Research Article

Antagonistic Effect of *Trichoderma* Species against Various Fruit Pathogens

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ABSTRACT

The present research deals with the study of antagonistic activities of different *Trichoderma* species were *in vitro* against *Colletotrichum capsici, Botryodiplodia, F.oxysporum,* causal agent of fruits rot of chilli, guava, tomato. By means of dual culture, colony interaction, culture filterate were also examined for fruit pathogens. Dual culture test showed that *Trichoderma harzianum* was effectively inhibited mycelia growth of the pathogens. *T.harzianum* showed the highest inhibition (69.0%) and mycelia over growth (55.6%). Colony interaction between soil fungi and *F. oxysporum* was showed better than other pathogens. In effect of culture filtrate was showed maximum inhibition of the pathogens. Chemical control of the test isolates was also done with zineb then thiophanatemethyl. Therefore *Trichoderma* species is a potential agent for biological control of fruit pathogens.

Keywords: *Trichoderma* species, Antagonism, *Colletotrichum capsici, B.theobromae, F. oxysporum.*

INTRODUCTION

The antagonistic microorganism of the rhizosphere are important determinants of plant health and soil fertility because of their participation in several key processes such as those involved in the biocontrol of pathogens, nutrient cycling and seedling establishment. As most of the soil borne plant pathogens are fungi have been used recently as biocontrol agent and their isolate become commercially available of late. This development is largely the result of the change in public attitude towards the use of chemical pesticides and fumigates[1]. The biological control of plant pathogens by antagonistic microorganism is a potential nonchemical means and is known to be a cheap effective eco-friendly method of the management of crop diseases.

Trichodermaspp. is the most studied biocontrol agent against plant pathogens because of their ability to reduce the population of soil borne plant pathogens *Trichodermaspp*. has proved to be useful in the control of phytopathogens affecting different crops. *Trichodermaspp* have shown biocontrol activity against damping–off and root rot diseases and have high yield of plant. Biological control of soil borne pathogens may resolve problems by introducing antagonistic fungi in the soil. *Trichodermaspp.* have been identified as most common fungal antagonistic. Several strains of *Trichoderma* have been found to be effective as biocontrol agent of various soil and seed borne plant pathogenic fungi.

Chilli is an important vegetable and spice crop worldwide and one of the most important vegetables in India. Chilli crop suffers from many diseases like damping off, foot rot, anthracnose, dieback fruit rot, wilt, leaf spots, powdery mildew among the diseases damping off diseases caused by *Rhizoctonia soloni, Fusarium oxysporum,* and *Alternaria alternate,* respectively, is the most prominent and prevailing ailment which has attained the economic importance. In recent year damping off diseases is causing increasingly the economic losses in chilli diseases play a vital role in reducing the yield of the chili. Out of the fungal diseases anthracnose incited by *Colletotrichum capsici* is very important. Guava (*Psidium guajava L.*) is an arbores cent shrub or small tree and is one of the popular fruit of the Punjab in Pakistan. It belongs to the family Myrtaceae and is one of the most gregarious of fruit trees. Guava decline caused by different pathogens *Botryodiplodiatheobromae, Fusarium oxysporum, Psidii, Phytophthoraparasitica* and *Fusarium solani Psidii* is a serious disease and causes considerable losses. Among the pathogens, *B.theobromae* and *F.oxysporum, Psidii* are predominant pathogens which are mainly responsible for decline.

The control of the disease by using only antagonist is effective, but its effectiveness increases when fungicides are used as soil drenching along with the antagonist. Tomato (*Lycopersiconesculentum L.)* is among the world's largest vegetable crops and known as healthy food, because of its special nutritive value and widespread production. It is one of the most important nursery-based vegetable crops cultivated for its fleshy fruits. Tomato plants are subjected to attack by several soil born fungal pathogens, which cause serious diseases as root rot and wilt. The natural control of several phytopathogens is based on the presence of suppressive soils where several biocontrol microorganisms belonging to *Trichodermaspp* are detected. *Trichoderma spp*. has proved to be useful in the control of phytopathogens affecting different crops. The aim of the present study was to evaluate *in vitro* biological control of *Trichoderma* species against fruit pathogens.

MATERIALS AND METHODS

Sample collection

The infected fruits were collected from the Mannargudi, local vegetables market at Thiruvarur district, Tamil Nadu South India. The collected samples were brought to the laboratory in sterilized polythene bags. *Trichoderma* species were isolated from soil samples by dilution plate technique and identified by the standard manuals[2].

