*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706*** **Volume 3 Number 1 (2014) pp. 96-103**
**http://www.ijcmas.com**


**Original Research Article**

# Antimicrobial activity of *Trichoderma harzianum* against bacteria and fungi

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**A B S T R A C T**

A study was undertaken in order to evaluate the antimicrobial activity of *T. harzianum* by well diffusion method and MIC determination. They produce or release variety of compounds that induce localized or systemic responses which explains its lack of pathogenicity to plants. The culture broth extract of *T. harzianum* in SDA media was investigated for antimicrobial activity. In the study undertaken invitro conditions *T. harzianum* showed antimicrobial on most of the test organisms, both bacteria and fungi. It showed maximum antagonistic activity on *A. terreus, A. fumigates, A. clavatus* and also on clinical isolates such as *Staphylococcus aureus*, *E. coli* & *Klebsiella*. The minimum inhibitory concentration of *T. harzianum* on fungal isolates ranges from 100 – 150 µl/ml and for bacterial isolates ranges from 50 – 100 µl/ml of media. *A. niger & A. clavatus* among fungal isolates and *Proteus* among bacteria was resistant to antimicrobial activity of *T. harzianum* extract. The use of *T. harzianum* as a biocontrol agent is gaining a new dimension in their application to resist other pathogenic organism. The antagonistic reaction that can lead to biological control include antibiosis, competition & hyper – parasitism. The results of the study indicate that *T. harzianum* is a source of ecofriendly biocontrol agent against the pathogenic microorganisms.

**Keywords**

*Trichoderma*; Biocontrol agent; MIC determination; Pathogenic.

## Introduction

*Trichoderma* is a genus of fungi that is present in all soils, where they are the most prevalent culturable fungi. Many species in this genus can be characterized as opportunistic avirulent plant symbionts. Cultures are typically fast growing at 25-30°C, but will not grow at 35°C. Colonies are transparent at first on media such as cornmeal dextrose agar (CMD) or white on richer media such as potato dextrose agar (PDA). Some species produce a characteristic sweet or coconut odour (Aneja, 2003). *Trichoderma harzianum* is a common soil, litter, and wood fungus. It possesses highly cellulolytic activity and is main agents of decomposition**.** Several strains of *Trichoderma* have been developed as biocontrol agents against fungal diseases of plants. The various mechanisms include antibiosis, parasitism, inducing host-plant resistance, and competition. Most biocontrol agents are

from the species *T. harzianum*, *T. viride* and *T. hamatum*. *Trichoderma* species are an efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. Different mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compound. Most plant species exploit soil with the help of beneficial microorganisms such as fungi and bacteria, some of which are important in nitrogen fixation and phosphate solubilization. Occurrence and distribution of phosphate solubilizing microorganisms (PSM) have been found in almost all the soils tested, although their populations vary with different soils, climate, and cropping history (Kucey, 1989).

Many recent findings suggest that plant development and biochemistry are strongly affected by *Trichoderma* strains (Mach *et al*., 1993). Nutrient competition, mechanical barriers, or pH changes are some of the antagonistic effects. Both fungi and bacteria are able to synthesize a wide range of metabolites with fungicidal and bactericidal capabilities. These antibiotics are an alternative biological protection to conventional fungicides (Brown 1996). Specific strains of fungi in the genus *Trichoderma* colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense response, which in the end leads to induced systemic resistance (ISR) in the entire plant. The capability of *Trichoderma* species to promote increased growth response was verified both in greenhouse experiments and in the hydroponic system. A 30% increase in seedling emergence was observed and these plants exhibited a 95% increase in root area. Similarly an increase in Phosphorous and Iron concentration was observed in *Trichoderma* inoculated plants. The *Trichoderma* species release antibiotics and other chemicals that are harmful to pathogens and inhibit growth (antibiosis). The potential use of the *Trichoderma* species as a biocontrol agent was suggested more than 70 years ago by Weindling (1932) who was first to demonstrate the parasitic activity of a member of this genus against soilborne fungal or bacterial pathogens. The mechanisms proposed to explain the biocontrol of plant pathogens by *Trichoderma* are presumptive.The suggested mechanisms for biocontrol are antibiosis, lysis, competition, and mycoparasitism (Cook and Baker 1983). These may act alone or in combination. *Trichoderma* species are also effective against various Gram positive and Gram negative bacterial species. They produce among 40 different metabolites of *Trichoderma harzianum* and ciprofloxacin and norfloxacin in cultures of *Trichoderma viridae* which are antibacterial in nature.

