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Biocontrol Efficacy of Trichoderma Koningii Against some Plant Pathogenic fungi

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ABSTRACT

In the present investigation, Trichoderma koningii was evaluated in vitro for its antagonistic activity against four fungal pathogen viz., Fusarium oxysporum, Fusarium solani, Alternari solani and Rhizoctonia solani causing vegetable diseases. T. koningii strongly antagonized with all pathogenic fungi in dual culture assay and gave maximum inhibition of mycelia growth (91.09%). Volatile metabolites produced by T. koningii exhibited highest growth inhibition rate (54.67%). The Metabolites released from T. koningii were tested in culture medium against all pathogens. Cell free metabolites of T. koningii inhibited the growth of all four pathogens and appeared to be fungicidal in its activity.

Keywords : T. koningii, fungal pathogens, Antagonistic activity.

INTRODUCTION:

Different species of Alternaria, Fusarium, Curvularia, Rhizoctonia, Colletotrichum are most common associates of fruit vegetables all over the world, causing pre and post infections and considerable quality losses. Fungicidal application as seed or soil treatment, however, has been found to be ineffective against these pathogens as the propagules are capriciously distributed in the soil and often beyond the reach of chemicals (Campbell, 1989). 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 (Larkin et al., 1998; Raupach and Kloepper, 1998). Biological control, therefore, holds promise as a strategy for disease management and it is environment friendly too. Different species of Trichoderma are well known to antagonize other pathogenic fungi and success has been achieved in a number of crop diseases. Trichoderma spp. is one of the most important biocontrol agent used for management of different diseases (Harman, 2004). The objective of the present investigation was isolation and screening of effective Trichoderma spp. against four fungal pathogens of vegetables.

MATERIAL AND METHODS

Isolation and identification of *Trichoderma* species:

Trichoderma species were isolated form soil and then identified using standard literature (Jha 2004). The purified and identified cultures of Trichoderma spp. were maintained on Potato Dextrose Agar (PDA) medium and stored at 4°C for further use. When T. koningii was exposed to different pH and temperature, maximum growth rate after 7 days of incubation period was observed at pH 7, and 30ºC temperature.

Dual culture Technique :

To determine the effect of Trichoderma spp. on mycelial growth of targeted pathogens, a dual culture method was used. The dual culture of T. koningii and targeted pathogens were 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 T. koningii was placed at the edge of each plate 10 mm from the periphery and on the opposite side 5mm disc of targeted fungal pathogens were placed on each plate. 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 T. koningii and targeted fungal pathogens seperataly. All the plates were incubated at 25±1˚C for about 7 days after inoculation. The radial growth of all fungi were measured, when the T. koningii in control plates show complete growth. The colony diameter of both T. koningii and targeted fungal pathogens 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):

$$\% \text{ Inhibition} = \frac{D1 - D2}{D1} \times 100$$

D1 = Colony diameter in the control.
D2 = Colony diameter in treated.

Detection of antifungal activity by Volatile metabolites from antagonistic fungi:

The effect of volatile metabolites released by T. koningii were evaluated against growth of targeted fungal pathogens. For antifungal activity of volatile, a petriplate containing PDA medium was inoculated with 5mm diameter plug of Trichoderma isolates growing on PDA. A second petriplate containing PDA was inoculated with a 5mm plug of the targeted pathogens in the center of the plate and inverted over the T. koningii culture. The two plates were sealed together with nescofilm and incubated at 28oC for 6 days. This ensured that both organisms were growing in the same atmosphere. For control instead of T. koningii 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).

Detection of antifungal activity by Non-volatile metabolites from antagonistic fungi:

To determine the effect of the non-volatile metabolites

on mycelial growth of pathogen, poisoned food technique was used. For the production of non-volatiles, three discs of mycelial agar plugs 6 mm obtained from edges of 7 days old culture of T. koningii were inoculated in 100 ml sterilized potato dextrose broth in 250 ml conical flasks and incubated at 25 ± 1oC on a rotary shaker at 100 rpm for 14 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. koningii. Collect the transparent supernatant containing non-volatile metabolites. For poisoned food assay the liquid formed non-volatile was added to molten PDA medium (at 40 ± 5oC) to obtain a final concentration of 10% (v/v). The medium was poured in Petri dishes at 20ml per plate and inoculated with 5mm mycelial plugs of the pathogens in the centre of the plates and incubated at 25 ± 2oC 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).

