Effect of Volatile and Non-volatile compounds from *Trichoderma spp*. against *Colletotrichum capsici* incitant ofAnthracnose on Bell peppers.

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Abstract: Volatile and non-volatile compounds produced from *Trichoderma sps*., Viz., *Trichoderma saturnisporum, Trichoderma harzianum, Trichoderma viride, Trichoderma reesei* were studied by poisoned food technique against *Colletotrichum capsici*, fungal pathogen responsible for anthracnose disease in Bell peppers (*Capsicum frutescence*). The results showed that all the selected *Trichoderma sps* has potential to inhibit the mycelial growth of *C.capsici*. The volatile compounds produced form all the selected *Trichoderma* species showed 30 to 67% inhibition of *C.capsici*, However non-volatile compounds or culture filtrate from *Trichoderma viride* at 3%-4% concentration shows complete mycelial inhibition of the test fungi. *Trichoderma harzianum, T.saturnisporum* and *T. reesei* also have the ability to control growth of *C.capsici* by 21 to 68% at a concentration of 50% culture filtrate. From the results it is clear that all the isolates taken were effective in controlling the pathogen *in-vitro*. . [Nature and Science 2010;8(9):265-269]. (ISSN: 1545-0740).

Key words: Volatiles, Non-Volatiles, *Trichoderma, Colletotrichum cpasici*, Bell peppers, Anthracnose, Fruit rot.

1. Introduction

Capsicum frutescence L. (Bell pepper) is one of the fruit vegetable belongs to the family *Solanaceae*, is an important constituent of many foods, adding flavor, colour, and is rich source of Vitamin 'C'. It is also well known for its neutraceutical properties. *Capsicum* is grown worldwide under various environmental and climatic conditions. In India it is cultivated in an area of about 7,33,800 hectares and is one of the largest fruit vegetable to be exported. In Karnataka state, the *Capsicum* is cultivated in an area of 3,284 hectares, with an annual production of 22,331tones (Anon., 1995). Several fungal diseases attack *Capsicum* crop among which, Anthracnose caused by *Colletotrichum capsici* (Sydow), Butler and Bisby, is most severe causing damage to fruits in the field as well as in storage resulting in loss of about 84 percent. *Colletotrichum* is capable of causing disease on virtually all parts of the pepper plant during any stage of plant growth. However, fruit lesions are the most economically important aspect of this disease. The characteristic feature of anthracnose symptom is formation of acervulli in concentric rings on disease lesion (**Figure 1**.). Since the synthetic fungicides are widely used by the farmers to eradicate pathogens but it results in environmental hazards and have harmful side effects on human beings and animals. The chemical fungicides not only develop fungicide resistant strains but also accumulate in food and ground water as residues. 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, one such strategy is use of Biocontrol agents (BCAs) to control fungal plant diseases. Among the BCAs, species of the genus *Trichoderma* is most promising and effective biocontrol agnet. *Trichoderma* as antagonist controlling wide range of microbes was well documented and demonstrated for more than seven decades ago (Weindling, 1934), but their use under field conditions came much later (Chet et al., 1997), and their mechanism of mycoparasitism is much more complex, involves nutrient competition, hyperparasitism, antibiosis, space and cell wall degrading enzymes.

In the present investigation four *Trichoderma* species namely *Trichoderma saturnisporum, Trichoderma harzianum, Trichoderma viride, Trichoderma reesei* was tested aginst *Colletotrichum capsici*. Both volatile and non-volatile (culture filtrate) compounds from *Trichoderma* were evaluated for growth of test fungus.

Fig.1 disease lesion showing acervulli in concentric rings on Capsicum fruit.

2. Materials and Methods

Fungal cultures:

Colletotrichum capsici was isolated from naturally infected *Capsicum* fruits and four *Trichoderma* species were isolated from rhizosphere soil from major *Capsicum* growing areas of Karnataka State, India. All the fungal cultures were isolated and maintained on Potato Dextrose Agar (PDA).

Effect of Volatile compounds from antagonist(s) on the radial growth of C.capsici:

The method used to test volatile compounds from *Trichoderma saturnisporum, Trichoderma harzianum, Trichoderma viride, Trichoderma reesei* on radial growth of *C.capsici* were followed as per the method given by Dennis and Webster (1971). The Two bottom portion of Petriplates containing PDA were inoculated with a 5mm disc of pathogen and antagonist respectively and both inoculated bottom plates were placed facing each other and sealed with cellophane adhesive tape. The petriplate containing PDA without antagonist serves as control. The observations on the radial growth of the test fungus were recorded after 7 days of incubation at 28±1ºC. The colony diameter of the test fungus in the treatment in comparison with that of check gave percent growth inhibition.