## Materials and Methods

### Isolation of Microorganisms

To isolate *Trichoderma* from soil, a *Trichoderma* selective medium was prepared (Mohammad Akrami *et al.* 2011). The basal medium consisted of 0.2 g $MgSO_4$ ($7H_2O$), 0.9 g $K_2HPO_4$, 0.15 g KCl, 1.0 g $NH_4NO_3$, 3.0 g D+ glucose anhydrous, 0.15 g rose bengal and 20g agar. These constituents were added to 950 ml of distilled water and autoclaved at 121°C for 30 min. The biocidal ingredients, 0.25g chloramphenicol, is

mixed in 50 ml of sterilized distilled water and added to the autoclaved basal medium where it cooled to 40 to 50°C. 10 grams of soil were suspended in 50 ml of sterile distilled water and agitated for 30 min at 200 rpm in a rotary shaker. Serial dilutions were made and 0.1 ml of each was spread on the *Trichoderma* selective medium plates with a glass rod. Three plates of each sample were prepared and incubated for 5 days at 30°C. *Trichoderma* isolates were collected and transferred onto CDA plates for maintaining pure culture (Aneja, 2003).

Infected plant samples from agricultural fields and infected seeds were collected using sterile container, needles and blades. The infected plant samples were cut into small pieces and placed on petriplate containing wet blotter paper in it, the infected seed samples were surface sterilized with mercuric chloride, washed sterile water and then these seeds were placed on petriplate containing wet blotter paper.

This set up was incubated at room temperature for 3 – 4 days. After incubation there different kinds of fungus was observed growing on the inoculated infected samples. These cultures were carefully picked separately and lactophenol cotton blue staining was performed and microscopically identified.
Urine samples were collected from different hospitals. These samples were streaked nutrient agar plates by quadrant streak method and the plates were incubated at 37˚C for 24 hrs. After incubation the colonies were observed and the identification of the isolates was made by appropriate staining, culturing on selective media and various biochemical tests.

**Antimicrobial activity of *Trichoderma harzianum***

The antimicrobial activity of this extract was determined by well diffusion method. Approximately $1.5x10^8$cells/ml was prepared and about 1.5 ml of each sample was uniformly spread on Muller Hinton media in glass petri dishes. The plates were left aside for 15 minutes. The wells of 6mm in diameter were punctured in the culture media using sterile cork borers. The stock solution 5, 10, 25, 50 & 100 µl was added to different wells. Care was taken not to over flow the extract. The plates were incubated at 37 ° C for 24 hours for bacteria in a straight position for fungi 25° – 28 ° C for 72 hours, control was also maintained. The diameter of zone of inhibition was measured in mm (Hwa Supchin *et al*., 2001). For fungi, spore suspension of the test organism was prepared using sterile water and spread on sterile CDA containing plates. The wells of 6mm in diameter were punctured in the culture media using sterile cork borers. The *Trichoderma* extract of different amount 5, 10, 25, 50 & 100 µl was added to different wells. These plates were incubated at 25 - 28º C for 3 – 4 days. The diameter of zone of inhibition was measured in mm (Hwa Supchin *et al*., 2001).

