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Assessment of Mycelia Extract from *Trichoderma harzianum* for its Antifungal, Insecticidal and Phytotoxic Importance

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Abstract

Trichoderma harzianum was cultured on potato dextrose broth media at 20°C in an incubator and mycelial cells were extracted with ethyl acetate to obtain the organic extract for *in-vitro* bio-activities including antifungal, insecticidal and phytotoxicity. Different strains of fungal pathogens including *Aspergillus flavus, Rhizopus stolonifer* and *Pythium ultimum* were used to assess the antifungal potential of *T. harzianum* extract. The inhibitory effect was found 82% for *A. flavus*, 77% for *P. ultimum* and 73% for *R. stolonifer* when compared with positive and negative control experiments. Aphids (*Macrosiphumrosae*) as a test insects were used to perform the insecticidal activity that showed potent activity with LC_{50} (38.88 μgml^{-1}). The herbicidal potential was evaluated against duck weed (*Lemna minor*) which showed that by using very high concentration (1000 μgml^{-1}) only 60% lethality was achieved. This pilot study revealed that the organic extract obtained from *T. harzianum* contains useful compounds having potential to be utilized in the development of fungicides and pesticides for the improvement of agricultural sector of the country.

Keywords: *T. harzianum*; Antifungal activity; Insecticidal activity; Phytotoxic activity; Fungal organic extracts

Introduction

Biological control is considered one of the important practices for pest management, as the use of fungicides and herbicides can cause diverse effect on non-targeted organisms [1]. Living microorganisms are applied to the crops; they grow there and inhibit the growth of pests by disturbing their life cycle this is due to the production of some of useful secondary metabolites. One of the important microorganisms among these is *Trichoderma harzianum*, a free living soil born fungus. Optimum temperature require for its growth is 25°C. Different strains of *Trichoderma* species are very well known for their potential as bio control agents and are used as pesticide and biofertilizer [2]. *Trichoderma* fungus is known to possess appreciable antagonistic effect on different phyto-pathogenic fungi [3]. This fungus biologically synthesize different compounds including glisoprenin, gliotoxin, gliovirin, viridian, hepteledic acid, trichoderm amides, polyketides, harzialactones and derivatives of α-amino acids [4]. This research was planned to assess the importance of organic extracts of *T. harzianum* against plant pathogenic fungi isolated from local crops, insects as well to find out if the extract possess any toxicity towards the plants.

Materials and Methods

Chemicals and reagents

The entire chemicals used were of analytical grade. The culture media for the growth of fungi was sterilized before inoculation in autoclave at 121°C for 15 minute.

Growth and extraction of the fungus

The culture of *T. harzianum* was isolated from the soil of district Charssada, Khyber Pakhtunkhwa. The pure culture of *T. harzianum* was obtained by sub culturing it on Potato Dextrose Agar (PDA) media. A small portion of pure culture on PDA was inoculated on Potato Dextrose Broth (PDB) at 25 ± 2°C for 15 days (Figure 1). After 15 days the cell culture was homogenised through blender and extracted with ethyl acetate (EtOAc) by following the standard growth protocol with slight modifications. The organic phase was separated from aqueous, dried over anhydrous $MgSO_4$ and the solvent was evaporated in vacuum under reduced pressure to obtain the extract as brown oil.

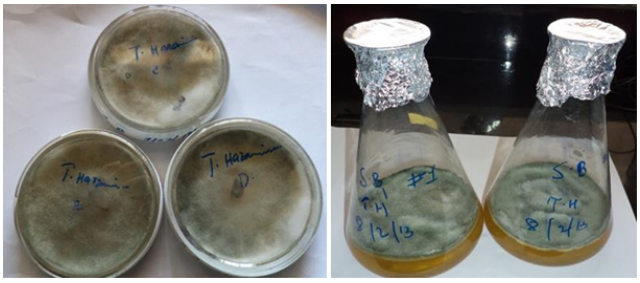

Figure 1: *Trichoderma harzianum* cultures a: Pure culture on PDA b: Culture on PDB.