Effect of non-volatile (culture filtrate) compounds from antagonist(s) on the radial growth of C.capsici:

The biocontrol agents were grown in Potato dextrose broth at 27 ºC with intermittent shaking at 150 rpm. The metabolites were collected after 12 days and filtered. The sterilized filtrate were amended in PDA to make 5,10,25 and 50% concentration in petriplates. The solidified agar plates in triplicates were inoculated at the centre with 6mm diameter mycelial disc of pathogen and incubated at 27 ºC for 7 days. The Plates without filtrate served as control. The Colony diameter was measured and percent inhibition of radial growth was calculated.

3. Results

Effect of Volatile compounds:

After 7 days of incubation, it is observed that volatile compounds from *Trichoderma harzianum* exhibited maximum growth inhibition (67%) of *C.capsici* when compare to the others. The *Trichoderm saturnisporum* and *Trichoderma reesei* exhibited 59.39% and 30.45% growth inhibition respectively. Whereas, *Trichoderma viride* shows least growth inhibition of test fungus which is about 8.81%.(Figure.2, Table.1).

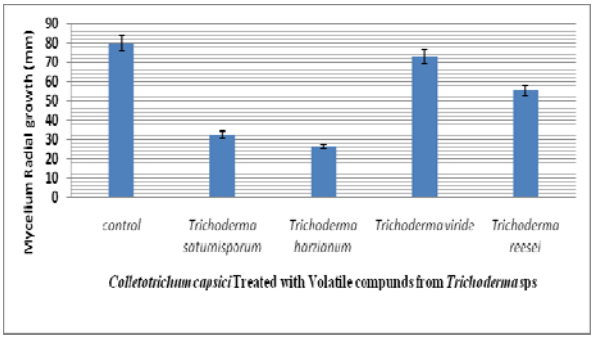

Figure.2 Effect of volatiles from Trichoderma on Radial growth of *C.capsici*

Table.1: Radial growth of *C.capsici* treated with volatile compounds from *Trichoderma* after seven days of Incubation.

Name of the organism	Radial growth of Mycelium (in mm)	Percent Growth Inhibition (in %)
Trichoderma saturnisporum	**32.36**	**59.39**
Trichoderma harzianum	**26.3**	**67**
Trichoderma viride	**72.7**	**8.81**
Trichoderma reesei	**55.43**	**30.45**
Control	**79.7**	

*The experiment was performed by maintaining three replicate per treatment.

Effect of Non-volatile compounds:

The culture filtrate (Non-volatile compound) from all the *Trichoderma* species exhibited growth inhibition except *Trichoderma harzianum* which is inhibiting the growth of test fungi (*C.capsici*) by 21.83% at 50% concentration. It is observed that culture filtrate from *Trichoderma* viride shows complete mycelial inhibition at a concentration of

5%. Whereas *Trichoderma reesei* and *Trichoderma saturnisporum* exhibited 68.37% and 44.27% (Figure.3, Table.2) mycelial inhibition of test fungus respectively when compared to control.

