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ECOFRIENDLY MANAGEMENT OF FUSARIUM FRUIT ROT OF CITRUS

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ABSTRACT

Fruit rot disease caused by *Fusarium pallidoroseum* is one of the major post-harvest disease of citrus and is adversely affects the fruit quality and the market value. In present study various antagonists and culture filtrate of antagonists were screened to know their bio efficacy in controlling the Fusarium fruit rot of citrus. *Trichoderma viride, T. harzianum, T. virens, T. atroviridae, T. asperellum,* and *T. fasciculatum*in significantly inhibit 100 % mycelial growth of *F. pallidoroseum in vitro* study, while in case of *in vivo* study lowest Fusarium rot severity was observed in fruits treated with *Trichoderma viride* both in pre- (15.30 %) and post-inoculation (17.08 %) methods at 8th day after inoculation. All the culture filtrate of antagonists found effective in inhibiting the mycelial growth of over control in *in vitro*. Lowest mycelial growth (29.63 mm) and significantly highest per cent mycelial growth inhibition was recorded in culture filtrate of *T. viride* (65.28 %). It was also found significantly most effective in reducing the Fusarium rot severity both in pre- (9.23 %) and post-inoculation (13.16 %) methods after 8th day of inoculation.

INTRODUCTION

Citrus is one of the most important fruits of the world and mostly cultivated widely in the tropical and sub-tropical regions. It ranks third among the sub-tropical fruits of the world with different varieties. Acid lime is one of the commercially important citrus fruit grown in India besides sweet oranges, mandarin and grape fruit.

Citrus fruits lose their market value due to damage caused by many fungi and bacteria. Akintobi *et al*. (2011) isolated the fungi associated with the spoilage of orange fruits. He reported 6 fungi isolates infecting citrus fruits *viz., Aspergillus flavus*, *A. niger*, *Fusarium solani*, *Penicillium digitatum*, *Rhizopus stolonifer* and *Candida tropicalis*. These pathogens by their prolific development destroy the fruits. Among all pathogens, the fruit rot incited by *Fusarium pallidoroseum* (Cooke.) Sacc. adversely affects the fruit quality, and ultimately reduces the market value. The Fusarium rot starts with softening of the tissues around the button of the fruit and a slight change in the peel color to beige or light brown. The internal part of the rotted fruit became either whitish-beige or violet pink (Nadel *et al*., 1987). The fruit rot caused by Fusarium incurred enormous yield losses and often observed in field and markets.

Use of fungicide on harvested fruits to manage the diseases is not desirable from health point of view, also continuous and indiscriminate use has led to the development of fungicide resistant strains of the pathogens. It also reduces the export quality due to high residues. An attempt was made to explore the possibility of using antagonists and cultural filtrates of antagonists for the management of Fusarium fruit rot of citrus.

MATERIALS AND METHODS

Antagonists

In vitro

Antagonistic effect of different bioagents *i.e. Trichoderma viride*, *T. harzianum*, *T. virens*, *T. atroviridae, T. fasciculatum, T. asperellum, Pseudomonas fluorescens, P. putida* and *Bacillus subtilis* were tested by dual culture technique for their antagonism against *Fusarium Pallidoroseum* (Dennis and Webster, 1971).

Seven days old culture of the bioagents and the pathogen were employed by following dual culture method. Mycelial disc of 5 mm diameter cut from the periphery of both antagonist and test pathogen and were placed at 50 mm apart from each other in Petriplates (90 cm) and in case of bacterial bioagents half portion of plates streaked and 5 mm diameter mycelial disc placed at centre of Petri plates. In control, only test pathogen was kept in the centre of Petri plate. Each treatment was replicated four times. The Petri plates were incubated at 27 ± 1^{0}C temperature in BOD incubator. The observations on mycelial growth (mm) and per cent growth inhibition of test fungi were recorded after 8 days of incubation. The standard procedure was adopted to evaluate the efficacy of antagonists and the percent growth inhibition of pathogen was calculated by the method suggested by Asalmol *et. al*. (1990).