Determination of antifungal activity

The antifungal activity of organic extract from *T. harzianum* was carried out by ager well diffusion method [5]. Three different pathogenic fungi (*A. flavus* and *P. ultimum* but *R. stolonifer*) isolated

from local soil were used as a test organisms. Stock solution (1000 μgml^{-1}) was prepared by dissolving 8 mg of organic extract from *T. harzianum* in 8 ml of DMSO (Dimethyl Sulfo Oxide). The stock was diluted to 10, 50, 100, 150 and 200 μgml^{-1} by adding sterilised distilled water. To each petridish having PDA media, three wells were made (8 mm in diameter) through sterilized borer. Equal amount (8 µL) of organic extract solution (8 µL) of each concentration was delivered into each well with the help of micropipette, and left for about 10 minutes for diffusion. After complete diffusion of test solution the media was inoculated by placing a PDA slant of pure culture of each test fungi. Blank (aqueous DMSO) and positive control (Diethene M45; 10 μgml^{-1}) was also run parallel. After inoculation the petridishes were sealed with Para film, labeled and incubated for seven days. Once the fungi growth and maturity was achieved the growth diameter of tested fungi was recorded in mm. Equation 1 is used for the calculation of percent inhibition.

$$I\% = \left(\frac{D_c - D_s}{D_c - 5}\right) \times 100 \quad (1)$$

Where I=Growth inhibition by of organic extract,

Dc=fungal growth in negative control,

Ds=fungal growth of sample, measurement done in mm.

Determination of phytotoxic activity

Phytotoxic effect of the crude was measured by percent lethality of *Lemna minor* plants. Stock solution (1000 μgml^{-1}) was made in DMSO and was further diluted to 10, 50, 100, 150, 200, 400, 600 and 800 μgml^{-1} and transfer to transparent cups. About 50 ml E-medium (water medium in which these plants grow) was pour to each cup. Ten fronds of *Lemna minor* were transferred to each cup. Blank (only DMSO) and positive control (Atrazine; 200 μgml^{-1}) was also run parallel under proper light supply at room temperature. After seven days the difference in colour of fronds from green to yellow was observed and affected fronds were counted. The FI_{50} (Concentration that can affect the growth of Fronds) was calculated via probit analysis [6].

Determination of insecticidal activity

Insecticidal activity was investigated against rose aphids using the procedure documented by Isman et al [7]. Stock solution (1000 μgml^{-1}) and further dilutions 10, 50, 100, 150 and 200 μgml^{-1} were prepared by dilution. The filter paper was cut of petridish size and solutions of different concentrations were absorbed on to it, air dried and placed in Petri dishes. Ten mature and good sized aphids were transferred to each petri plate along with fresh rose leaves as natural feed. The mortality count was done at the interval of 3 hours and 12 hours. The mortality (%) was calculated by Equation 2 and LC_{50} was calculated by probit analysis.

$$Mortality\ \% = \left(\frac{ITs - Ib}{Ti}\right) \times 100 \quad (2)$$

I_{Ts}=Insects killed by experimental solution,

I_b=Insects killed in blank experiment,

T_i=Total numbers of insects.

Statistical analysis

All the experiments were carried out in triplication following CRD experimental layout. The data collected was statistically analyzed using MS Excel and presented in Table 1 as mean ± S.D. The statistical descriptive comparison of more values was analyzed by ANOVA following LSD at 5% significance level.

Results

Antifungal activity

Each concentration of mycelial extract of *T. harzianum* showed different level of inhibitory affect against pathogenic fungi *A. flavus* and *P. ultimum* but *R. stolonifer* showed 0% inhibition up to 100 μgml^{-1} (Figure 2). Maximum inhibition was shown by *A. flavus* (82%) followed by *P. ultimum* (77%) and *R. stolonifer* (73%) when compared to positive control (Table 1).

