The Andhra Agric. J 59(4): 605-608, 2012

Antagonistic Potential of Sympatric *Trichoderma* Isolates Against *Fusarium oxysporum* f. sp. *ciceri*

Y Parvathi Devi, A K Patibanda, J Krishna Prasadji, and M Lal Ahmed
Department of Plant Pathology, Agricultural College, Bapatla 522101, Andhra Pradesh

ABSTRACT

Five out of twenty six interactions (19%) resulted in static growth of both the interacting isolates When twenty six isolates of *Trichoderma* were allowed individually to interact with *Fusarium oxysporum.* f. sp. *ciceri* in dual culture. In three out of twenty six interactions (12%), *Fusarium oxysporum* f. sp. *ciceri* overgrew *Trichoderma*. Sixty nine per cent of *Trichoderma* isolates were found to be antagonistic to *Fusarium oxysporum* f. sp. *ciceri*. Eighty six per cent of *T. harzianum* isolates (six out of seven isolates) and 73 per cent of *T. virens* isolates (eight out of eleven isolates) were antagonistic to *Fusarium oxysporum* f. sp. *ciceri*.

Key words : Antagonism, Dual culture, *Trichoderma*, Variability.

The increasing awareness of fungicide related hazards has emphasized the need of adopting biological control for management of soil borne plant pathogens. Species of *Trichoderma* are well documented fungal biocontrol agents against certain soil borne pathogenic fungi (Papavizas, 1985). In this regard the first requirement of biological control is the identification and deployment of highly effective strain(s) to manage soil borne plant pathogenic fungi. In the present investigation, variability in the *in vitro* antagonistic potential of twenty six *Trichoderma* isolates was assessed against *Fusarium oxysporum.* f. sp.*ciceri,* the causal agent of chickpea wilt.

MATERIAL AND METHODS

Twenty six sympatric isolates of *Trichoderma* spp., *viz*., *T. harzianum* (Th 2 to Th 8), eleven isolates of *T. virens* (Tv 1 to Tv 11), two isolates of *T. longibrachiatum* (Tl 1 and Tl 2) and one isolate each of *T. aureoviride, T. citrinoviride*, *T. piluliferum*, *T. polysporum*, *T. pseudokoningii* and *T. reesei* (Ta, Tc, Tpil, Tpol, Tpsi and Tr respectively) isolated from different cropping systems of Guntur district, Andhra Pradesh and available in the Department of Plant Pathology, Agricultural College, Bapatla, Guntur Dt., Andhra Pradesh, were evaluated for their antagonistic activity against *Fusarium oxysporum* f. sp. *ciceri* (collected from Pulse Pathology Section, RARS, Lam, Guntur) following dual culture technique (Morton and Straube, 1955). Observations were recorded upto six days after inoculation on the growth of test isolates (diameter in cm) based on which per cent inhibition was calculated for comparison.

RESULTS AND DISCUSSION

When observations were recorded on the growth of both the interacting fungi in dual cultured plates, three stages of interactions were observed.

i) Pre-interaction stage, where both the antagonist and pathogenic isolate grew towards each other in dual culture plate without establishing physical contact and without showing any effect on the opposing fungus in the dual culture plate.

ii) Interaction stage, where in inhibition in the growth of one or both the isolates was observed with or without physical contact between the two isolates in dual culture.

iii) Post-interaction stage, where prolonged incubation resulted in either static growth of both the interacting fungi or overgrowth of *Trichoderma* / pathogenic fungus on the opposing test fungus.

Pre-interaction stage:

None of the twenty six *Trichoderma* isolates had any impact on the growth of *Fusarium oxysporum* f. sp. *ciceri* in dual culture up to two days of incubation as evidenced from the insignificant differences in comparison to monocultured *Fusarium oxysporum* f. sp. *ciceri* control plate (Table 1). Similarly, except Tpol 1 (on day 1) and Tv 2 (on day 2), all other test *Trichoderma* isolates grew normally with insignificant differences in their radial growth in comparison to respective monocultured control

plates (Table 1). At this time, *i. e*., up to two days after inoculation, a gap of 1.9 to 4.7 cm existed between the two test fungi in different interactions (Table 1). This indicated that up to two days physical contact was not established between the two test fungi, and neither *Fusarium oxysporum* f. sp. *ciceri* nor *Trichoderma* had any impact on the opposite fungus.

