	13	13
Sarcina lutea	+	-	-	+	9	12	10
Enterobacter dissolvens	-	-	-	-	+	9	+

+ indicates slight inhibition (< 9 mm) zone
- indicates no inhibition zone

Discussion

The results show that *T. harzianum* (IMI 339496) yield more active extracts than those of the other *Trichoderma* spp. investigated in this study and the most suitable media were medium M1, M5, M6 and M7. *Trichoderma longibrachiatum* (IMI 339495) extracts also showed substantial antibacterial and antifungal properties while *T. koningii* (IMI 342183) showed much lower activity against all the test organisms. The remaining strains of fungi had little or no activity despite changing the medium.

The extracts showed higher antifungal potency than antibacterial activity. When *T. longibrachiatum* was cultured in all the media, no activity was observed against *E. dissolvens* and the highest activity was against *N. corylii*. *T. longibrachiatum* showed higher activity when cultured or grown in medium M5 (22 mm) and M7; still the activity of the extracts from M4 to M7 were fairly high (12 mm) against *N. Corylii*. The activity of the extracts from *T. longibrachiatum* were however generally less active against most organisms than *T. harzianum* (IMI 339496) in most of the media utilised in this study.

T. koningii (IMI 342183) extracts had higher activity when cultured in medium M5, with the highest inhibition observed against *N. corylii* which had an inhibition zone of 16 mm. This activity was much lower compared with that recorded against *N. corylii* using extracts from *T. longibrachiatum* (T3) and *T. harzianum* (IMI 339496) when cultured in medium M5.

Table 2. Zones of inhibition in millimeters for the culture broth extracts from *Trichoderma longibrachiatum* (IMI 339495) in different fermentation media.

Media Test organism	M1	M2	M3	M4	M5	M6	M7
Paecilomyces variotii	-	-	9	12	18	11	18
Penicillium notatum	-	-	9	11	16	12(16i)	16
Nematospora corylii	11	10	10	13	22	11(20i)	18
Mucor miehei	-	-	-	-	12	10	12
Bacillus brevis	9	9	9	9	11	12	11
Bacillus subtilis	9	+	9	+	11	12	11
Sarcina lutea	-	-	-	-	+	12	+
Enterobacter dissolvens	-	-	-	-	-	-	-

i indicates incomplete inhibition
+ indicates slight inhibition(< 9 mm)zone
- indicates no inhibition zone

Table 3. Zones of inhibition in millimeters for the culture broth extracts from *Trichoderma koningii* (IMI 342183) in different fermentation media.

Media Test organism	M1	M2	M3	M4	M5
Paecilomyces variotii	+	-	+	-	9
Penicillium notatum	+	-	-	-	12
Nematospora corylii	9	+	10	9	16
Mucor miehei	-	-	-	+	+
Bacillus brevis	+	+	+	+	12
Bacillus subtilis	+	+	+	-	11
Sarcina lutea	-	-	-	-	12
Enterobacter dissolvens	-	-	-	-	-

+ indicates slight inhibition (< 9 mm) zone
- indicates no inhibition zone

The antibacterial activity of the *Trichoderma* extracts tested at 100 µg per disk was lower than that recorded for the antibiotics, ampicillin as sodium salt (A1) and ampicillin trihydrate, tested at concentrations of 5 µg and 10 µg per disk respectively (Table 5). The ampicillin inhibited the *B. brevis* and *B. subtilis* with inhibition zones ranging from 30-40 mm compared with 8-25 mm for the extracts obtained from *Trichoderma* isolates. Tetracycline (T) inhibited the growth of *E. dissolvens* with inhibition zones of 20 and 25 mm when tested at 5 µg and 10 µg per disk, respectively. In addition the antibiotic clotrimoxazole (CL) exhibited much higher antifungal properties when tested at 5µg and 10µg than the bioactivity of all the *Trichoderma* crude extracts (100 µg) against the fungal test organisms used in the study.

Table 4. Zones of inhibition in millimeters for the culture broth extracts from *T. harzianum* (IMI 339497, 342184, 342185) and *T. koningii* (IMI 339493, IMI 342180 and IMI 342182) in medium M1.

	T. harzianum			T. koningii		
Test organism	IMI 339497	IMI 342184	IMI 342185	**IMI** 339493	IMI 342180	IMI 342182
P. variotii	+	-	-	9	+	-
P. notatum	+	-	-	10	+	-
N. corylii	12	+	+	12	9	+
M. miehei	-	-	-	-	+	-
B. brevis	+	+	-	10	+	+
B. subtilis	+	+	-	10	9	+
S. lutea	-	-	-	-	-	-
E. dissolvens	-	-	-	-	-	-

\+ indicates slight inhibition (< 9 mm) zone
\- indicates no inhibition zone

Table 5. Diffusion assay of some known antibiotics against a few selected target organisms.