Zone of fungal growth ± S.D (mm)							
Concentrations (μgml^{-1})							
Tested fungi	**Pos.**	**10**	**50**	**100**	**150**	**200**	**Neg.**
P. ultimum	12.7 ± 2 (87%)	47 ± 3.6 (31%)	38 ± 5 (44%)	37 ± 3 (52.5%)	24 ± 3.6 (68%)	18 ± 1 (77%)	65 ± 3 (0%)
A. flavus	15.0 ± 4 (84%)	37.7 ± 4 (47%)	28 ± 2 (62%)	24 ± 2 (71%)	20 ± 1 (75.8%)	16 ± 4 (82%)	67 ± 3 (0%)
R. stolonifer	12.0 ± 1 (82%)	68 ± 1.2 (0%)	68 ± 1.0 (0%)	68 ± 0.8 (0%)	23.6 ± 2 (72%)	21 ± 1 (73%)	68 ± 1 (0%)

Table 1: Anti-fungal activity of organic extract from *T. harzianum.*

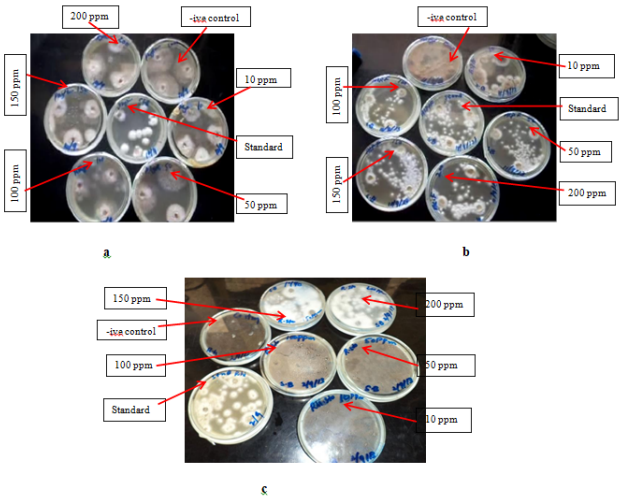

Figure 2: Antifungal activity a: *P. ultimum* b: *A. flavus* c: *R. stolonifer.*

Phytotoxic activity

Positive control (Atrazine) exhibited 100% lethality against *Lemna minor* whereas maximum fronds inhibition (60%) was found at 1000 μgml^{-1} of organic extract while overall FI_{50} was found to be 1786 μgml^{-1} (Figure 3). The results for phytotoxic activity are shown in Figure 4. Such results suggest that the organic extract of the *T. harziamun* is devoid of any compound or if contain is in minor proportion with respect to other metabolites that can cause negative impact on the plant growth (Figure 5).

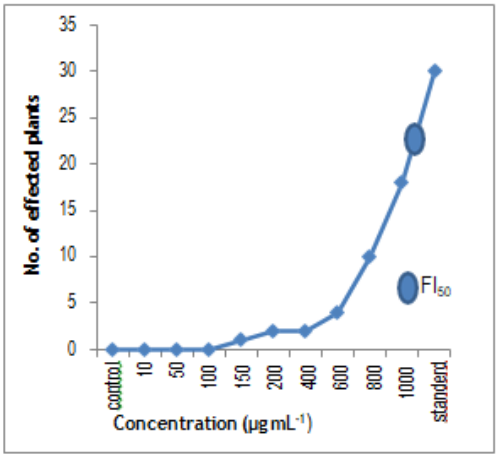

Figure 3: FI_{50} of *Lemna minor* observed in different concentration of *T. harzianum* extract.

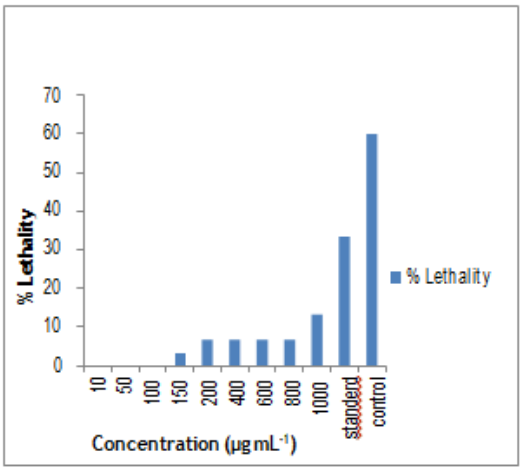

Figure 4: % Lethality of *Lemna minor* observed in different concentration of *T. harzianum* extract.