**MIC Determination**

The crude extract of *Trichoderma harzianum* was used as stock. A series of test tube were taken which contains 10ml of molten Nutrient agar medium with its temperature being maintained at 45-50° C. From the stock 5-200 µg/ml of crude extract was dispensed into different tubes. The media was then allowed to solidify in the form of slants and were seeded with test organisms. MIC for the fungal test

organisms were performed using SDA medium, control tubes were also maintained. The tubes were kept in slanting position for 30 minutes to obtain SDA slants. The slants were seeded with test organisms. The inoculated slants were incubated at 37° C for 24 hours for bacteria and for fungi 25 - 28 °C for 72 hours. The concentration of the *Trichoderma harzianum* extract at which there is no growth of organism was recorded and considered as MIC of the extract of that organism. The results were expressed in terms of growth of organisms (+) or inhibition of growth (-). The lowest concentration inhibiting fungal growth was noted as MIC (Rath *et al*., 1999).

## Results and Discussion

The concentrations at which the fungal species *Aspergillus fumigatus, Aspergillus flavus, Aspergillus candidus, Cladosporium, Rhizopus* were found to be inhibited was 100µl/ml, whereas *for Aspergillus niger, Fusarium graminarium, Fusarium semitectum, Aspergillus terreus* were found to inhibit by the *Trichoderma harzianum* extract at 150 µl/ml (Table 1 and 2).

Based on the biochemical tests the Bacteria were *E.coli, Klebsiella, Staphylococcus aureus, Proteus.* The fungi include *A. flavus, A. clavatus, A. terreus, A. fumigatus, A. candidus, A. niger, Fusarium graminiarum, F. semitechtum, Rhizopus, Cladosporium*. In our studies the strain of *Trichoderma* also showed a various degree of inhibition to various plant pathogens (Table 3 and 4). When *Trichoderma harzianum* tested for their antagonistic activity against the bacterial species (*Staphylococcus aureus, Proteus, E. coli, Klebsiella*) the isolates were found to be affective at various concentration (Fethi Bel Haj 2008, Parshikov *et al.* 2002). The concentrations at which the bacterial species *Staphylococcus aureus, E coli, Klebsiella* were found to be inhibited by the *Trichoderma harzianum* extract were 100 µl/ml of *T. harzianum* extract *A niger* & *A clavatus* among fungal isolates and *Proteus* among bacteria were resistant to antimicrobial activity of *T harzianum* extract.

*Trichoderma* produce or release a variety of compounds that induce localized or systemic resistance responses, and this explains their lack of pathogenicity to plants. These root-microorganism associations cause substantial changes to the plant proteome and metabolism. Plants are protected from numerous classes of plant pathogen by responses that are similar to systemic acquired resistance and rhizobacteria-induced systemic resistance. Pesticide and organic compound that are widely used to control plant pathogen do not degrade completely and leave toxic residue. Hence scientist has turned on to fungus to their use as a controlling agent. Several various experiment have already been reported showing fungal inhibiting various other plant pathogen. *Trichoderma harzianum* isolate showed strong antagonism against fungal species (*Aspergillus niger, Aspergillus fumigates, Aspergillus flavus, Aspergillus terreus, Aspergillus candidus, Fusarium graminarium, Fusarium semitectum, Cladosporium, Rhizopus*). The same phenomenon was observed by Nashwa M.A. Sallam *et al.,* (2008); Jegathambigai *et al*., (2009). The main theme of our experiment is to show at what concentration does the fungus inhibits or shows the maximum inhibition of growth of other pathogen. When *Trichoderma harzianum* was inoculated against different plant pathogens that are

