Research Article

Antimicrobial Activity of Trichodermaol against Phytopathogens

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ABSTRACT

Trichoderma viridae was isolated from the soil; it was co-cultured with *Fusrium oxysporum* in PDA broth and incubated at room temperature for 7days for the production and extraction of trichodermaol. Trichodermaol showed good sporicidal and inhibitory action (mycelia growth) on *Alternaria, Fusarium*, and *Cladosporium* species at a concentration of 250µg/ml. It also possessed antibacterial activities against the plant bacterial pathogenic genus *Xanthomonas, Pseudomonas, Erwinia* and *Corybacterium* species.

Keywords: Trichodermaol, Antifungal, Antibacterial, Phytopathogens, BCAs.

INTRODUCTION

Soil borne diseases caused by bacteria, fungi and nematodes create a major problem in many crops. Annual yields have been estimated to be an average of 30-35% less than they would be in the absence of pests (Zechendrot, 1995). The traditional method used to protect crops from diseases have been largely based on the use of chemical pesticides but chemical methods are not economical in the long run because they pollute the soil, atmosphere, ground water and leave residues that lead to the development of resistant strain among target organisms with repeated use (Nasety *et al*.,2000). A reduction or elimination of synthetic pesticides application in agriculture is archive the use of new tools Bio-Control Agents (BCAs). BCAs used alone to control or integrated with reduced dose of chemicals minimised the harmful impacts of the chemical pesticides on the environments (Harman and Kubicek, 1998). Till date number of BCAs have been registered and available as commercial products including the strain belonging to bacterial genera *Agrobacterium, Pseudomonas, Streptomyces, Bacillus* , fungal genera *Giocladium, Trichoderma, Ampelomyces, Beaveria, Metarhizium, Conithyrium* and certain viral strain (Ninale *et al*.,2008).

Trichoderma sp. is common fungi found in almost any soil. It interacts with other plant pathogenic fungi and inhibits their growth. The antagonistic nature of fungal species from the genus *Trichoderma* was first demonstrated by Weindling (1932). He suggested their potential use as bio-control agents for plant diseases. Even though the *Trichoderma* sp. have been studied for their antimicrobial potential against plant pathogens for more than two decades their information is extremely limited. In this work the antimicrobial activity of trichodermaol from *Trichoderma* sp. was investigated.

MATERIALS AND METHODS

Isolation and Identification of *Trichoderma* sp.

Soil samples were collected in sterile polythene bags from different types of agricultural field. One gram of soil sample was suspended in 100ml of sterile distilled water (10^{-2}). One ml of sample was transferred into a sterile Petriplate in aseptic condition and pour plate method was performed with malt extract agar. The plates were incubated at room temperature for 5 to 7 days.

After incubation suspected green coloured powdery sporulated colonies were subculture in potato dextrose agar. The fungus was identified according to their colony morphologies, microscopic

appearances of mycelium and spore structures by lacto-phenol cotton blue mount.

Production and purification of Trichodermaol

About 500ml of potato dextrose broth was prepared in 1000ml flask and sterilized at 121.C for 15 minutes. The medium was inoculated with 10mm of agar block of 5days old culture of *Trichoderma viridae* and *Fusarium oxysporum*. The flask was incubated at room temperature with 12hour of light and dark condition per day up to 5days. Finally the culture was acidified with concentrated HCl to pH 2.5 and extracted with ethylacetate. The extract was dried over sodium anhydrous and concentrated the trichodermaol. The concentrated extract was dissolved in acetone and recrytallized. Five milligram of the compound was dissolved in 3ml methanol and analysed by UV spectrophotometer.

Effect of non-volatile phase of trichodermaol against phytopathogenic fungus (Carpenter, 1942)

Various concentration of trichodermaol (50, 100, 150, 200 and 250µl) were incorporated into 100ml of malt extract agar at 60ºC after sterilization and poured into the Petriplate allowed for solidification. The plates were inoculated with 6mm of agar block of plant pathogenic fungus. The plates were incubated at room temperature and the radius of fungal colonial growth was recorded at 3rd, 5th and 7th day of incubation.