Antibiotics	CL		A1		A2		T	
Conc. (μg)	**5**	**10**	**5**	**10**	**5**	**10**	**5**	**10**
P. variotii	10	11	-	-	-	-	-	-
P. notatum	25i	30i	-	-	-	-	-	-
N. corylii	11	10	-	-	-	-	-	-
M. miehei	30	34	-	-	-	-	-	-
B. brevis	-	-	40	46	30	40	10	10
B. subtilis	9	12	19	22	21	25	21	25
S. lutea	9i	11i	25	30	22	27	10	14
E. dissolvens	-	-	-	-	-	-	21	25

i indicates incomplete inhibition

Conclusion

It is concluded from the data from the study that *T. harzianum* (IMI 339496) showed very high activity against all the test organisms and especially *N. corylii* than the other eight *Trichoderma* strains studied. The extracts of *T. harzianum* (IMI 339496) and *T. longibrachiatum* (IMI 339495) showed high antifungal showed high antifungal and antibacterial activity against the various test organisms. It is imperative that the active metabolites in extracts be isolated so that the components of the extracts responsible for the activities are identified. Work on this is in progress and the metabolites involved will be reported in due course. Finally, *T. harzianum* species (IMI 339496) is a potential candidate for the production of useful antimicrobial agents against pathogenic bacteria, fungi and yeasts.

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References

1. Rifai MA. A revision of the genus *Trichoderma. Mycological Paper Commonwealth Mycological Institute.* 1969; 116: 1-56.
2. Aytoun RSC. The genus *Trichoderma*: Its relationship with *Armillaria mellea* (Vahl. ex . Fries) Quel and *Polyporzus schiveinitzii* Fr. together with preliminary observations on its ecology in woodland soils. *Translation Botany Society Edinburg.* 1951; XXXVI: 99-114.
3. Danielson RM and Davey CD. The abundance of *Trichoderma* propagules and the distribution of species in forest soils. *Soil Biology Biochemistry.* 1973; **5:** 485-494.
4. Elad Y; Chet I and Katan J. *Trichoderma harzianum*: A biological control agent effective against *Sclerotina rolfisii* and *Rhizoctonia solani. Phytopathology.* 1980; **70:** 119-121.
5. Elad Y; Hader Y; Hader E; Chet I and Henis Y. Biocontrol of *Rhizoctonia solani Trichoderma harzianum* in carnation. *Plant disease.* 1981; **65:** 675-677.
6. Hader Y; Chet I and Henis Y. Biological control of *Rhizoctonia solani* damping off with wheat bran culture of *Trichoderma harzianum. Phytopathology.* 1979; **27:** 131-135.
7. Lewis JA and Papavizas GC. Reduction of inoculum of *Rhizoctonia solani* in soil by germlings of *Trichoderma hamatum. Soil Biology Biochemistry.* 1987; **19:** 195-201.
8. Trufmann P and Keane PJ. *Trichoderma koningii* as a biological control agent for *Sclerotina sclerotiorum* in Southern Australia. *Soil Biology Biochemistry.* 1990; **229:** 43-50.
9. Sivan A; Ucko O and Chet I. Control of Fusarium crown rot of tomato by *Trichoderma harzianum* under field conditions. *Plant disease.* 1987; **71:** 587-592.
10. Henis Y; Ghaffer A and Baker R. Factors affecting suppressiveness to *Rhizoctonia solani* in soil. *Phytopathology.* 1979; **69:** 1164-1169.
11. Chet I and Baker R. Isolation and biocontrol potential of *Trichoderma hamatum* from soil naturally suppressive of *Rhizoctonia solani. Phytopathology.* 1981; **71:** 286-290.
12. Liu S and Baker R. Mechanism of biological control in soil suppressive to *Rhizoctonia solani. Phytopathology.* 1980; **70:** 404-412.
13. Turner WB and Aldridge DC. Fungal metabolites II. New York, Academic Press. 1983; 592-631.
14. Dunlop RW; Simon A; Sivasithamparam K and Ghisalberti EL. *Journal of Natural Products.* 1989; **52:** 67.
15. Papavizas GC. *Trichoderma Gliocladium*: Biology, ecology and potential for biocontrol. *Annual Review of Phytopathology.* 1985; **23:** 23-54.
16. Collins RP and Halim AF. Characterization of the major aroma constituents of the fungus *Trichoderma viride. Journal of Agriculture and food chemistry.* 1972; **20**: 47-438.
17. Simon A; Dunlop RW; Ghulsalberti EL and Silvasithamparam K. *Trichoderma koningii* produces a pyrone compound with antibiotic properties. *Soil. Biology and Biochemistry.* 1988; **20**: 263-264.
18. Biles CL and Hill JP. Effects of *Trichoderma harzianum* on sporulation of *Conchliobolus sativus* on excised wheat seedlings leaves. *Phytopathology.* 1988; **78:** 656-659.
19. Tronsomo A; Dennis C. The use of *Trichoderma* species to control strawberry fruit rots. *Netherland Journal of Plant Pathology.* 1977; **83:** 449-455.
20. Ayer WA and Dufresne C, *Bulletin Society Chim Belgium.* 1986; **95:** 699.
21. Onsando JM. A biological control approach of armillaria root rot of tea, *Armillaria mellea.* Ph.D. Thesis, Kenyatta University ,Nairobi. 1991; 1-148.
22. Schneider G. Neue testsysteme zur untersuchung antagonistischen und mycophilen pilzen. Ph.D thesis. University of Kaiserslautern, Germany. 1997; 1-90
23. Barry AL; Coyle MB; Thornsberry C; Gerlach EH; Hawkinson RW. Methods of measuring zones of inhibition with the Bauer-Kirby disk susceptibility test. *Journal of Clinical Microbiology.* 1979; **10:** 885-889.