Interaction stage:

Continued incubation up to five days after inoculation resulted in establishment of physical contact between the two test isolates in sixteen interactions. In the remaining ten interactions a gap of 0.2 to 1.0 cm existed between the test fungi (Table 1). Further, observations on the radial growth of *Fusarium oxysporum* f. sp. *ciceri* and *Trichoderma* five days after inoculation indicated change in the growth of individual test fungus in dual cultured plate in comparison to respective monocultured control plates. Hence this stage is considered as interaction stage and the gap between the two test isolates in dual cultured plate is considered as zone of inhibition. Campbell (1989) opined that such an inhibition zone could be considered as a clue for the production of antibiotics and thereby screening and selecting effective antagonists. However, Patibanda and Sen (2004) interpreted inhibition zone as an interaction effect rather than antagonist's antibiosis alone and suggested continued incubation.

When observations were recorded on the radial growth of *Fusarium oxysporum* f. sp. *ciceri* five days after inoculation in dual cultured plates, except in interactions involving Tpil, Tpsi, Tv 1 and Tv 7, in all other interactions growth of *Fusarium oxysporum* f. sp. *ciceri* was affected. Based on per cent inhibition in growth of *Fusarium oxysporum* f. sp. *ciceri* and over growth, all the interactions were categorized into four groups (Table 2):

1. Growth of *Fusarium oxysporum* f. sp. *ciceri* was static at zone of inhibition when interacted with Th 5, Tv 4, Tv 5, Tpol 1 and Tpsi.
2. Formation of inhibition zone followed by overgrowth of *Trichoderma* isolate when interacted with Th 3, Tv 1, Tv 10, Tl 1 and Tr 1.
3. *Fusarium oxysporum* f. sp. *ciceri* colony overgrew by *Trichoderma* without inhibition zone in interactions involving Ta, Tc, Th 2, Th 4, Th 6, Th 7, Th 8, Tv 3, Tv 6, Tv 8, Tv 9 and Tv 11.
4. *Fusarium oxysporum* f. sp. *ciceri* overgrew on *Trichoderma* colony in interactions with Tv 7, Tl 2 and Tpil.

At this stage, it was also interesting to observe effect of *Fusarium oxysporum* f. sp. *ciceri* on the radial growth of *Trichoderma* isolates in dual cultured plates when compared to their respective control plates. Based on the observations on the radial growth of *Trichoderma* isolates in dual cultured plates five days after inoculation, the interactions were grouped in to three (Table 3). (i) The growth of *Trichoderma* increased significantly in comparison to check (in Tpol 1) (ii) Static growth of *Trichoderma* at interaction zone in Tc, Th 4, Tl 1, Tv 1, Tv 4, Tv 5, Tv 6 and Tv 8 and (iii) Significant decrease in *Trichoderma* growth in comparison with check in Ta, Th2, Th3, Th5, Th6, Th7, Th8, Tl2, Tpil, Tpsi, Tr1, Tv2, Tv3, Tv7, Tv9, Tv10 and Tv11. Thus the present investigation revealed variation in the reaction of *Fusarium oxysporum* f. sp. *ciceri or* individual *Trichoderma* isolate in terms of growth in 'interaction stage'.

Post-interaction stage:

While interaction stage signified effect of one or both the interacting test fungal isolate on another with or without establishing physical contact, post-interaction stage signified the final outcome of the interactions. Continued incubation of dual cultured plates for another day, *i. e*., up to six days after inoculation revealed four types of interactions: (i) Static growth of both the fungi at the point of contact with inhibition zone in between the cultures was observed in *Fusarium oxysporum* f. sp. *ciceri* –Tpol / Tpsi / Tv 4 / Tv 5 interactions, (ii) Overgrowth of *Trichoderma* on *Fusarium oxysporum* f. sp. *ciceri* colony with formation of inhibition zone prior to over growth was observed in *Fusarium oxysporum* f. sp. *ciceri* –Th 3 / Tl 1 / Tr 1 / Tv 1 / Tv 2 / Tv 10 interactions, (iii) Overgrowth without zone of inhibition was observed in *Fusarium oxysporum* f. sp. *ciceri* –Ta / Tc / Th 2, / Th 4 / Th 6 / Th 7 / Th 8 / Tv 3 / Tv 6 / Tv 8 / Tv 9 / Tv 11 interactions, (iv) Overgrowth of *Fusarium oxysporum* f. sp. *ciceri* on *Trichoderma* colony was observed in *Fusarium oxysporum* f. sp. *ciceri* -Tl2 / Tpil / Tv7 interactions.

