ANTAGONISTIC POTENTIALITY OF VARIOUS ISOLATES OF *Trichoderma* spp. AGAINST *Fusarium oxysporum* f.sp. *ricini* CASTOR WILTS PATHOGEN

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ABSTRACT

A variety of soil micro organisms have demonstrated activity in the control of various soil borne plant pathogens. The known groups of bio-control fungi, such as Trichoderma and Gliocladium spp. have been used to control a variety of fungal pathogens viz., Fusarium, Rhizoctonia, Pythium, Sclerotinia, Sclerotium etc. Various species of Trichoderma were identified with the help of their morphology and microscopic structure. Out of ten isolates, seven isolates of Trichoderma harzianum (Th_1-Th_7) and three of Trichoderma viride (Tv_1-Tv_3) were gained. The antagonistic potential of isolates of Trichoderma species were screened in vitro against castor wilt pathogen Fusarium oxysporum f. sp. ricini by dual culture technique as well as volatile technique. The results showed the significant differences in growth and per cent growth inhibition of pathogen. All the isolates significantly inhibited the growth of pathogens. The highest radial growth inhibition of pathogen was observed by isolate Th_2 (77.78 %) followed by Th_1 (75.92 %), Th_4 (74.07 %), Th_3 (71.11 %), Tv_2 (71.11 %), Tv_3 (70.00 %) and least effective was Th_5 isolate with 64.81 per cent inhibition. In volatile technique, production of volatile metabolites caused different levels of mycelial inhibition of Fusarium oxysporum f. sp. ricini. The highest radial growth inhibition of pathogen was observed by Tv_3 (67.07 %) followed by Tv_2 (67.04 %), Th_7 (64.81 %) and Th_6 (62.22 %) and least effective was Tv_1 with 44.44 per cent inhibition.

KEY WORDS: Antagonistic potentiality, castor, isolation, Trichoderma spp, Fusarium oxysporum

INTRODUCTION

Biological control of plant pathogens was an outgrowth of research on soil borne pathogens and on the ecology of the rich microbial flora and found in the *rhizosphere*. This work began during the second half of the 20th century and now forms the basis for a unique plant pathological province of biological control, based heavily on microbial and microbe plant interactions (Nelson, 1989). A variety of soil micro organisms have demonstrated activity in the control of various soil borne plant pathogens. The known groups of bio-control fungi, such as *Trichoderma* and *Gliocladium* spp. have been used to control a variety of fungal pathogens *viz., Fusarium, Rhizoctonia, Pythium, Sclerotinia, Sclerotium* (Harman, 1991; Taylor *et al.,* 1994 and Lewis *et al.,* 1996).

Castor wilt is serious problem in Gujarat state and caused heavy losses in past and also causing in present. Mono-cropping resulted in the endemic development of *Fusarium* wilt, which becomes a limiting factor of castor cultivation in the state resulting in considerable yield losses in castor hybrids (Dange *et al.*, 1997). The biological

control is one of the methods by which damage could be minimized up to great extent. The microorganisms isolated from the root or *rhizosphere* of a specific crop may be better adapted to that crop and may provide better control of diseases than organisms originally isolated from other plant species. The screening of such locally adapted strains has yielded improved bio-control in some cases (Cook, 1993).

MATERIALS AND METHODS

Isolation of isolates of *Trichoderma spp*.

The isolation of *Trichoderma* species were made by soil dilution plate technique (Johnson and Curl, 1972). From each soil sample, 10 g of closely associated *rhizosphere/rhizoplane* soil was mixed thoroughly with 90 ml sterile distilled water to prepare stock solution and serially diluted up to 10^{-5} (Harris and Sommers, 1968). One ml of suspension from the soil dilutions were plated on solidified *Trichoderma* Selective Medium (TSM) and gently shaken to spread evenly. These petriplates were incubated at $28^{o} \pm 1^{o}$C temperature for one week with periodic observation for the development of colonies of *Trichoderma* species. The early growing colonies of different morphology were examined critically, picked-up and transferred to Potato Dextrose Agar slants. Finally the cultures were purified and maintained on PDA slants at low temperature (5^{o}C) in refrigerator in the Department of Plant Pathology, C P College of Agriculture, S D Agricultural University, Sardarkrushinagar, for further activities. The isolates were identified with the help of their microscopic structure and compared with taxonomic keys of *Trichoderma* species (Rifai, 1969 and Cook and Baker, 1983). For the further confirmation, all the isolates were identified at MPUAT, Udaipur.