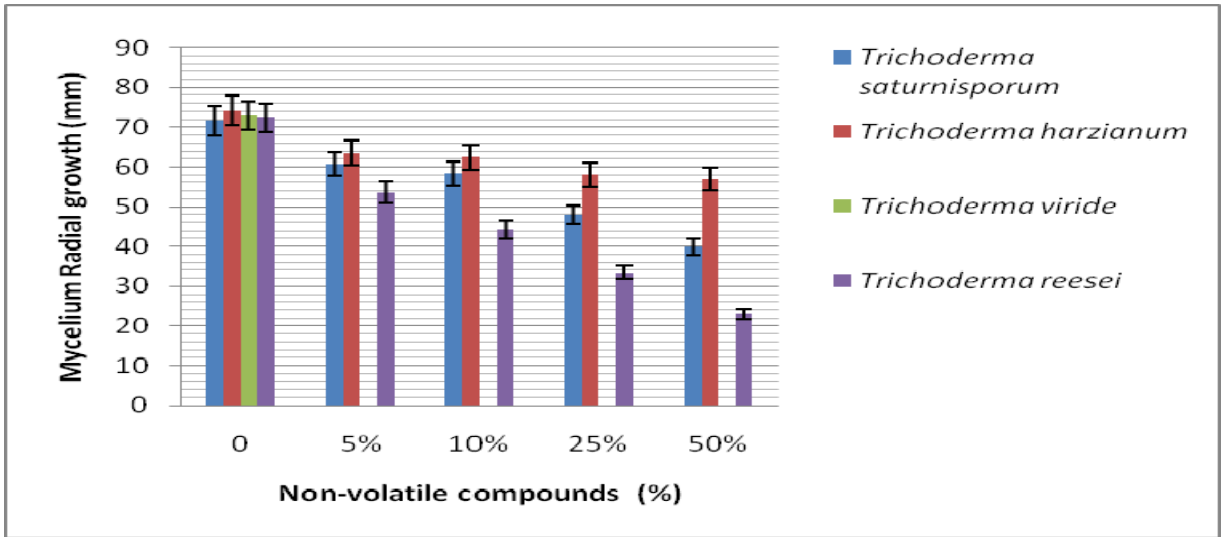

Figure.3 Effect of Culture extract (Non-volatile compounds) of *Trichoderma* spp on radial growth of *C.capsici*

Table.2: Radial growth of *C.capsici* treated with different concentration of *Trichoderma* Culture extract (Non-volatile compounds) after 7 days of incubation.

Name of the organism	Radial mycelium growth (in mm)				
	Concentration of Culture filtrate (in %)				
	0%	**5%**	**10%**	**25%**	**50%**
Trichoderma saturnisporum	71.6	60.8	58.4	47.9	39.9
Trichoderma harzianum	74.2	63.5	62.5	58	56.9
Trichoderma viride	72.9	0	0	0	0
Trichoderma reesei	72.4	53.7	44.3	33.4	22.9

*The experiment was performed by maintaining three replicate per treatment.

4. Discussions

Trichoderma have long been recognized as agents for the control of plant disease and for their ability to increase plant growth and development. The antagonistic nature is may be due to antibiosis, nutrient competition and cell wall degrading enzymes. In the present study, we tested *Trichoderma* species for their production of volatile and non-volatile compounds that inhibit the growth of *Colletotrichum capsici in-vitro*. From the results it is evident that volatile compounds from *Trichoderma harzianum* suppress the mycelial growth of *C.capsici* and found effective when compare to others. The earlier studies also revealed that antimicrobial metabolites produced by *Trichoderma* is effective against a wide range of fungal phytopathogens eg., *Fusarium oxysporum, Rshizoctonia solani, Curvularia lunata, Bipolaris sorokiniana and Colletotrichum lagenarium,Colletotrichum acutatum, Colletotrichum gloeosporioides* (yan *et al* 2006, Svetlana *et al* 2010). Also it is found that there is large variety of volatile secondary metabolites produced by *Trichoderma* such as Ethylene, Hydrogen cyanide, Aldehydes and Ketones which play an important role in controlling the plant pathogens (Vey *et al* 2001).

The non-volatile secondary metabolites from *Trichoderma* species were found more effective in suppressing the mycelial growth of *C.capsici* when compared to volatile compounds. It is observed that the non-volatile compounds from *Trichoderma viride* completely inhibits the radial mycelial growth of *C.capsici* at a concentration of 5%. Later we further reduced the concentration of culture filtrate and found that it is completely inhibiting at a concentration between 3%- 4%. As it is showing complete inhibition at a very low concentration *in-vitro*, there is need to give more importance for further studies *in-vivo* is in progress. It is also observed that with an increase in concentration of culture filtrates of all the *Trichoderma* species, the radial mycelial growth of test pathogen was proportionally decreased. The same with volatile compounds the greater the amount of volatile compounds less is the radial growth. It is reported and well documented that *T.viridae* were used as potential antagonist for controlling many fungal plant pathogens such as *Colletotrichum acutatum, Colletotrichum falcatum, Fusarium oxysporum* (Porras *et al.*, Deshmukh *et al* 2010, Ryota *et al* 2010). Although species of *Trichoderma* is used extensively to control many plant pathogens but no significant work has been done to control anthracnose of bell pepper caused by *C.capsici*. In the present study an attempt was made to test volatiles and non-volatiles produced from *Trichoderma* to control the growth of *C.capsici in-vitro*. Based on the present investigation a new strategy will be developed for controlling Anthracnose on *Capsicum frutescence in-vivo*.

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