Volatile phase effect of trichodermaol against phytopathogenic fungus (El Hassan *et al*., 2007)

Whatman No 1 filter paper disc (3cm diameter) was coated with (250µl/disc) trichodermaol and fixed inside the lid of Petriplate containing malt extract agar already inoculated with test fungus. The plates were closed and the bottom plate with top lid wrapped by two layer of parafilm. The plates were invertly incubated at room temperature for 9 days.

Assessment of sporicidal activity of trichodermaol

Various concentration of trichodermaol (50, 100, 150, 200 and 250µl) was incorporated into 1ml sterile of potato dextrose broth in eppendrof tubes. A loopful of targeted fungal spore suspension (10^3spores/ml) was inoculated into the tubes. The tubes were incubated at room temperature for 6hours, then observed the number of germination and recorded.

Antibacterial activity of trichodermaol

Broth culture of *Corybacterium machiganense, Erwinia amylovora, Pseudomonas lisi* and *Xanthomonas oryzae* were swabbed on Muller Hinton agar plates. Created 6mm of well into the agar medium by using cork borer. The wells were loaded with various concentration of (50, 100, 150, 200 and 250µl) trichodermaol. The plates were incubated at 37ºC for 24 hours and recorded the zone of clearance.

RESULTS AND DISCUSSION

The idea of sustainable agricultural practices and environmental protection has enhanced the importance of biocontrol agents. The fungus *Trichoderma* typically considered soil borne organism associated with the roots of plant, has potency to control plant diseases caused by microbes (Harmen *et al*., 2004). However, the present investigations are mainly focused on the production, purification and antimicrobial potency of an antimicrobial compound trichodermaol from *Trichoderma viridae*.

The isolated *Trichoderma* sp. was identified based on their colony appearances, mycelia morphology and spore shape and arrangements. *Trichoderma viridae* is whitish green colony, microscopic view showed long thick sterile hyphae, side branches are short and thick, grouped phialides in 3 or 4 and conidia is freeze edged spherical. UV spectrophotometer analysis of the ethyl acetate extract of *Trichoderma viridae* show peak values at 235nm, 278nm and 346.7nm which is identified as trichodermaol (Fig.1). Trichodermaol is a

anthraquinone derivative, it is chemically 1, 2, 3, & 4α hexahydromono anthraquinone. The similar compound was previously reported by Adachi *et al*. (1983) from dual culture of *Trichoderma* sp. with *Fusarium* sp. Donnelly and Sherdan (1986) reported that combined culture of *Trichoderma* sp. with *Fusarium* sp. formed pachihasin, chrysophenol and emodin like compounds; *Fusarium* sp. alone did not produce the trichodermaol compound in liquid medium reported by Adachi *et al*. (1983).

The higher concentration of trichodermaol compound was inhibiting the germination of spores of phytopathogenic fungi, range of inhibition of growth from 50 to 75% at the trichodermaol concentration of 200 to 250µg /1ml (Table 1). The compound effectively suppressed the vegetative growth of *Cladosporium cladosporoides, Alternaria alternata, Fusarium oxysporum* and *Fusarium solani* but less action against *Verticillium* sp. (Table.2). Ubalua *et al.* (2007) reported that an isolate of *T.viridae* effectively controlled the spore germination of *Rhizopus* sp. and *Aspergillus* species. Similarly the *Cladosporium cladosporoides, Alternaria alternate, Fusarium oxysporum,* and *Fusarium solani* spore germination was effectively inhibited by trichodermaol but less inhibition against spores of *Verticillium* sp.

The volatile phase of trichodermaol was highly retarding the growth of *Alternaria alternate* on 9th day of post inoculation. Viridofungin and 6-pentolyl adinopensone are the volatile substances occurred in *Trichoderma* culture; they inhibiting the growth of *Fusarium moniforme*, *Cory. michganense* and *Erwinia amylovora* previously reported by El Hasan *et al.* (2007, 2009). Antibacterial activity of trichodermaol produced greatest zone of inhibition (27mm) against *Pseudomonas lisi* and *Xanthomonas campestris*, 15mm zone with *Erwinia amylovora* and 12mm zone of clearance against *Corynebacterium machiganense* (Fig.1). These results suggested that the compound trichodermaol extracted from the *Trichoderma viridae* may be used as a biocontrol agent to control fusarium wilt, fusarium rot, late blight of potato, wet rot of potato, citrus canker, anthraconase of tomato and tomato wilt.

