

Antagonistic Activity Of *Trichoderma Koningiopsis* (Th003) Against *Alternaria* Spp

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Abstract: *The fungi Alternaria is a phyto-pathogen cause leaf blight disease to variety of plants. Alternaria alternata and Alternaria helianthi was isolated from Dalbergia sissoo L. and Helianthus annuus L. respectively. Test antagonists grow faster than the pathogen and produced inhibition zones thereby limiting the growth of the pathogen. These antagonistic interactions influence the incidence and severity of the disease caused by the pathogen. When the two Alternaria spp. were tested by dual culture method in combination with T. koningiopsis, the result recorded found that A. helianthi (79.26 %) was most susceptible and revealed high percent of inhibition of mycelia growth while A. alternata (62.38%) showed lowest percent inhibition of mycelia growth.*

In well diffusion method, A.helianthi (64.40 %) showed minimum percent inhibition of mycelial growth than A. alternata (69.84 %). In volatile metabolites screening both the species revealed lowest percent inhibition of mycelia growth than the other three methods of testing. As the biocontrolling agent T. koningiopsis has the ability to produce non-volatile metabolites, antifungal activity of non-volatile metabolites was tested against Alternaria spp. by Agar diffusion method. Between the Two Alternaria spp. A. alternata was found to be more susceptible to non volatile metabolites of T. koningiopsis and showed 79.26 % inhibition of mycelia growth over control, whereas A. helianthi showed lowest percent inhibition than A. alternata (71.30 %).

I. INTRODUCTION

Variety of plants has affected by the *Alternaria* leaf blight which caused by the various species of *Alternaria* genus. *Dalbergia sissoo* and *Helianthus annuus* are infected by the *Alternaria alternata* and *Alternaria helianthi* respectively. Various chemical fungicides were used to control the leaf blight of *Alternaria*. But excess used of these which caused the hazardous health problems that's why is a need to search an alternative method for their control. Due to environmental concerns there is considerable interest in finding alternatives to chemical pesticides for suppression of soil borne plant pathogens and plant-parasitic nematodes (Shaikh and Nasreen 2013). Fungal disease control is achieved through the use of fungicides which is hazardous and toxic to both people and domestic animals and leads to environmental pollution.

Therefore, a more balanced, cost effective and eco-friendly approach must be implemented and adopted farmers. In order to overcome such hazardous control strategies, scientists, researchers from all over the world paid more attention towards the development of alternative methods which are, by definition, safe in the environment, non-toxic to humans and animals and are rapidly biodegradable (Ragab *et al.*, 2012). Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods. *Trichoderma* spp. is now the most common fungal biological control agents that have been comprehensively researched and deployed throughout the world (Rajendiran *et al.*, 2010).

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researched and deployed throughout the world. Several fungal cell wall degrading enzymes like chitinase and glucanase seems to play an important role in the antagonistic action of *Trichoderma* against a wide range of fungal pathogens (Agarwal *et al.*, 2011). *Trichoderma* spp. Species of the genus *Trichoderma* are considered as potential biological control agents (BCAs), and the modes of action include mycoparasitism, antibiosis, competition, enzyme activity and induced plant defence (Fatima *et al.*, 2015).

The species of *Trichoderma* have shown efficiency in the biological control of many foliar diseases. In Senna, the leaf production is severely affected by many foliar diseases. Among them, the leaf spot disease caused by *Alternaria alternata* results in serious yield loss by reducing the leaf biomass and 78% reduction in the senno side yield. The disease incidence is inversely proportional to decrease in the senno side content of the plant. Thus, the application of biological control agents seems to be one of the most promising approaches (Tagaram *et al.*, 2015). The objective of the present investigation was isolation and screening of effective *Trichoderma* spp. against five fungal pathogens of fruit vegetables.