This indicated that in type 1 interaction, neither of the test fungal isolate could overpower the other in dual culture. In type 2 and 3 inetractions, *Trichoderma* could overpower *Fusarium oxysporum* f. sp. *ciceri* whereas in type 4 interaction, *Fusarium oxysporum* f. sp. *ciceri* could overpower and overgrew on *Trichoderma* colony.

Table 1. Growth of *Trichoderma* species (T) and *Fusarium oxysporum* f. sp. *ciceri* (Foc) in dual culture plates.

Treatments	1 DAI				2 DAI				3 DAI				4 DAI			
	Foc	T	Total	Gap	Foc	T	Total	Gap	Foc	T	Total	Gap	Foc	T	Total	Gap
Ta	0.6	1.1	1.7	5.4	1.2	3.4	4.6	2.5	1.8	5.2	7.0	0.0	1.8	5.6	7.4	OG
Tc	0.6	1.1	1.7	5.3	1.2	2.7	3.9	3.1	1.9	6.6	8.5	OG	1.9	5.1	7.0	OG
Th2	0.6	1.6	2.2	4.9	1.2	3.1	4.3	2.7	1.7	5.2	6.9	0.1	1.7	5.2	6.9	OG
Th3	0.6	1.4	2.0	5.1	1.2	3.8	5.0	2.0	1.8	5.2	6.9	0.0	1.8	5.2	6.9	OG
Th4	0.6	1.1	1.7	5.3	1.2	3.1	4.3	2.7	2.2	5.0	7.1	OG	2.5	5.0	7.5	Zi+ OG
Th5	0.6	1.0	1.6	5.4	1.2	2.5	3.7	3.3	2.1	4.8	6.9	0.1	2.6	5.1	7.7	Zi+ OG
Th6	0.6	1.4	2.0	5.1	1.2	3.9	5.1	1.9	1.8	5.2	7.0	0.0	1.8	5.2	7.0	OG.
Th7	0.6	1.1	1.7	5.4	1.2	3.4	4.6	2.4	1.9	5.1	7.0	0.0	1.9	5.1	7.0	OG
Th8	0.6	1.0	1.6	5.4	1.2	3.3	4.5	2.6	1.9	5.0	6.9	0.1	1.9	6.4	8.3	OG
Tl1	0.6	1.4	2.0	5.1	1.2	3.6	4.8	2.2	1.9	4.6	6.5	0.5	1.9	4.8	6.7	Zi+ OG
Tl2	0.6	0.9	1.5	5.5	1.2	2.3	3.5	3.6	2.2	4.2	6.4	0.6	2.2	4.7	6.9	F- OG
Tpil	0.6	0.8	1.4	5.7	1.2	1.1	2.3	4.7	2.6	3.5	6.1	0.9	2.6	3.7	6.3	F- OG
Tpol1	0.6	0.9	1.5	5.6	1.2	2.5	3.7	3.4	2.0	5.0	7.0	0.0	2.4	4.8	7.2	Zi
Tpsi	0.6	0.7	1.3	5.7	1.2	1.8	3.0	4.0	2.6	4.0	6.5	0.4	2.6	3.9	6.5	Zi
Tr1	0.6	1.2	1.8	5.2	1.2	3.5	4.7	2.3	1.8	5.2	7.0	0.0	1.8	5.2	7.0	Zi+ OG
Tv1	0.6	0.6	1.2	5.9	1.2	1.7	2.9	4.1	2.6	4.4	7.0	0.0	2.6	4.4	7.0	Zi+ OG
Tv2	0.6	0.8	1.4	5.6	1.2	3.1	4.3	2.8	1.9	4.9	6.8	0.2	1.9	5.2	7.1	Zi+ OG
Tv3	0.6	1.0	1.6	5.4	1.2	3.3	4.5	2.6	1.8	5.2	7.0	0.0	1.9	5.3	7.2	OG
Tv4	0.6	1.0	1.6	5.5	1.2	2.1	3.3	3.7	2.5	4.5	7.0	0.0	2.5	4.6	7.1	Zi
Tv5	0.6	0.9	1.5	5.6	1.2	2.3	3.5	3.6	2.3	4.3	6.5	0.4	2.3	4.7	7.0	Zi
Tv6	0.6	1.6	2.2	4.9	1.2	2.2	3.4	3.6	2.0	4.0	5.9	1.0	2.1	4.3	6.4	OG
Tv7	0.6	0.6	1.2	5.8	1.2	1.4	2.6	4.4	2.7	3.7	6.4	0.6	2.7	3.9	6.6	F-OG
Tv8	0.6	1.3	1.9	5.1	1.2	2.8	4.0	3.0	2.5	4.2	6.7	0.3	2.5	4.2	6.6	OG
Tv9	0.6	1.5	2.1	5.0	1.2	3.5	4.7	2.4	1.9	5.3	7.1	OG	1.9	5.3	7.1	OG
Tv10	0.6	0.8	1.4	5.7	1.2	2.6	3.8	3.2	1.8	4.9	6.7	0.3	1.9	4.9	6.8	Zi+OG
Tv11	0.6	1.0	1.6	5.4	1.2	3.5	4.7	2.3	1.8	5.2	7.0	0.0	1.9	5.3	7.1	OG