Isolation and identification test organism

Infected fruits of chilli, tomato, guava were collected from the local market and brought into the laboratory. They were then cut in to small pieces (0.5 to 1 cm length). The tissue containing the infected regions were surface sterilized with 0.1 per cent mercuric chloride solution. The cut pieces of infected fruits bits as well as infected tissues were taken in that solution and kept for about 30-40 seconds. Then they were carefully removed and washed in repeated changes of sterilized distilled water at least for five times. Finally the bits were blotted to dry. These bits were then plated on to the solidified PDA medium. At least 4 bites were plated. The plates were then incubated at 30˚C ± 2˚ C for five days in the laboratory. The organisms that grow from the infected specimen over PDA medium. The fungi were identified by using wet mount technique[3].

Pathogenicity Test

The pathogenicity test was performed using cut fruits test. The fresh healthy fruits of samples were collected and washed with sterile distilled water. Fruits surface was tested and wounded. The one drop of the conidial suspension was placed on the wounded region. The inoculated fruits were incubated in damp chamber. Damp chamber were prepared in enamel trays. The trays were thoroughly washed and sterilized with alcohol. Then three to four layer of sterilized filter paper were placed and moistened with sterilized distilled water. Sterilized glass rods were used as support for the fruits. The fruits were placed vertically over the glass rods. Holes were made in opposite around the fruits with the help of cork borer (0.5mm). Pure culture of the pathogens were inoculated into the holes with help of sterilized forceps. Conidial suspension of pathogens were also inoculated into fresh chilli fruits at random with the help of sterilized disposable syringe and needle. The trays were covered with polythene paper and pin holes were made for the passage of then the trays were incubated in the laboratory for a week. The appearance of the diseases symptoms and the development were recorded. The symptoms were compared with the symptoms of the disease as observed in the field[4].

Antibiotic Interaction

Dual culture

Colony interaction between the test pathogens and soil fungi were studied in *in vitro* in dual culture experiments. The individual test organisms, namely *Colletotrichum capsici, Botryodiplodia theobromae, Fusarium oxysporum* and in individual species of the soil fungi viz *A. flavus, A. fumigate, A niger, A. terreus, A .luchuensis, T.harzianum, Penicillium sp., T.harzianum, T.koningii* were grown separately on PDA medium. Then the agar blocks (5mm thickness) cut from the actively growing margin of the individual species of soil fungi and test organisms were inoculated juxtaposed to each other approximately 3cm apart, on potato dextrose agar medium in petriplates. Three replicates for each set were maintained. Control was set in single and dual inoculated cultures of the fungus. The position of the colony margin on the back of the disc was recorded daily. Assessments were made when the fungi had achieved an equilibrium after which there was no further alteration in the growth Since both of the organisms were mutually inhibited, the assessment was made for both organisms[5].

Culture Filtrate method

The biocontrol agent was grown in potato dextrose broth at 27º C with intermittent filtrate was amended in PDA to make 5%, 10% and 15% concentration in petriplates. The solidified agar plates in triplicates were inoculated at the centre with 6 mm diameter mycelia disc of pathogen and incubated at 27º C for 7 days. The plates without filtrate served as control. The colony diameter measured and percent inhibition of radial growth was calculated.[6]

$$\%\ of\ Inhibition\ of\ Growth = \frac{Growth\ in\ control - Growth\ in\ treatment}{Growth\ in\ control} \times 100$$

Effect of chemical fungicide on the growth pathogens

Fungicidal activity of commercial fungicide Zineb, Thiophanatemethyl was tested against *C.capsici, B.theobromae, F.oxysporum,* by using disc diffusion method.

The PDA medium was prepared and sterilized at 121º C for 15 minutes and allow it to cool approximately 50ºC. Then the medium was poured into the sterile petriplate. After solidification the isolated pathogen *C .capsici, Botryodiplodia theobromae, F.oxysporum* were swabbed on the agar plate with help of sterile cotton buds.

Disc preparation

The whatmann NO. 1 filter paper was used to disc preparation.

The disc size was 6mm the commercially available chemical fungicides Zineb Thiophanatemethyl fungicide 0.5 gm diluted in 10 ml of sterile distilled water and added into the disc and the disc were maintained in hot air oven at 45º C till reach required concentration.