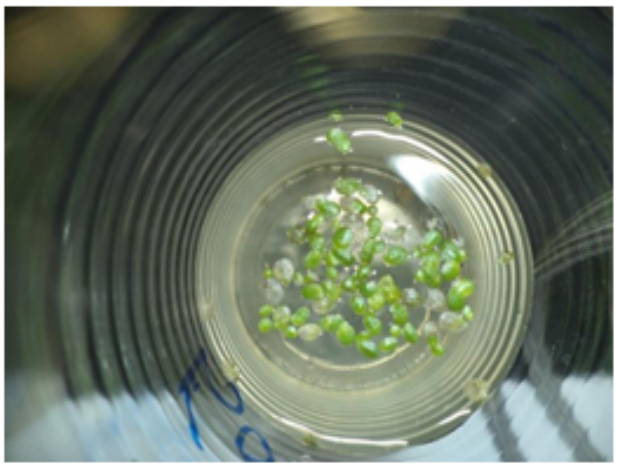

Figure 5: *Lemna minor* plants.

Insecticidal activity

Time dependent difference in mortality rate of aphids was observed where the maximum mortality (93%) was recorded after 12 hours at 200 μgml^{-1} concentration. Result for insecticidal activity is shown by Figure 6. LC_{50} was calculated as 213 μgml^{-1} for 3 hours and 38.88 μgml^{-1} for 12 hours (Figure 7). It is clearly visible from the following graph that even 50 μgml^{-1} killed more than 50% insects population after 12 hours showing the presence of potential aphidicidal metabolites in the mycelial organic extract of *Trichoderma harzianum* (Figure 8).

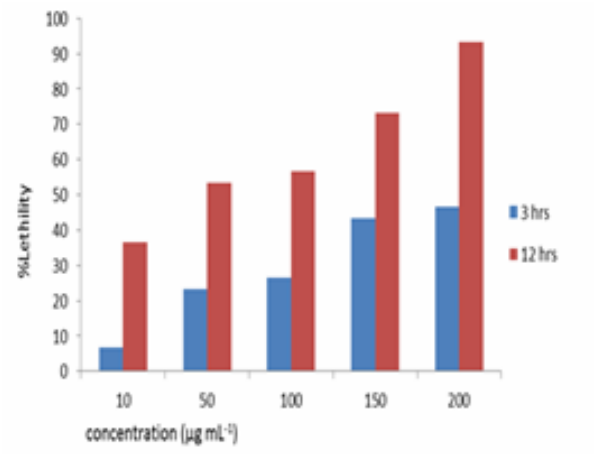

Figure 6: Mortality (%) of the aphids where the positive control (permithrine) showed 100% killing within one hour of application while negative control (EtOAc) showed 0% mortality.

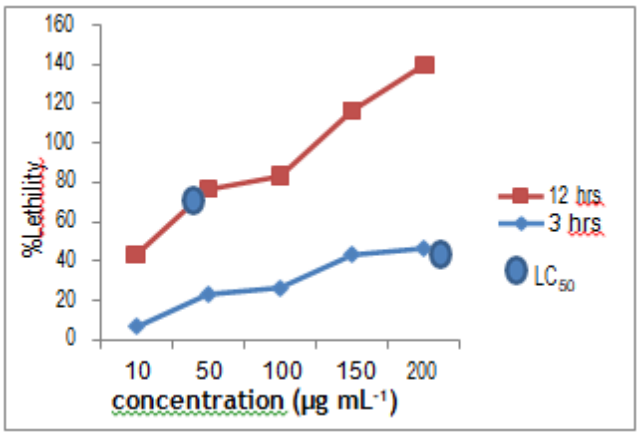

Figure 7: LC_{50} of aphids caused by different concentration of *T. harzianum* extract.

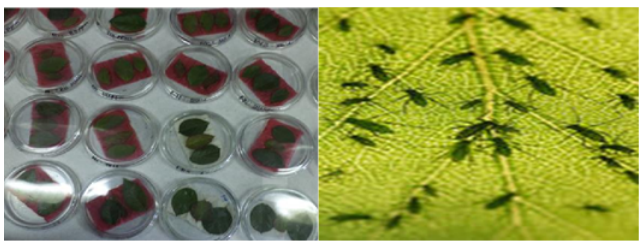

Figure 8: Aphidicidal activity a: Aphidicidal activity b: Aphids.