In vivo

Antagonists studied *in vitro* were further screened to test their antagonism in controlling the Fusarium fruit rot of citrus following pre- and post-inoculation methods suggested by

Singh (2011). In pre-inoculation method the healthy semi-mature fruits were first inoculated with spore suspension (10^6 spores/ml) of seven days old culture of different antagonists separately and after 12 hrs, the fruits were inoculated at the same site with spore suspension (10^6 spores/ml) of seven days old culture of test pathogens and vice-versa in case of post inoculation method. Control was maintained separately with pathogen. The inoculated citrus fruits were placed separately in sterilized polythene bags. One fruit was kept in one bag. A piece of sterilized moist absorbent cotton swab was placed inside the bag to create humidity and mouth of the bag was loosely tied with rubber band. The bagged fruits were kept at 27 ± 1°C in BOD incubator for eight days. Each treatment was replicated four times. The disease severity was recorded on the basis of per cent infection in citrus fruits after 4th and 8th days of inoculation.

Culture filtrate

Six species of *Trichoderma* cultured in conical flask containing Potato Dextrose Broth (PDB) and incubated in BOD incubator for 21 days. The Liquid culture filtrates of six *Trichoderma* spp. viz. *Trichoderma viride, T. harzianum, T. virens, T. atroviride, T. fasciculatum, T. asperellum* were collected separately after 21days through Whatman filter paper No. 42.

In vitro

Bio-efficacy of different culture filterates were studied *in vitro* by following Poisoned Food Technique method (Nene and Thapliyal, 1979) against Fusarium fruit rot pathogen (*Fusarium pallidoroseum*) with 50 % v/v. Required quantity (50 ml) of each culture filterates under study was mixed thoroughly in sterilized 50 mL PDA media filled in 250 ml flask separately under aseptic condition. The medium was supplemented with streptomycin sulphate @ 50 ppm to prevent bacterial contamination. The poisoned medium was then poured in sterilized Petri plates (20 mL) and allowed to solidify. The plates were then inoculated with five mm diameter disc of seven days old culture of *Fusarium pallidoroseum* by placing in the centre of the plate. Control was maintained for each set where fungal disc were placed on PDA medium without culture filtrate. Each treatment was replicated thrice. The inoculated plates were then incubated at 25 ± 1°C temperature in BOD incubator.The percent growth inhibition was calculated by the method suggested by Asalmol *et. al*. (1990).

In vivo

Pre-inoculation

Culture filtrates studied *in vitro* were used for further investigation to test their efficacy in controlling Fusarium fruit rot of citrus following both pre- and post-inoculation methods suggested by Singh (2011). The healthy semi-mature fruits were first treated with 21 days old culture filtrate of different *Trichoderma* spp. separately and after 12 hrs, the fruits were inoculated with spore suspension (10^6 spores/ml) of seven days old culture of *Fusarium Pallidoroseum*. Control was maintained separately with only pathogen. The inoculated citrus fruits were placed separately in sterilized polythene bags. One fruit was kept in one bag. A piece of sterilized moist absorbent cotton swab was placed inside the bag to create humidity and mouth of the bag was loosely tied with rubber band. The bagged fruits were kept at 27 ± 1°C in BOD incubator for eight days. Each treatment was replicated four times. The disease severity was recorded on the basis of per cent infection in citrus fruits after 4th and 8th days of inoculation.

Post Inoculation

The procedure described in pre-inoculation was followed except that the fruits were first inoculated with pathogen and then after 12 hrs. with culture filtrate of bio-agents separately and seitz filter to remove the spores.

