Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 394-398
Received: 15-05-2017
Accepted: 14-06-2017

Alka
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

RK Patil
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

BK Prajapati
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Correspondence
Alka
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Effect of *Trichoderma* Spp. and its culture filtrate antagonists on growth and management of Rhizopus rot of tomato fruit *in vitro* and *in vivo*

Alka, RK Patil and BK Prajapati

Abstract
The present study on various antagonists and its culture filtrate was carried out to know their efficacy in managing the Rhizopus rot of tomato *in vitro* and *in vivo*. *In vitro*, all the six antagonists significantly helped in inhibiting the mycelial growth of *R. oryzae* over control. Lowest mycelium growth (22.75 mm) with highest mycelial growth inhibition was recorded in *T. asperellum* (74.72 %), while *T. virens* gave minimum mycelial growth inhibition (62.50 %) after 7th days of incubation. In case of *in vivo* study, *T. asperellum* found significantly superior both in pre- (0.45 %) and post-inoculation (0.45 %) treatments over control (99.27 %) after 5th day of inoculation. The culture filtrate of all the antagonists found effective in inhibiting the mycelial growth of *R. oryzae* over control. Complete mycelial growth inhibition was recorded in *T. asperellum* and *T. viride* on 5th day after incubation in *in vitro*. While *in vivo* study, the culture filtrate of *T. asperellum* and *T. viride* found most effective both in pre- (0.45 & 0.45 %) and post-inoculation (0.45 & 0.45 %) treatments in managing Rhizopus rot severity on 5th day after inoculation.

Keywords: antagonists, culture filtrate, rhizopus rot, tomato

1. Introduction
Tomato (*Lycopersicon esculentum* Mill) is one of the most important vegetable crop cultivated all over the world rank second after potato. The harvested tomato fruits always succumb to the infection by various pathogens causing various fruit rots. Post-harvest diseases of tomato caused by fungi are responsible for incurring the substantial yield losses to the tune of 40 per cent of their market value (Okoli and Erinle, 1989) [11]. These eight genera of fungi are responsible for causing various rots to the tune of 4 to 9 per cent (Sharma and Choudhary, 2004) [18]. Tomato fruits are highly perishable in nature and it is very difficult to store for longer period, therefore, it needs immediate marketing and utilization.
The important pathogens causing post-harvest diseases of tomato are *Alternaria, Aspergillus, Rhizopus*, etc., which make the fruit not only to lose its appearance but also make them to become soft and watery (Ratnam and Nema, 1967) [16]. Biological control of plant pathogens through microorganisms has been considered as a potential tool for management of post–harvest diseases in recent years and search for potential bio-agents has been increased (Balai and Singh, 2013). *Trichoderma* spp. are now the most common fungal bio-control agent that have been comprehensively studied and deployed throughout the world.
The aim of the present study was to investigate the bio-efficacy of antagonists and their culture filtrate in management of Rhizopus rot of tomato incited by *Rhizopus oryzae.*

Material and Methods
Antagonists
The bioagents viz. *Trichoderma viride*, *T. harzianum*, *T. virens*, *T. asperellum*, *T. atroviride* and *T. fasciculatum* were screened by dual culture technique for their antagonism against Rhizopus rot pathogen. The cultures of fungal antagonists were maintained on PDA medium.

In vitro
The bio-assay of antagonists was performed in PDA in Petri plates by dual culture method (Dennis and Webster, 1971) [5]. Paired cultures were incubated at 25 $\pm$ 1^{0}C in BOD incubator for 7 days. Petri plates inoculated only with test pathogens served as control. Three replications of each treatment were undertaken. Observations on per cent growth inhibition were recorded after 7 days of incubation. The per cent growth inhibition (PGI) was calculated by following the method suggested by Asalmol *et al*., (1990) [1].

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

Where,
I = Inhibition per cent
C = Colony diameter (mm) in control
T = Colony diameter (mm) in treatment

In vivo
Antagonists studied *in vitro* were evaluated further to test their antagonism in managing Rhizopus fruit rot of tomato following both pre- and post-inoculation methods.

Pre- inoculation
The healthy, semi-riped, uniform size tomato fruits of GT-2 were randomly pin-pricked and then dipped in the spore suspension of different Trichoderma spp. (10^7 spores/ml) of seven days old culture of various antagonists separately and after 12 hr. the fruits were inoculated at the same site with spore suspension (10^7 spores/ml) of seven days old culture of test pathogen. Control was maintained separately only with pathogen. Each treatment was replicated four times. Observations on percent disease severity were recorded after 5th days of inoculation with the help of standard assessment key (Plate No. 1).