Discussion

The present study revealed that ethyl acetate extract of the cell culture of *T. harzianum* exhibited potent antifungal activity against plant pathogenic fungi as it produce certain metabolites and enzymes that are useful for fungicides development. *A. flavus* produce aflatoxins which are carcinogenic compounds [8]. The organic extract of *T. harzianum* inhibits its growth by 82%. Various enzymes produced process antifungal potential against pathogenic fungi. *P. ultimum* cause soil born plant diseases. Different species of *T. harzianum* secrete extracellular enzymes i.e., Chitinase, Laminase that restrict the growth of *P. ultimum* [9]. The present study showed inhibition of this pathogen up to 77%. Our results are in strong agreement with their findings.

Phytotoxicity was carried out against *Lemna minor* plants, a sensitive plant show quick response to different chemicals. The FI_{50} (1786 μgml^{-1}) suggest that the extract lack plant hazard compounds. So *T. harzianum* can be used for the development of agro chemicals, fungicides and other pesticides. Furthermore the culture of *T. harzianum* can be used as plant growth promoter as its metabolites activate the defense system of the plant attacked by pathogens [10]. Different types of insects like cockroaches, aphids, weevils, moths are harmful to different plants and food stuffs. The crude extract of *T. harzianum* was screened against aphids for measuring insecticidal activity. The LC_{50} (38.8 μgml^{-1}) indicate that the extract may contain certain metabolites process activity against insects. Different fungal species are toxic to insects. The spore suspension of *T. harzianum* caused 60% reduction in cockroach population [11]. Aphids cause leaf disease in cucumber plants; the fungus Verticillium was applied to effected plants. After 15 days of inoculation the aphid population was highly reduced [12].

Conclusions

The organic extract of *T. harzianum* exhibited potent activities against pathogenic *Macro siphumrosae* and phyto-pathogenic fungi including *A. flavus*, *R. stolonifer* and *P. ultimum*. While, very minor plant toxicity was noticed against *Lemna minor* even at very high concentration. Further studies towards the isolation and identification of potent metabolites responsible for biological activities would lead to the development of more responsive insecticides as well as fungicides. Very minor plant toxicity can also made it more useful for agricultural practice.

References

1. Brimner TA, Boland GJ (2003) A review of the non-target effects of fungi used to biologically control plant diseases. Agriculture, Ecosystems & Environment 100: 3-16.
2. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) Trichoderma species opportunistic avirulent plant symbionts. Nat Rev Microbiol 2: 43-56.
3. Vinale F, Marra R, Scala F, Ghisalberti EL, Lorito M, et al. (2006) Major secondary metabolites produced by two commercial Trichoderma strains active against different Phytopathogens. Applied Microbiology 43: 143-148.
4. Hasan EA, Walker F, Schone J, Buchenauer H (2006) Detection of Viridiofungin A and other antifungal metabolites excreted by Trichoderma harzianum active against different plant pathogens. Eur J Plant Pathol 124: 457-470.
5. Dahham SS, Ali MN, Tabassum H, Khan M (2010) Studies on antibacterial and antifungal activity of pomegranate (Punicagranatum L.). American-Eurasian J Agric & Environ Sci 9: 273-281.
6. Finney DJ, Stevens WL (1948) A table for the calculation of working probits and weights in probit analysis. Biometrika 35: 191-201.

7. Isman M, Arnaen B, Philogene R, Morands P (1987) Insecticidal of plant origin. J Ame Chem Soc 44: 387.
8. Diener UL, Cole RJ, Sander T, Payne GA, Lee LS, et al. (1987) Epdemiology of Aflatoxin formation by Aspergillus Flavus. Ann Rev Phytopathol 25: 249-270.
9. Haggag WM (2011) Biodiversity, biological and molecular investigations of biocontrol by the genus Hypocrea/Trichoderma spp. European Journal of Scientific Research 65: 281-292.
10. Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ, et al. (2008) A novel role for Trichderma secondary metabolites in the interactions with plants. J Physio Mol Pl Path 72: 80-86.
11. Wahid OA, Elabanna SM (2012) Evaluation of the insecticidal activity of Fusariumsolani and T. harzianum against cockroaches; periplaneta americana. African Journal Microbiology 6: 1024-1032.
12. Goettel MS, Eilenberg J, Glare TR (2005) Entomopathogenic fungi and their role in regulation of insect populations. Comprehensive Molecular Insect Science 6: 361-406.