RESULTS AND DISCUSSION

Antagonists

In vitro

The results presented in table 1 showed that all the antagonists significantly inhibited the mycelial growth of *F. pallidoroseum* over control. No mycelial growth of *Fusarium Pallidoroseum* was produced in petriplates inoculated with *T. viride, T. harzianum, T. virens, T. atroviridae, T. fasciculatum* and *T. asperellum* While *Bacillus subtilis* showed minimum mycelial growth inhibition (42.44 %) followed by *Pseudomonas putida* (50.30 %) and *Pseudomonas flourescens* (61.99%). The results of present investigation corroborate with the results obtained by Krishna and Kumar (2013). They screened fourteen different antagonists against post-harvest rot disease in citurs. Among them *T. viride* and *T. harzianum* gave highest per cent growth inhibition of *F. solani*. (78.68 & 81.57 %) *in*

Table 1: Effect of antagonists on per cent growth inhibition of *Fusarium pallidoroseum in vitro*

Sr. No.	Antagonists	Mycelial Growth(mm)8 DAI	Per cent Growth Inhibition (PGI)
1	*Trichoderma viride*	0.00	100
2	*Trichoderma harzianum*	0.00	100
3	*Trichoderma virens*	0.00	100
4	*Trichoderma atroviridae*	0.00	100
5	*Trichoderma fasciculatum*	0.00	100
6	*Trichoderma asperellum*	0.00	100
7	*Pseudomonas fluorescens*	31.30	61.99
8	*Pseudomonas putida*	40.92	50.30
9	*Bacillus subtilis*	47.40	42.44
10	Control	82.35	-
	S.Em.±	0.39	-
	C.D. at 5%	1.49	-
	C.V. %	3.93	-

Table 2: Effect of antagonists on severity of Fusarium fruit rot *in vivo*

Sr.No.	Antagonists	Fusarium Rot Severity (%) Pre-Inoculation 4th day	8th day	Post-Inoculation 4th day	8th day
1	*T. viride*	5.27	15.30	6.52	17.08
2	*T. harzianum*	6.30	16.40	7.62	17.40
3	*T. virens*	7.50	18.32	8.75	19.55
4	*T. atroviridae*	7.37	16.95	7.97	18.97
5	*T. fasciculatum*	7.87	20.25	9.00	21.12
6	*T. asperellum*	8.47	21.62	9.57	23.42
7	*P. fluorescens*	9.40	23.07	12.02	26.12
8	*P. putida*	9.35	21.67	11.45	25.87
9	*B. subtilis*	9.50	24.15	14.32	28.55
10	Control	25.97	48.45	27.67	59.82
	S. Em. ±	0.29	0.35	0.37	0.55
	C. D. at 5%	0.84	1.01	1.09	1.59
	C.V. %	6.01	3.09	6.60	4.29

Table 3: Effect of culture filtrate of bio-agents on per cent growth inhibition of *Fusarium pallidoroseum in vitro*

Sr. No.	Antagonists	Mycelium Growth(mm) 8 DAI	Per cent Growth Inhibition (PGI)
1	*Trichoderma viride*	29.63	65.28
2	*Trichoderma harzianum*	35.60	58.29
3	*Trichoderma virens*	40.33	52.75
4	*Trichoderma atroviridae*	53.33	37.52
5	*Trichoderma fasciculatum*	44.06	48.37
6	*Trichoderma asperellum*	45.33	46.89
7	Control	85.36	-
	S.Em.±	0.84	-
	C.D. at 5%	2.56	-
	C.V. %	3.07	-

Table 4: Effect of culture filtrate of bio-agents on the severity of Fusarium fruit rot of citrus *in vivo*