Post-inoculation
The procedure detailed in pre-inoculation was followed except that the fruits were first inoculated with the pathogen and then with antagonists.

Culture Filtrate Study
Six species of *Trichoderma* cultured in conical flask on Potato Dextrose Broth (PDB) and incubated for 21 days in BOD incubator at 25 $\pm$ 1^0C. The Liquid culture filtrate of six *Trichoderma* spp. *viz. T. viride, T. harzianum, T. virens, T. atroviride, T. fasciculatum* and *T. asperellum* was collected separately after 21days of incubation by filtering through Whatman filter paper No. 1 to remove mycelial mat and Seitz filter was used to separate the spores

In vitro
Bio-efficacy of different culture filtrates (500µl/ml) of fungal bio-agents were studied *in vitro* by following Poisoned Food Technique method (Nene and Thapliyal, 1979) [9] against Rhizopus rot pathogen (*R. oryzae*) with 50 % v/v. The medium was supplemented with streptomycin sulphate @ 50 ppm to prevent bacterial contamination. Control was maintained for each set where fungal disc were placed on PDA medium without culture filtrate. Each treatment was replicated for three times. The inoculated plates were then incubated at 25 ± 1^0 C temperatures in BOD incubator for 7 days. The percent growth inhibition was calculated by following the method suggested by Asalmol *et al*. (1990) [1].
Observations on per cent growth inhibition were recorded after 7 days of inoculation by following the procedure mentioned earlier.

In vivo
Liquid culture filtrates of Trichoderma spp. studied *in vitro* were evaluated further to test their bio-efficacy in controlling Rhizopus rot of tomato following both pre- and post-inoculation methods.
Culture filtrates studied *in vitro* were used for further investigation to test their efficacy in controlling Rhizopus rot of papaya following both pre- and post-inoculation methods. The healthy, semi-riped, uniform size tomato fruits were first dipped in 21 days old culture filtrate of *Trichoderma* spp. separately and after 12 hrs, the fruits were inoculated with spore suspension (10^7 spores/ml) of seven days old culture of test pathogens and *vice-a-versa* in case of post inoculation method. Control was maintained separately only with pathogen inoculation. Each treatment was replicated three times. The disease severity was recorded on the basis of per cent infection in tomato fruits on 7th day after inoculation.

Results and Discussion
Antagonists *In vitro*
The results presented in Table No. 1 revealed that all the antagonists showed varying degree of mycelial growth inhibition of *R. oryzae* over control. Significantly lowest mycelial growth (22.75 mm) with highest growth inhibition was recorded in *T. asperellum* (74.72 %) followed by *T. viride* (27.13 mm) (69.86 %) and *T. harzianum* (29.88 mm) (66.80 %) after 7th day of incubation. While *T. virens* gave lowest mycelial growth inhibition (62.50 %).
The growth inhibition of the *R. oryzae* could be due to fast growing nature of *Trichoderma* spp., as well as secretions of harmful extra-cellular compounds like antibiotics *i.e.* gliotoxin, glyoviridin and cell wall degrading enzymes such as glucanases, endochitinases, chitinases and mycoparasitism.
Results similar to the present findings were reported by Katatny and Emam (2012) [6]. They studied antagonistic effect of *Trichoderma harzianum* against *Fusarium*, *Alternria*, *Aspergillus* and *Rhizopus* spp. causing post-harvest rotsin tomato. *Trichoderma harzianum* found most competent antagonist to control post-harvest rots of tomato *in vitro*.
Mokhtar and Dehimat (2014) [7] tested *in vitro* and *in vivo* ability of *T. harzianum* to control the *Rhizopus* soft rot of tomato fruits (*Lycopersicon esculentum*). The results of direct confrontation (*in vitro*) of *T. harzianum* against *R. stolonifer* on PDA medium, showed 43.66 per centinhibition in the mycelial growth of *R. stolonifer* by hyphae of *T. harzianum*in the fourth day of the experiment.
Patil (1992) [13] evaluated the antagonistic activity of three bio-agents (*T. viride*, *T. harzianum* and *B. subtilis*) *in vitro* to control the *Rhizopus* fruit rot of mango fruits. The results revealed that*T. harzianum* and *T. viride* inhibited the mycelial growth of *R. oryzae*after 72 hours of incubation.