II. MATERIAL AND METHODS

Isolation and identification of *Trichoderma koningiopsis*:

Trichoderma koningiopsis was isolated from the soil by serial dilution technique. Therefore, 1 g of soil was dissolved in 10 ml of sterile double distilled water and mixed well to get 1:10 dilution. From this dilution 1 ml was mixed in 9 ml of sterile double distilled water and mixed well for 1:100 dilution. In the same manner 1:1000 dilutions was prepared. Out of these various soil dilution; 1ml was inoculated in fresh PDA plates and incubated for $28 \pm 2^\circ\text{C}$ for 7 days. After incubation period the growth of different fungal agents was observed microscopically and species of *Trichoderma koningiopsis* was identified by using soil manual of Jha 2004. Identified *Trichoderma koningiopsis* culture was further purified by secondary screening and sub culturing and stored in slant with three replicate at 4°C in refrigerator.

DUAL CULTURE TECHNIQUE

To determine the effect of *Trichoderma koningiopsis* on mycelia growth of *Alternaria spp.*, a dual culture method was used. The dual culture of *Trichoderma koningiopsis* and *Alternaria spp.* was studied on Potato dextrose agar (PDA). 20 ml of PDA medium was poured in plates (9 cm) and was allowed to solidify. Discs (5mm diameter) of mycelium cut from the margin of 6 days old culture of each *Trichoderma koningiopsis* were placed at the edge of each plate 10 mm from the periphery. Then disc of 5mm diameter of mycelium cut from the growing edge of 7 days old cultures of *Alternaria spp.* were placed on each plate, opposite to the mycelial discs of *Trichoderma koningiopsis*. In control plates, a sterile disc Whatman No.1 filter paper of 6mm diameter was placed at opposite side of targeted fungal pathogens in complete aseptic condition. Three replications were maintained for each *Trichoderma spp.* and targeted fungal pathogens separately.

All the plates were incubated at $25 \pm 1^\circ\text{C}$ for about 7 days after inoculation. The radial growth of all fungi was measured, when the *Trichoderma koningiopsis* in control plates show complete growth. The colony diameter of both *Trichoderma koningiopsis* and *Alternaria spp.* were measured at two locations, right angle to each other and the average diameter was calculated. Percent inhibition of mycelial growth of targeted fungal pathogens over control was calculated by following equation given by Vincent (1947).

$$I = \frac{100(C-T)}{C}$$

Where,

I = Inhibition of mycelia growth.

C = Mycelial growth in Control.

T = Mycelial growth in treated.

WELL-DIFFUSION METHOD

Antagonistic activity was carried out by using *Trichoderma koningiopsis* against two *Alternaria spp.* of plants by well diffusion method (Sivakumar *et al.*, 2000). 100 ml potato dextrose broth was inoculated with three disc of 6 mm diameter obtained from the 7 days old culture of *Trichoderma koningiopsis*. The inoculated medium was incubated at $28 \pm 2^\circ\text{C}$ on orbital shaker at 80 rotations per minute (rpm) for 10 days. At the end of incubation period, the culture filtrate was harvested by filtering through two layers of muslin cloth. This obtained culture filtrate was concentrated by freeze drying.

Fresh PDA plates were prepared which had four well of 10 mm in diameter prepared with the help of cork borer situated at 1 cm away from the periphery and equidistant from each other. Then the culture filtrate 0.1 ml was placed in each well of the PDA plates and immediately afterwards, a 5 mm diameter of mycelia disc of targeted pathogen was placed at the center of Petri plates. In control plates, autoclaved culture filtrate was used instead of normally prepared *Trichoderma koningiopsis* filtrate. Then all inoculated plates were incubated at $28 \pm 2^\circ\text{C}$ for 7 days. Observation were made for measurement of radial growth of targeted pathogen and percent inhibition of average mycelial growth was calculated in relation to growth in control by using formula of Vincent (1947).