Sr.No.	Antagonist	Fusarium Rot Severity (%) Pre-Inoculation 4th day	8th day	Post-Inoculation 4th day	8th day
1	*T. viride*	4.03	9.23	6.40	13.16
2	*T. harzianum*	5.70	11.26	7.80	15.63
3	*T. virens*	8.30	13.43	8.43	19.33
4	*T. fasciculatum*	14.16	23.10	15.73	26.50
5	*T. asperellum*	10.70	16.33	12.30	22.50
6	*T. atroviridae*	12.40	20.53	13.76	23.33
7	Control	31.86	82.06	36.63	87.76
	S. Em. ±	0.47	0.61	0.68	0.48
	C. D. at 5%	1.44	1.85	2.09	1.45
	C.V. %	6.64	3.51	8.27	2.79

vitro. Singh (2011) screened five antagonist *viz., Trichoderma viride, T. harzianum, T. virens, Pseudomonas fluorescens* and *Bacillus subtilis* against *F. moniliforme* infecting banana fruits by dual culture method. All the antagonists significantly helped in inhibiting the mycelial growth of *F. moniliforme* over control. Significantly highest mycelial growth inhibition was recorded in *T. harzianum* (53.06 %) followed by *T. virens* (50.09 %) and *T. viride* (49.72 %) after 7 days of inoculation.

Pseudomonas fluorescens gave minimum mycelial growth inhibition (22.07 %). Senthil *et al*. (2011) evaluated nine different antagonists against post-harvest rots of grapes. Among them, *Trichoderma viride* was found most effective in inhibiting the mycelial growth of *Aspergillus carbonarius, Penicillium expansum* and *Fusarium moniliforme* by 10.1, 26.8 and 88.8 per cent, respectively *in vitro*. Rajput *et al*. (2013) also reported that out of eight antagonists *T. viride* and *T. harzianum* isolates showing maximum growth inhibition of *Alternaria alternate* pathogen and appeared to be the most superior over all the antagonists tested in dual culture technique.

In vivo

The results presented in Table 2 revealed that all the antagonists found significantly superior in reducing the Fusarium fruit rot severity after 4th and 8th day of inoculation in pre-and post-inoculation treatments. In pre-inoculation *Trichoderma viride* was found significantly superior in reducing the Fusarium fruit rot severity (5.27 and 15.30 %) on 4th and 8th day after inoculation, respectively followed by *T. harzianum* (6.30 & 16.40 %). While *T. virens* (7.50 & 18.32 %), *T. fasciculatum*

(7.87 and 20.25 %) and *T. asperellum* (8.47 &21.62 %) showed mediocre effect in controlling the rot on 4th and on 8th day after inoculation, respectively over control (25.97 & 48.45 %). *Bacillus subtilis* found least effective in reducing the Fusarium rot severity (9.50 & 24.15 %) followed by *P. fluorescens* (9.40 & 23.07 %), respectively over control (25.97 & 48.45 %).

The trend similar to that observed in pre-inoculation was noted in post-inoculation treatment. *Trichoderma viride* was found significantly superior in reducing the Fusarium rot severity (6.52 & 17.08 %) over control (27.67 & 59.82 %) at 4th and 8th day after inoculation (Table 2), the next best treatment in order of merit was *Trichoderma harzianum* (7.62 & 17.40 %). While *Trichoderma atroviridae* (7.97 & 18.97 %), *T. virens* (8.75 & 19.55 %), *T. fasciculatum* (9.00 & 21.12 %) and *T. asperellum* (9.57 & 23.42 %) showed mediocre effect on 4th and 8th days after inoculation, respectively. *Bacillus subtilis* found least effective in controlling the Fusarium rot severity (14.32 & 28.55 %) followed by *P. flourescens* (12.02 & 26.12 %) on 4th and 8th day after inoculation, respectively.

The results of present investigations are in agreement with the results obtained by Pratella and Mari (1993). They reported that application of *T. viride, T. harzianum, Gliocladium roseum* and *Paecilomyces varioti* as spray treatment, partially controlled *Botrytis cinerea* in strawberry and kiwi fruit, *Fusarium oxysporum* in potatoes and *Alternaria citri* in lemon. Padmodaya and Reddy (1996) also found *Trichoderma viride* (71.05 %) highly inhibitory to *Fusarium* sp. causing wilt in tomato followed by *T. harzianum* (66.42 %). Xiao *et al*. (2007) reported that *T. harzianum* antagonize *Rhizoctonia solani, F. moniliforme, C. capsici* and *S. rolfsii***.** The pathogen colonies were either overgrown or invaded by *Trichoderma* sp. leading to the inhibition of pathogen growth, along with spore concentration debasement and reduction.