**Table.1** Antifungal activity of *Trichoderma harzianum* by well diffusion method

| Sl.No | Test organism | Amount of *T .harzianum* extract (µl) Zone of inhibition (cm) | | | | |
|---|---|---|---|---|---|---|
| | | 5 | 10 | 25 | 50 | 100 |
| 1. | *Aspergillus fumigatus* | - | 0.9 | 1.5 | 2.2 | 2.9 |
| 2. | *Aspergillus niger* | - | - | - | - | - |
| 3. | *Aspergillus clavatus* | - | 0.5 | 1.2 | 2.1 | 2.3 |
| 4. | *Aspergillus terreus* | - | - | 3.3 | 3.7 | 4.2 |
| 5. | *Aspergillus flavus* | - | - | 0.8 | 1.5 | 2.1 |
| 6. | *Aspergillus candidus* | - | 0.9 | 1.5 | 1.8 | 2.2 |
| 7. | *Cladosporium* | 1.3 | 1.4 | 1.7 | 1.9 | 2.2 |
| 8. | *Rhizopus* | 0.5 | 1.2 | 1.5 | 1.8 | 2.1 |
| 9. | *Fusarium graminiarum* | 0.7 | 0.9 | 1.3 | 1.6 | 1.8 |
| 10. | *Fusarium semitectum* | 0.6 | 1.1 | 1.5 | 1.9 | 2.0 |

**Table.2** Minimum inhibitory concentration of *T. harzianum* on fungi

| Sl.no | Test organism | Minimumm inhibitory concentration (µl / ml of media ) | | | | |
|---|---|---|---|---|---|---|
| | | 10 | 50 | 100 | 150 | 200 |
| 1. | *Aspergillus fumigatus* | + | + | - | - | - |
| 2. | *Aspergillus niger* | No inhibition within this range of concentration | | | | |
| 3. | *Aspergillus clavatus* | + | + | + | - | - |
| 4. | *Aspergillus terreus* | + | + | + | - | - |
| 5. | *Aspergillus flavus* | + | - | - | - | - |
| 6. | *Aspergillus candidus* | + | + | - | - | - |
| 7. | *Cladosporium* | + | + | - | - | - |
| 8. | *Rhizopus* | + | + | - | - | - |
| 9. | *Fusarium graminiarum* | + | + | + | - | - |
| 10. | *Fusarium semitectum* | + | + | + | - | - |

**Table.3** Antibacterial activity of *T. harzianum* by well diffusion method

| Sl. No | Test organism | Amount of *T .harzianum* extract (µl) Zone of inhibition (Cm) | | | | |
|---|---|---|---|---|---|---|
| | | 5 | 10 | 25 | 50 | 100 |
| 1. | *Staphylococcus aureus* | 1.0 | 1.0 | 1.2 | 1.5 | 2.0 |
| 2. | *Proteus* | 0.8 | 1.0 | 1.2 | 1.2 | 1.8 |
| 3. | *E. coli* | 1.0 | 1.2 | 1.4 | 1.6 | 1.9 |
| 4. | *Klebsiella* | 0.9 | 1.2 | 1.5 | 1.7 | 1.9 |

**Table.4** Minimum inhibitory concentration of *T. harzianum* on bacteria

| Sl.No | Test organism | Minimum inhibitory concentration (µl / ml of media ) | | | | |
|---|---|---|---|---|---|---|
| | | 10 | 50 | 100 | 150 | 200 |
| 1. | *E. coli* | + | + | - | - | - |
| 2. | *Klebsiella* | + | + | - | - | - |
| 3. | *Staphylococcus aureus* | + | + | - | - | - |
| 4. | *Proteus* | + | + | + | - | - |

been isolated from different source of infected plants by standard blot method. The inoculated organism showed a varying degree of inhibition.

It is concluded from the data that *T. harzianum* showed very high antagonist activity against *A terreus* and *A fumigatus* and among the clinical isolates of bacteria, *Staphylococcus aureus* and *E. coli* was found to be sensitive to *T. harzianum* extract. The MIC ranges from 100 – 150 µl/ml for fungal isolates and 50 - 100µl/ml for bacterial isolates. *A. niger* among fungal isolates and *Proteus* among bacteria were resistant to antimicrobial activity of *T. harzianum* extract. *T. hazianum* is an effective biocontrol agent on agricultural crops without causing damage to the plants. In future *T. harzianum* becomes an important biocontrol agent. Farmers can use this on fields instead of chemical fertilizers to cut down their expense on chemical fertilizers.

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