Collection and maintenance of *Fusarium oxysporum* f.sp. *ricini* causing castor wilt

The pure culture of *Fusarium oxysporum* f. sp. *ricini* of most dreadful disease, castor wilt was procured from Main Castor-Mustard Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The culture was maintained on Potato Dextrose Agar (PDA) slant's and preserved at low temperature (4°C) in refrigerator for further study.

Antagonistic properties of isolates of *Trichoderma* spp. against *F. oxysporum* f. sp. *ricini*

All the isolates were evaluated against the test pathogen for their antagonism and antibiosis properties in dual culture technique and volatile metabolites production, respectively under *in vitro* conditions.

1. Dual culture method

The method adopted by Morton and Strouble (1955) was followed. Mycelial discs of 5 mm diameter cut from the margin of young vigorously growing three day-old cultures of both test pathogen and antagonists were placed at opposite points in petriplates 40 mm apart. Both the pathogen and antagonists were inoculated at the same time. Medium used was PDA and for each treatment three petriplates were incubated at 28 ± 1^{o}C. Side by side control plates were maintained for each isolate and the pathogen temperature. The linear growth of the pathogen, *F. oxysporum* from the centre of the disc towards the centre of the plate was recorded after the control plates were completely covered by the pathogen and the growth inhibition of pathogen by antagonist over control was calculated by using the formula given by Vincent (1947).

$$PGI = \frac{C-T}{C} \times 100$$

Where,

PGI = Per Cent Growth Inhibition
C = Radial Growth in Control (mm)
T = Radial Growth in Treatment (mm)

2. Effect of volatile metabolites

The effect of volatile metabolites produced by the *Trichoderma* isolates was studied as per techniques described by Dennis and Webster (1971b). Different isolates of *Trichoderma* spp. were inoculated in the centre of the petriplates containing solidified sterilized PDA by placing fresh 5 mm disc (3 days old) with the help of a needle. After that the lid of each plate was replaced by the bottom of a plate containing PDA inoculated with 5 mm disc of the pathogen. The two plates were sealed together with adhesive tape and incubated at 28 ± 1°C. Each treatment had three replicate plates. Control was maintained by pairing the PDA solidified bottom plates with pathogen inoculated on solidified PDA top plates. Observations were recorded by measuring the colony diameter of the pathogen at 24 h of intervals and compared with that of the control plates.

RESULTS AND DISCUSSION

Isolation of *Trichoderma* spp. isolates from rhizosphere

On the basis of early growing colonies of different morphology, ten isolates of *Trichoderma* spp. were obtained on *Trichoderma* Selective Medium (TSM) from the *rhizosphere* of castor plant by soil dilution plate technique (10^{-5} dilution) after incubation period of one week at 28° ± 1°C. Out of 20 soil samples collected from castor wilt field and 8 isolates of *Trichoderma* species i.e. seven isolates of *Trichoderma harzianum* (Th_1- Th_7) and three of *Trichoderma viride* (Tv_1-Tv_3) were obtained. The results are in accordance with the methodology adopted by Sivan and Chet (1989), D'souza *et al.* (2001), Vyas and Mathur (2002), Kavitha *et al.* (2004), Sangle and Bambawale (2004) and Kapil and Kapoor (2005). All the cultures were also identified at MPUAT, Udaipur (Rajasthan) for further confirmation.

Antagonistic potentiality of isolates of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *ricin*

Dual culture technique

The antagonistic potential of isolates of *Trichoderma* species were screened *in vitro* against castor wilt pathogen *Fusarium oxysporum* f. sp. *ricini* by dual culture technique. The results (Table 1) showed significant differences in growth and per cent growth inhibition of pathogen. All the isolates significantly inhibited the growth of pathogen. The highest radial growth inhibition of pathogen was observed by isolate Th_2 (77.78 %) followed by Th_1 (75.92 %), Th_4 (74.07 %), Th_3 (71.11 %), Tv_2 (71.11 %), Tv_3 (70.00 %) and least effective was Th_5 isolate with 64.81 per cent inhibition. The comparison of *Trichoderma* species isolates revealed the most effective myco-parasitic potentiality with inhibition of 66.29 to 77.78 per cent by the isolates of *Trichoderma harzianum* followed by *Trichoderma viride* with 64.44 to 71.11 per cent respectively. The results are in confirmation with that all isolates collected from different soil samples are not equally antagonistic to pathogen. The variation in hyper-parasitic potential of different isolates of *Trichoderma* against soil borne pathogens has been reported (Maity and Sen, 1985, Prasad and Rangeswaran, 1999; Sarkar and Sharma, 2001) and the species of strains of *Trichoderma* were differently selective against different fungi has also been recorded (Dennis and Webster, 1971a; Wells *et al.,* 1972). The fast growing isolates caused more inhibition of the pathogen probably due to myco-parasitism and competition for nutrients.