Detection of antifungal activity by Volatile metabolites from antagonistic fungi: (Dennis and Webster., 1971). The effect of volatile metabolites released by *Trichoderma koningiopsis* were evaluated against growth of targeted *Alternaria spp.* For antifungal activity of volatile, a Petri plate containing PDA medium was inoculated with 5mm diameter plug of *Trichoderma koningiopsis* growing on PDA. A second Petri plate containing PDA was inoculated with a 5mm plug of the targeted pathogens in the center of the plate and inverted over the *Trichoderma koningiopsis* culture. The two plates were sealed together with nescofilm and incubated at 28°C for 6 days. This ensured that both organisms were growing in the same atmosphere. For control instead of *Trichoderma koningiopsis* one plug of PDA was placed on Agar surface. All the experiments in vitro were arranged as Randomized complete design with three replications. The surface areas of

the colonies of targeted pathogens were recorded compared with controls and the percentage of growth inhibition was calculated by using Vincent (1947).

III. DETECTION OF ANTIFUNGAL ACTIVITY BY NON-VOLATILE METABOLITES FROM ANTAGONISTIC FUNGI

To determine the effect of the non-volatile metabolites on mycelia growth of pathogen poisoned food technique was used. For the production of non-volatiles, three discs of mycelia agar plugs (6 mm diameter) obtained from edges of 7 days old culture of *T.koningiopsis* were inoculated in 100 ml sterilized potato dextrose broth (PDB) in 250 ml conical flasks and incubated at $25 \pm 1^\circ\text{C}$ on a rotary shaker at 100 rpm for 10 days. The control conical flasks were inoculated with sterile PDA plugs respectively. After incubation, the culture was filtered through Millipore filter for removing spores for collecting non-volatile metabolites from *T.koningiopsis*. Collect the transparent supernatant containing non volatile metabolites. For poisoned food assay the liquid formed nonvolatile was added to molten PDA medium (at $40 \pm 4^\circ\text{C}$) to obtain a final concentration of 10% (v/v). The medium was poured in Petri dishes at 20 ml per plate and inoculated with 5 mm mycelial plugs of the pathogens in the centre of the plates and incubated at $25 \pm 2^\circ\text{C}$ for 7 days or until the colony reached the plate edge in control plate. Triplicates were maintained for each treatment and radial growth of the pathogen was recorded by using formula of Vincent (1947) (Shaikh and Nasreen, 2013).

IV. RESULTS

When the two *Alternaria spp.* were tested by dual culture method in combination with *T. koningiopsis*, *A. helianthi* was found to be most susceptible and revealed high percent of inhibition of mycelia growth of 79.26 %, while *A. alternata* was revealed lowest percent inhibition of mycelia growth as 62.38 % (Table 1).

Antagonistic activity of *T. koningiopsis* tested by well diffusion method, which revealed that *A.helianthi* showed lowest percent inhibition of mycelial growth (64.40 %) than the *A. alternata* (69.84 % Table 2). The biocontrol agent has ability to produce volatile metabolites which were evaluated against *Alternaria spp.* adopting the method (Dennis and Webster, 1971). In this screening both the species revealed lowest percent inhibition of mycelia growth than the other three methods of testing (Table 3). As the biocontrol has the ability to produce non- volatile metabolites, antifungal activity of non- volatile metabolites was tested against *Alternaria spp.* by Agar diffusion method. Between the Two *Alternaria spp.* *A. alternata* found to be more susceptible to non volatile metabolites of

T. koningiopsis and showed 79.26 % inhibition of mycelia growth over control, whereas

A. helianthi showed lowest percent inhibition than *A. alternata* (71.30 % Table 4)(Plate 1).

Fungal pathogen	Control	Growth of pathogen in mm	% of Inhibition
<i>Alternaria alternata</i>	51 \pm 0.5 mm	19.33 \pm 0.2 mm	62.09 %
<i>Alternaria helianthi</i>	39 \pm 0.6 mm	12.33 \pm 0.3 mm	68.38 %

Table 1: Antagonistic activity of *Trichoderma koningiopsis* by Dual culture method