After disc preparation the disc were placed on the PDA medium. The test plates were repeated count in triplicates.

The plate were stored in incubated at 27° C ± 2° C for 48 hours. After incubation, the result were recorded.

Statistical analysis

Random sampling was used for the entire test. The data of all the parameter were statistically analyzed and expressed as Mean ± S.D.[7]

RESULTS AND DISCUSSION

Fungal pathogens were isolated from the infected fruits such as chilli, guava, tomato in the laboratory. Infected skin parts of the fruits cut into small pieces and surface sterilized with 0.1 % mercury chloride. After incubation the plates were observed for mycelial growth. Individual fungal colonies were separated and transferred to fresh potato dextrose agar and the pure cultures were maintained. Fungal species were identified by lacto phenol cotton blue technique using the manual of soil fungi. Among the different fungal colonies, three predominant colonies were selected for further study namely, *Colletotrichum capsici, Botryodiplodia theobromae, Fusarium oxysporum.*[8]

Antagonistic effect

Dual culture method

Compared to *T.koningii* the *Trichoderma harzianum* species was effectively control all the pathogens. Especially *Fusarium oxysporum* was effectively controlled by *Trichoderma harzianum* and measured as (35.5±0.7 mm) followed by *Botryodiplodia theobromae* (30±0.12 mm) *C.capsici* (20±0.8 mm)

Antibiotic Interaction

Colony interaction between soil fungi and C .capsici

The maximum percentage inhibition of *Collectotrichum capsici* was done by using *Trichoderma harzianum* (66.7±0.35mm) followed by *T.koeningii* (63.6±0.35mm), *P.javanicum* (47.6±0.20 mm), *A. sulphureus* (36.8±0.23mm), *A. terreus sp* (35.3±0.23 mm) and *penicillum* (31.5±0.13mm) (Table-1).

Colony interaction between soil fungi and *Botryodiplodia theobromae*

The maximum percentage inhibition of *Botryodiplodiaspp*. was noticed in *T.harzianum* (66.0 ±0.7mm) followed by *T. koningii* (63 .5±0.23mm), *A. niger* (60.0 ±0.32 mm), *A. flavus* (57.9 ±0.21 mm), *A. sulphureus* (55.0 ±0.22 mm) *P. javanicum* (53.3 ±0.24 mm), *A. terreus* (45.4 ±0.2 mm), *A. luchuensis* (37.5 ±0.9 mm), *Penicillum* (34.5 ±0.9 mm) and *A. fumigates* (31. 2±0.15mm).

The mycelium of *T. hazianum*and *T .koningii* were found growing over the pathogens. (Table -2)

Colony interaction between soil fungi and *Fusariumoxysporum*

The maximum percentage inhibition of was *F.oxysporum* identified in *T.harzianum*, (71.2±0.12 mm) followed by *T.koningii*, (69.0±0.20 mm), *A.niger* (68.7±0.18 mm), *Penicillum* (68.0±0.23 mm), *A. sulphureus* (66.7±0.25 mm), *P.javanicum* (53.3±0.28 mm) *A.luchuensis* (50.0±0.25mm), *A.fumigatus* (47.0 ±0.14 mm), *A. flavus* (42.9 ±0.26 mm), *A.terreus* (33.0±0.15mm).

The mycelium of *T.harzianum*, and *T.koninjii* were found growing over the pathogens (Table-3).

Effect of culture filtrate of fungi on the growth of test pathogens

The maximum percentage of inhibition of growth of *Collectotrichum capsici Botryodiplodia, Fusariumoxysporum* as on the potato dextrose agar medium amended with 25% of the culture filtrate of *T.harzianum* (68.2±0.16 mm), followed by *T.koningii* (60.7±0.25mm), *A.niger* (59.6±0.19mm), *P.javanicum* (48.8±0.20mm) and *A.luchuensis* (44.1±0.16mm) Comparatively the culture filtrate *T.koningii* was more effective than other species of fungi. The dominant of culture filtrate of *Trichoderma* were more effective than other species of fungi. Compared to *T. harzianum*, was showed better control of *Colletotrichumcapsici, Botryodiplodi, F. oxysporum* (Table -4,5,6).