*Pathogen and antagonist discs were placed at a distance of 7cm apart in plates.

OG – Over growth F-OG – F O ciceri over grown on *Trichoderma* Zi – Zone of inhibition

Table 2. Categories of *Trichoderma* spp. – *F. oxysporum* f. sp. *ciceri* (Foc) interactions based on Foc growth

Category	Interacted isolates
Growth of Foc was static at zone of inhibition	Tv 4, Tv 5, Tpol 1, Tpsi
Formation of inhibition zone followed by overgrowth of *Trichoderma*	Th 3, Th 5, Tv 1, Tv 2, Tv 10, Tl 1, Tr 1
Foc colony overgrew by *Trichoderma* without inhibition zone	Ta, Tc, Th 2, Th 4, Th 6, Th 7, Th 8, Tv 3, Tv 6, Tv 8, Tv 9, Tv 11
Foc overgrew on *Trichoderma* colony	Tv 7, Tl 2, Tpil

Table 3. Categories of *Trichoderma* spp. – *F. oxysporum* f. sp. *ciceri* interactions based on *Trichoderma* growth

Category	Isolate
Increased *Trichoderma* growth	Tpol 1
Static *Trichoderma* growth	Tc, Th 4, Tl 1, Tv 1, Tv 4, Tv 5, Tv 6, Tv 8
Decreased *Trichoderma* growth	Ta, Th 2, Th 3, Th 5, Th 6, Th 7, Th 8, Tl 2, Tpil, Tpsi, Tr 1, Tv 2, Tv 3, Tv 7, Tv 9, Tv 10, Tv 11

In the present investigation wherein twenty six isolates of *Trichoderma* were allowed to individually interact with *Fusarium oxysporum* f. sp. *ciceri*, all the interactions were of antagonistic type and none were neutral or mutual. Five out of twenty six interactions (19%) resulted in static growth of both the interacting isolates, *i.e.,* neither could overpower the other. Similarly only three out of twenty six interactions (12%) resulted in *Fusarium oxysporum* f. sp. *ciceri* overgrowing on *Trichoderma*. In other words, only 69% of *Trichoderma* isolates were found to be antagonistic to *Fusarium oxysporum* f. sp. *ciceri*. Eighty six per cent of *T. harzianum* isolates (six out of seven isolates) and 73% of *T. virens* isolates (eight out of eleven isolates) were found antagonistic to *Fusarium oxysporum* f. sp. *ciceri*.

LITERATURE CITED

Campbell R 1989 *Biological control of microbial plant pathogens*. *Cambridge University Press*, 218.

Morton D J and Straube W H 1955 Antagonistic and stimulatory effect of soil microorganisms upon *Sclerotium rolfsii. Phytopathology,* 45: 417-420.

Papavizas G C 1985 *Trichoderma* and *Gliocladium*: Biology, ecology and potential for biocontrol. *Annual Review of Phytopathology*, 23: 23-54.

Patibanda A K and Sen B 2004 *In vitro* screening of *Aspergillus niger* van Teigh against *Fusarium oxysporum* f. sp. *melonis*, muskmelon wilt pathogen. *Journal of Biological Control,* 18: 29-34.

(Received on 20.09.2011 and revised on 16.01.2012)