Fungal pathogen	Control	Growth of pathogen in mm	% of Inhibition
<i>Alternaria alternata</i>	63 \pm 0.7 mm	19.00 \pm 0.3 mm	69.84 %
<i>Alternaria helianthi</i>	44 \pm 0.4mm	15.66 \pm 0.5 mm	64.40 %

Table 2: Antagonistic activity of *Trichoderma koningiopsis* by Well diffusion method

Fungal pathogen	Control	Growth of pathogen in mm	% of Inhibition
<i>Alternaria alternata</i>	59 \pm 0.2 mm	23.66 \pm 0.4 mm	59.89 %
<i>Alternaria helianthi</i>	40 \pm 0.3 mm	23.33 \pm 0.5 mm	41.37 %

Table 3: Antagonistic activity of *Trichoderma koningiopsis* by Volatile metabolites

Fungal pathogen	Control	Growth of pathogen in mm	% of Inhibition
<i>Alternaria alternata</i>	45 \pm 1.2 mm	9.33 \pm 0.8 mm	79.26 %
<i>Alternaria helianthi</i>	36 \pm 1.3 mm	10.33 \pm 0.6 mm	71.30 %

Table 4: Antagonistic activity of *Trichoderma koningiopsis* by Non- volatile metabolites

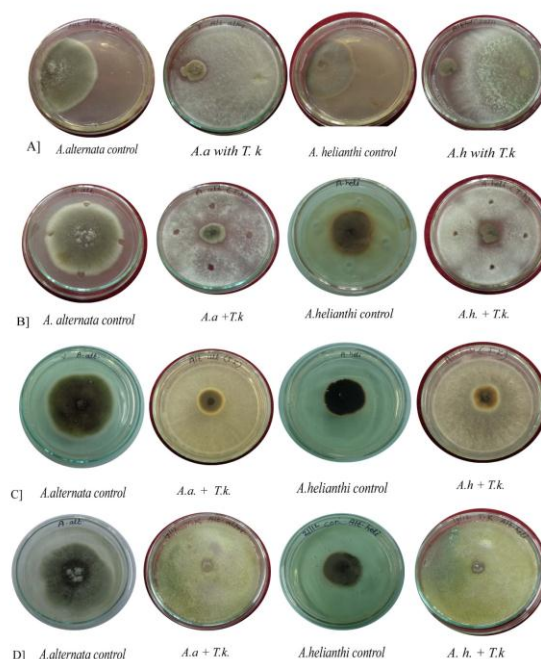


Plate 1: Antagonistic activity of *T. koningiopsis* against *A. alternata* and *A. helianthi*. Fig. A) Dual culture method, B) Well diffusion method, C) Volatile metabolites activity D) Non volatile metabolites activity.

Figure 1

V. DISCUSSION

Our results revealed that *T. koningiopsis* which obtained from the rhizosphere, has been reported as the best biocontrolling agent for controlling the *Alternaria* blight disease of plants caused by the *Alternaria spp.* *T. koningiopsis* treatments reduced the mycelia growth of *Alternaria spp.* It is very important, especially the chemical methods are not economical in the long run, because they cause pollution in the atmosphere, damage the environment, leave harmful residues and can lead to the development of resistant strains among the target organisms with repeated use (Shaikh and Nasreen 2013).

Biological control of plant diseases is eco- friendly approach that utilizes antagonistic microorganisms as a potential, non- chemical means of disease management. One of the goals for the use of biocontrol in agriculture is to avoid the pitfalls associated with overuse of synthetic pesticides, including the development of resistance amongst pest populations (Tapwal *et al.*, 2011).

In the present work *T.koningiopsis* was found to be the best biocontrolling agent for *Alternaria spp.* It was found to be effective against two *Alternaria spp* i.e *Alternaria alternata* and *Alternaria helianthi*. Volatile and non- volatile compounds produced by *T. koningiopsis* had drastically reduced the mycelia growth and conidial production of test pathogens which is helpful in controlling the disease.

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