Effect of chemical fungicides on the growth of test pathogens

Effect of zineb

Zineb was amended with potato dextrose agar medium in various concentrations viz 10, 20, 50 and 60 ppm. The percentage inhibitions of *Colletotrichum capsici, Botryodiplodia, Fusarium oxysporum* were as expressed in terms of ppm concentration (55.1±0.2mm), (60.9±0.35mm), (67.1±0.37mm), (72.9±0.39 mm) respectively and also increase the concentrations of fungicide and percentage of inhibition also increased. (Table-7)

Effect of Thiophanatemethyl

Thiophanatemethyl was suspended with potato dextrose agar medium in various concentration viz, 10, 20, 40 and 60 ppm. The percentage inhibitions of *Colletotrichum capsici, Botryodiplodia theobromae*, *Fusarium oxysporum* were expressed in terms of ppm concentration as (31.9±0.7 mm) , (39.7±0.8 mm), (45.8±0.17 mm), (50.1±0.17 mm) respectively increased the percentage of inhibition when the concentration of fungicides was increased and percentage of inhibition also increased. Compared to Thiophanatemethyl, zineb had exhibited maximum inhibition of tested pathogens namely *Colletotrichum capsici, Botryodiplodia theobromae, Fusarium oxysporum* (Table-7).

Hence our study clearly indicated that, antagonistic effect of *T. harzianum* was better than *T.koningii* and the tested fruits pathogens. Among the pathogens tested, *F.oxysporum* (Tomato) was effectively controlled. Comparative to soil fungi culture filtrate test, *T.harzianum*

was exhibited maximum control effect on the tested pathogens.

From the commercial fungicides aspects, zineb was showed maximum zone of inhibition of the tested pathogens.

Control of soil borne plant diseases is possible through the use of antagonistic microorganism as well as with the use of fungicides in the form of soil drenches.

Table 1: Antagonistic effect of *Trichoderma* species against *C. capsici*

Incubation (days)	Average diameter of mycelial growth (mm)				
	T.harzianum	*T.koningii*	*R .soloni*	*C .capsici*	Percentage of incubation
3	50.0±0.12	45.0±0.8	30.0±0.7	45.2±0.15	41.7±0.13
5	55.0±0.20	50.0±0.18	25.0±0.12	40.0±0.17	40.0±0.17
6	60.0±0.28	55.0±0.25	20.0±0.19	30.0±0.25	36.0± 0.25

Table 2: Antagonistic effect of *Trichoderma* species against *Botryodiplodiatheobromae*

Incubation (days)	Average diameter of mycelial growth (mm)				
	T.harzianum	*T.koningii*	*B.theobromae*	*R .soloni*	Percentage of inhibition
3	40.0±0.15	35.5±0.7	45.2±0.8	30.0±0.7	38.4 ±0.8
5	50.0±0.25	40.0±0.15	40.0±0.15	25.0±0.12	38.3±0.12
6	56.5±0.28	45.5±0.28	30.0±0.25	20.2±0.15	35.5±0.25

Table 3: Antagonistic effect of *Trichoderma* species against *F. oxysporum*

Incubation (days)	Average diameter of mycelial growth (mm)				
	T.harzianum	*T.koningii*	*F.oxysporum*	*R.soloni*	Percentage of inhibition
3	45.0±0.17	40.0±0.16	40.2±0.16	35.0±0.7	48.4±0.22
5	50.0±0.25	45.0±0.17	45.0± 0.17	30.0±0.7	48.3±0.22
6	55.5±0.26	50.0±0.25	35.0±0.7	25.2±0.4	40.5±0.18

Table 4: Effect of culture filtrate of soil fungi on the growth rate of *C.capsici*

Name of culture filtrate	Concentration	Growth rate (mm)	Percentage of inhibition
A. niger	5	25±0.7	48.8±0.7
	10	19±0.12	49.1 ±0.12
	15	17±0.20	51.7 ±0.25
	20	15±0.25	60.1±0.36
A .flavus	5	24±0.7	41.5± 0.16
	10	21±0.12	49.7±0.25
	15	17±0.17	52.9±0.27
	20	15±0.22	55.3±0.35
Penicillium	5	28±0.7	48.8±0.7
	10	25±0.12	47.1±0.15
	15	23±0.25	45.2±0.25
	20	21±0.21	51.7±0.35
T.harzianum	5	21±0.7	37.9±0.9
	10	19±0.12	47.1±0.13
	15	15±0.20	51.9±0.20
	20	13±0.18	72.9±0.25