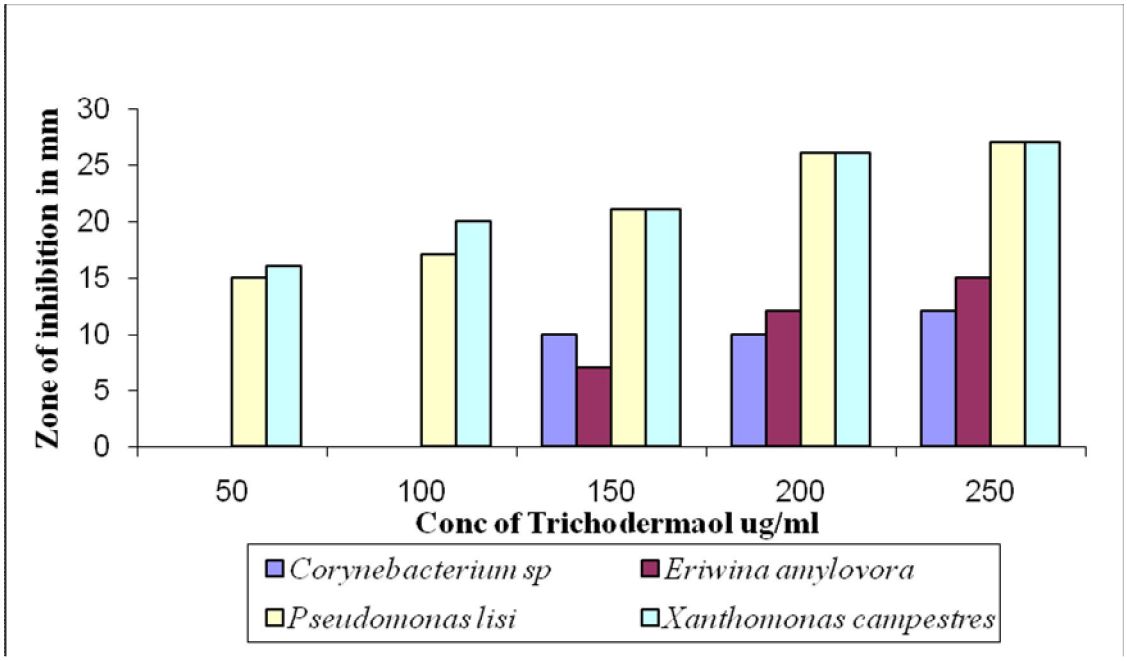

Fig. 1: Antibacterial activities of Trichodermaol

Table 1: Non volatile effect of trichodermaol against various fungal plant pathogens

Conc of Trichodermaol µg/ml	*Alternaria altenata*			*Cladososporium cladosporoitus*			*F.oxysporum*			*F.solani*			*Verticillium sp*		
	Day(s) of incubation														
	3	5	7	3	5	7	3	5	7	3	5	7	3	5	7
	Vegetative fungal size in mm														
50	4.0	4.0	6.0	0	13.0	19.0	0	6.0	8.0	0	0	0	0	0	6.0
100	4.0	9.0	12.0	8.0	14.0	21.0	9.0	9.0	10.0	5.0	6.0	7.0	0	0	6.0
150	7.0	10.0	12.0	16.0	16.0	22.0	12.0	12.0	24.0	8.0	9.0	22.0	0	0	12.0
200	7.0.	12.0	19.0	24.0	20.0	24.0	14.0	13.0	31.0	11.0	17.0	31.0	0	0	13.0
250	12.0	13.0	25.0	24.0	36.0	46.0	18.0	18.0	33.0	21.0	32..0	38.9	0	0	15.0

Table 2: Sporicidal effect of trichodermaol against phytopathogenic fungal spores

Conc of Trichodermaol ug/ml	*Alternaria*	*Cladososporium*	*F.oxysporum*	*F.Solani*	*Verticillium*
50	1+	1+	1+	1+	1+
100	2+	2+	2+	2+	2+
150	3+	3+	3+	3+	3+
200	4+	3+	3+	4+	3+
250	4+	4+	3+	4+	3+

1+ - ≤ 25%, 2+ - ≥ 25% and ≤ 50%, 3+ - ≥ 50% and ≤ 75%, 4+ - ≥ 75% and ≤ 100%

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