Volaile technique

Different isolates of *T. harzianum* and *T. viride* were found to be varied in production of volatile metabolites, as they caused different levels of mycelial inhibition of *Fusarium oxysporum* f. sp. *ricini* (Table 2). The highest radial growth inhibition of pathogen was observed by Tv_3 (67.07 %) followed by Tv_2 (67.04 %), Th_7 (64.81 %) and Th_6 (62.22 %) and least effective was Tv_1 with 44.44 per cent inhibition. Isolates Tv_3 and Tv_2

were statistically at par with Th_7 and Th_6, but significantly differed with remaining isolates. The comparison of *Trichoderma* species isolates revealed the most effective antibiosis potentiality by the isolates of *T. viride* (44.44 to 67.07 %) followed by *T. harzianum* (45.55 to 64.81 %). There was no clear correlation was observed between the production of volatile metabolites and the growth inhibition of pathogen caused by various isolates in dual culture. The highest 58.82 per cent inhibition was noted from *T. viride* species followed by *T. harzianum* (55.76 %).

The production of a large variety of volatile secondary metabolites by *Trichoderma e.g.,* ethylene, hydrogen cyanide, alcohols, aldehydes and ketones play an important role in biocontrol (Vety *et al.*, 2001). The inhibition of pathogen by the volatile antibiotic compounds of antagonists is more advantageous, as they may diffuse through air filled pores in soil and actual contact between pathogen and antagonists may not be necessary for inhibition of pathogen. The antagonistic properties of *Trichoderma* species are well known and several workers had reported similar effects of volatile compounds to inhibit the growth of various soil borne pathogens (Dennis and Webster, 1971b; Upadhyay and Mukhopadhyay, 1983; Padmodaya and Reddy, 1996).

CONCLUSION

In dual culture technique, all the isolates significantly inhibited the growth of pathogen. The highest radial growth inhibition of pathogen was observed by isolate Th_2 (77.78 %) followed by Th_1 (75.92 %), Th_4 (74.07 %), Th_3 (71.11 %), Tv_2 (71.11 %), Tv_3 (70.00 %) and least effective was Th_5 isolate with 64.81 per cent inhibition. In Volatile technique production of volatile metabolites caused different levels of mycelial inhibition of *Fusarium oxysporum* f. sp. *ricini*. The highest radial growth inhibition of pathogen was observed by Tv_3 (67.07 %) followed by Tv_2 (67.04 %), Th_7 (64.81 %) and Th_6 (62.22 %) and least effective was Tv_1 with 44.44 per cent inhibition.

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Table 1: Effect of antagonistic potentiality of isolates of *Trichoderma spp*. on growth of *F.oxysporum* f. sp. *ricini* in dual culture technique

Sr. No.	Isolates	Mycelial Growth of Pathogen (mm)	Per Cent Inhibition in Mycelial Growth of Pathogen
1.	Th_1	21.67*	75.92**
2.	Th_2	20.00	77.78
3.	Th_3	26.00	71.11
4.	Th_4	23.33	74.07
5.	Th_5	31.67	64.81
6.	Th_6	28.00	68.89
7.	Th_7	30.33	66.29
8.	Tv_1	30.00	66.66
9.	Tv_2	26.00	71.11
10.	Tv_3	27.00	70.00
11.	Control	90.00	-
	S.Em.±	1.52	-
	C.D. at 5%	4.35	-
	C.V.%	8.04	-

** Mean of three replications* *** Treatments means with the letter/letters in common are not significant by Duncan's New Multiple Range Test at 5 per cent level of significance*

Table 2: Effect of antagonistic potentiality of isolates of *Trichoderma* spp. on growth of *Fusarium oxysporum* f. sp. *ricini* in volatile technique

Sr. No.	Isolates	Mycelial Growth of Pathogen (mm)	Per Cent Inhibition in Mycelial Growth of Pathogen
1.	Th_1	49.00*	45.55**
2.	Th_2	41.33	54.07
3.	Th_3	45.00	50.00
4.	Th_4	41.00	54.44
5.	Th_5	36.67	59.26
6.	Th_6	34.00	62.22
7.	Th_7	31.67	64.81
8.	Tv_1	50.00	44.44
9.	Tv_2	29.67	67.04
10.	Tv_3	32.33	67.07
11.	Control	90.00	-
	S.Em.±	1.65	-
	C.D. at 5%	4.72	-
	C.V.%	6.70	-

** Mean of three replications* *** Treatments means with the letter/letters in common are not significant by Duncan's New Multiple Range Test at 5 per cent level of significance*

[MS received: June 21, 2013] *[MS accepted: August 12, 2013]*