Table 5: Effect of culture filtrate of soil fungi on growth rate of *Botryodiplodiatheobromae*

Name of culture filtrate	Concentration (%)	Growth rate (mm)	Percentage of inhibition
A.flavus	5	24±0.7	41.5±0.15
	10	21±0.15	49.1±0.1
	15	17±0.20	52.9±0.16
	20	15±0.20	55.3±0.25
A.niger	5	25±0.7	48.8±0.6
	10	19±0.12	49.1±0.25
	15	17±0.25	51.7±0.36
	20	15±0.25	60.1±0.37
P.javanicum	5	28±0.31	48.8±0.7
	10	25±0.36	47.1±0.11
	15	23±0.24	45.2±0.14
	20	21±0.31	48.8±0.36
T.harzianum	5	21±0.7	37.9±0.7
	10	19±0.12	47.1±0.11
	15	15±0.25	51.9±0.13
	20	13±0.20	72.9±0.36

Table 6: Effect of culture filtrate of soil fungi on the growth rate of *F.oxysporum*

Name of culture filtrate	Concentration (%)	Growth rate (mm)	Percentage of inhibition
Penicillium	5	28. ±0.8	48.8±0.7
	10	25.±0.11	47.1±0.13
	15	23.±0.9	45.2±0.14
	20	21.±0.22	51.2±0.25
A .niger	5	25±0.5	48.8±0.7
	10	19±0.7	49.1±0.9
	15	17±0.9	51.7±0.15
	20	15±0.15	60.1±0.25
A.flavus	5	24±0.8	41.5±0.16
	10	21±0.11	49.7±0.17
	15	1 7±0.13	52.9±0.25
	20	15±0.17	55.3±0.27
T.harzianum	5	21±0.8	37.9±0.7
	10	19±0.15	47.1±0.6
	15	15±0.20	51.9±0.25
	20	13±0.29	72.9±0.28

Table 7: Effect of chemical fungicide on the growth rate of test pathogens

Name of the fungicides filtrate	Concentration (ppm)	Growth rate(mm)	Percentage of inhibition
Control	0	0	
Zinep	10	20±0.7	55.1±0.16
	20	18±0.8	60.9±0.17
	40	15±0.9	67.1±0.25
	60	11±0.7	72.9±0.28
Control	0	0	
Thiophanatemethyl	10	15±0.6	37.5±0.8
	20	13±0.7	39.7±0.12
	40	1 ±0.3	45.8±0.16
	60	9±0.8	50.1±0.25

SUMMARY AND CONCLUSION

In our study, antagonistic activities of different *Trichoderma* species were performed *in vitro* against *C.capsici, B.theobromae, F. oxysporum,* causal agent of fruits rot of chilli, guava, tomato. Dual culture test showed that *Trichodermaharzianum* effectively inhibited mycelial growth of the pathogens. *T.harzianum* showed the highest inhibition (69.0%) and mycelial over growth (55.6%). Colony interaction between soil fungi and *F.oxysporum* was showed better than other pathogens. In effect of culture filtrate was showed maximum inhibition of the pathogens. Chemical control of the test isolates was also done with zineb, Thiophanatemethyl as the fungal agent. The zineb had exhibited maximum inhibition of tested pathogens namely *Colletotrichum capsici, Botryodiplodia theobromae, Fusarium oxysporum* the growth of the isolates significantly[9].

Biological control of plant disease with antagonists is accomplished by destroying existing pathogen inoculums, excluding the pathogen from the host plant or suppressing or displacing the pathogen after infection has occurred. *Trichoderma harzianum* is a parasite of other fungi and can rapidly colonize plant roots, thereby competing pathogens for nutrient and space. *Trichoderma harzianum* also promotes plant growth in the absence of pathogens. Biological control by antagonistic organism was a potential non-chemical tool for crop protection against phytopathogens. *T.harzianum* showed best antagonistic effect for the control of guava, chilli, tomato. Nonetheless, this study and the result are particularly useful for identifying likely candidates for biocontrol and for making educated guesses concerning the mechanisms by which they reduce pathogens damage.

This would be possible when a better fundamental knowledge on *Trichoderma* species as fruits pathogens with better understanding of molecular level interactions is achieved. Hence, our research may have extended economic and environmental impacts when further in depth investigation into the molecular plant-microbe interactions of their system is undertaken[10].

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