RESULTS:

When all the pathogenic fungi were tested in combination with T. koningii among them Rhizoctonia solani was found to be most susceptible and revealed highest percent of inhibition of mycelia growth of 91.09%. While Fusarium oxysporum was most resistant and revealed lowest percent inhibition of mycelial growth as 32.14% in combination with T. koningii (Table.1). The biological agent has the ability to produce volatile metabolites which were evaluated against different fungal pathogens of vegetables by adopting the method (Dennis and Webster, 1971). In this screening, when T. koningii was tested, Rhizoctonia solani was found to be more susceptible and showed 54.67%, where as Alternaria solani showed least percent inhibition of mycelia growth as 22.89% over control (Table.2). As the Biological agents has the ability to produce Non-volatile metabolites, Antifungal activity of non-volatile metabolites were tested against all tested fungal pathogens by Agar diffusion method. Among all the fungal pathogens of Alternaria solani was found to be more susceptible to non-volatile metabolites of T. koningi and showed 78.57% inhibition of mycelial growth over control. Whereas Rhizoctonia solani showed minimum percent inhibition of mycelial growth as 72.18% (Table.3).

Table 1. Dual culture technique.

Bioagents	Pathogens	Radial Growth of Bioagents(cm)	Growthof Pathogens (cm)	%of Inhibition
T. koningi	F.oxysporum	5.6±0.02	3.8±0.09	32.14%
	F. solani	7.7±0.04	1.13±0.05	85.32%
	A. solani	7.3±0.04	1.5±0.08	79.45%
Control (pathogens)	R. solani	8.2±0.05	0.73±0.05	91.09%
		-	9.0	-

Each value is an average of 3 replicate samples, + Standard error.

Table 2.Volatile compounds produced by *T. koningii* against five fungal pathogens.

Treatments	Radial Growth of Pathogens (cm)	% inhibition
T. k +Fusarium oxysporum	3.63±0.06	49.08%
T. k + Fusarium solani	3.6±0.09	43.12%
T. k + Alternaria solani	2.93±0.05	22.89%
T. k + Rhizoctonia solani	3.2±0.09	54.67%
Control	9.0	-

Each value is an average of 3 replicate samples + Standard error.

Table 3. Effect of non-volatile compounds produced by antagonist(s) on the radial growth of five pathogens.

Treatments	Radial Growth of Pathogens (cm)	% inhibition
T. k +Fusarium oxysporum	1.26±0.10	75.09%
T. k + Fusarium solani	1.23±0.02	73.26%
T. k + Alternaria solani	0.66±0.05	78.57%
T. k + Rhizoctonia solani	1.26±0.05	72.18%
Control	9.0	-

Each value is an average of 3 replicate samples + Standard error.

DISCUSSION:

Our results revealed that T. koningii, which obtained from the rhizosphere soil, have been reported as the best antagonists for controlling the vegetable diseases caused by fungal pathogens under laboratory conditions. T. koningii inhibited the growth of the targeted organisms through its ability to grow much faster than the pathogenic fungi thus efficiently for space and nutrients. Culture filtrate from T. harzianum Rifai and T. pseudokoningii Rifai strains inhibited the growth of postharvest pathogens of some fruits (Odebode., 2006). Trichoderma species produce both volatile and non-volatile metabolites that adversely affect growth of different fungi (Bruce et al 1984; Corley et al 1994; Horvath et al 1995; Moses et al., 1975). Different workers reported the antagonistic activity of different Trichoderma Isolates against different phtyopathogenic fungi such as R. solani, F. oxysporum and Sclerotium rolfsii (Deshmukh and Raut, 1992; Xu et al., 1993; Askew and Laing, 1994). Different mechanism are said to be involved i.e. competition, production of antibiotics inhibiting fungal growth by producing volatile and non- volatile compounds as reported by (Michrina et al., 1995). No previous report of antagonistic activity T. koningii against these vegetable pathogenic fungi are available.

In conclusion, the T. koningii is found to be effective against all four pathogenic fungi. Volatile and Non-volatile compounds produced by Trichoderma viride drastically reduced the mycelial growth and conidial production of test pathogens which is helpful in disease reduction by checking the survival and spread by pathogen. Use of T. koningii needs further elucidation.

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