Culture filtrate

In vitro

The results presented in Table 3 revealed that all the culture filtrates of antagonists found effective in inhibiting the mycelial growth of *F. pallidoroseum* over control. Lowest mycelial growth (29.63 mm) and highest mycelial per cent growth inhibition (65.28 %) was recorded in culture filtrate of *T. viride*. The next best treatment was *T. harzianum* (35.60 mm) (58.29 %) followed by *T. virens* (40.33 mm) (52.75 %) and *T. fasciculatum* (44.06 mm) (48.37 %) on 8th day after incubation. While lowest mycelial growth inhibition was recorded in culture filtrate of *T. atroviridae* (53.33 mm) (37.52 %). These results are consonance with the results obtained by Adebesin *et al*. (2009) revealed that *Trichoderma asperellum* (NG-T161) alone or in combination with the pathogens at 50 per cent (v/v) inhibited the mycelial growth of *Fusarium oxysporum* and *Colletotrichum musae* to the tune of 49.7 and 60.3 per cent respectively, in banana fruits. Rajendiran *et al*. (2010) studied inhibitory effect of culture filtrate of *Trichoderma viride* (50 % v/v) on mycelium growth inhibition of *A. niger*, *A. fumigatus* and *A. flavus* reported 64, 49 and 48 per cent inhibition, respectively. The results also corroborate with the results obtained by Kataty and Emam (2012). They studied the effect of different concentrations of culture filtrate of *T. harzianum* on the growth of *Fusarium* sp. and *Alternaria* sp. causing tomato rot. The undiluted culture filtrate (100 %) of *T. harzianum* completely inhibited the spore germination of all the test fungi except *Rhizopus* sp

DAI - Days after inoculation

In vivo

Pre-inoculation

The results presented in Table 4 revealed that culture filtrate obtained from *Trichoderma viride* was found most superior in reducing the Fusarium fruit rot severity (4.03 & 9.23 %). The next best treatment in order of merit was *T. harzianum* (5.70 & 11.26 %) followed by *T. virens* (8.30 & 13.43 %) on 4th and 8th day after inoculation, respectively. While the culture filtrate of *T. asperellum* (10.70 & 16.33 %) showed mediocre effect in managing Fusarium fruit rot severity on 4th and 8th day after inoculation, respectively. The culture filtrate of *T. fasciculatum* (14.16 & 23.10 %) found least effective in reducing Fusarium fruit rot on 4th and 8th day after inoculation, respectively.

Post-inoculation

The trend observed in Pre- inoculation treatment was noted in post- inoculation (Table 4). The culture filtrate of *T. viride* found significantly superior over all other treatments in reducing the Fusarium rot severity (6.40 & 13.16 %) followed by *T. harzianum* (7.80 & 15.63 %) and *T. virens* (8.43 & 19.33 %) over control (36.63 & 87.76 %). While culture filtrate of *T. asperellum* (12.30 & 22.50 %) gave medicore effect in controlling the Fusarium rot severity on 4th and 8th day after inoculation, respectively. The culture filtrate of *T. fasciculatum* (15.73 & 26.50 %) found least effective in reducing the Fusarium rot severity at 4th and 8th day after inoculation, respectively.The results of present investigations are in conformity with the results obtained by Mortuza and Ilag (1999). They reported that conidia and culture filtrates of *Trichoderma* spp. could be effectively reduced the rotting in banana fruits when inoculated artificially with *Lasiodiplodia theobromae*. El-Katatny and Emam (2012) also found that, culture filtrate of *Trichoderma harzianum* (T3 & T24) greatly inhibited spore germination of the test post-harvest pathogenic fungi (*Geotricum candidum*, *Penicillium steckii, Rhizopus* sp., *Fusarium* sp., and *Aspergillus* sp.).

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