In vivo
All the antagonists were found significantly superior in reducing the Rhizopus fruit rot severity after 5th day of inoculation in pre-and post-inoculation treatments (Table No. 1).

Pre inoculation
Among the antagonists, *Trichoderma asperellum, T. viride* and *T. harzianum* were found significantly superior in reducing the Rhizopus rot severity (0.45 %) on 5th day after inoculation followed by *T. fasciculatum* (4.77 %). While *T. atroviride* (35.85 %) and *T. virens* found least effective in reducing the rot severity (61.49 %) on 5th day after inoculation over control (85.11%) Post- inoculation
Trichoderma asperellum and *T. viride* were found significantly superior in reducing the rot severity (0.45 %) followed by *T. harzianum* (23.78 %). While *T. virens* (57.78 %) and *T. atroviride* (67.49 %) found least effective in reducing the Rhizopus rot severity on 5th day after inoculation

over control (85.11 %). Further, it was observed that among two methods of inoculation, pre- inoculation method was found superior in managing the Rhizopus rot as compare to post-inoculation.

The results of present study are in agreement with the results obtained by Salman (2005) [17]. He reported Rhizopus rot of tomato fruits can be effectively managed through application of *Trichoderma harzianum.*

Results similar to the present investigation were obtained by Padmodaya and Reddy (1996). They reported that *T. viride* found highly inhibitory to *Fusarium* sp. causing wilt in tomato followed by *T. harzianum.*

Pratella and Mari (1993) [14]. reported that when *T. viride, T. harzianum, Gliocladium roseum* and *Paecilomyces varioti* applied as spray treatment to fruits, partially controlled *Botrytis cinerea* in strawberry and kiwi fruits, *Fusarium oxysporum* in potatoes and *Alternaria citri* in lemon.

Trichoderma viride found most effective in controlling the apple rots caused by *Botrytis* sp., *Rhizoctonia* sp., *Sclerotinia* sp., *Pythium* sp., and *Fusarium* sp. (Batta, 2004) [3].

Damaram (2012) [4] evaluated five antagonists *viz., Trichoderma viride, T. harzianum, T. virens, Bacillus subtilis* and *Pseudomonas fluorescens* against *Fusarium pallidoroseum*. All the antagonists were found significantly superior in reducing the Fusarium fruit rot over control. Among them *Trichoderma harzianum* was found significantly superior in reducing the Fusarium fruit rot severity (10.25 %) followed by *T. virens* (14.25%) on 8th day after both in pre- and post-inoculation treatments. *Bacillus subtillis* found least effective in reducing the rot severity (25.25%).

Culture Filtrate *In vitro*

Liquid culture filtrate of six *Trichoderma* spp. *viz., T. viride, T. harzianum, T. virens, T. atroviridae, T. fasciculatum* and *T. asperellum* were evaluated to test their antagonistic activity against *R. oryzae* by poisoned food technique method. The results presented in Table 2. Revealed that all the culture filtrates of antagonists found significantly superior in inhibiting the mycelial growth of *R. oryzae* over control. No mycelial growth was recorded in *T. asperellum* and *T. viride* followed by *T. harzianum* (25.25mm, PGI-71.19). The culture filtrate of *T. atroviride* (33.53 mm, PGI- 62.74) and *T. virens* (36.00 mm, PGI-60.00) found least effective in inhibiting the mycelial growth of *R. oryzae.*

The growth inhibition of the *R. oryzae* with liquid culture filtrates of *Trichoderma* spp. could be due to the secretions of harmful extra-cellular compounds like antibiotics *i.e.*, gliotoxin and glioviridin and cell wall degrading enzymes such asglucanase, endochitinase and chitinase.

Results similar to the present investigation were reported by Naresh (2014) [8]. He reported that culture filtrate of *Trichoderma viride* and *T. harzianum* found effective in inhibiting the mycelial growth of *F. solani*. Lowest mycelial growth (33.56 mm) and highest mycelial percent growth inhibition (60.54 %) was recorded in *T. harzianum* and it was at par with *T. viride* (35.80 mm) (57.91 %).

Rajendiran *et al*., (2010) [15] reported maximum per cent growth inhibition of *A. niger* (64%), *A. fumigatus* (49%) and *A. flavus* (48%) in 50 per cent culture filtrate of *Trichoderma viride*.

In vivo

Pre-inoculation

The results presented in Table 3 revealed that 21 days old culture filtrate obtained from *T. asperellum*, and *T. viride* were found significantly superior recording lowest Rhizopus rot severity followed by *T. harzianum* (6.94 %) on 5th day after inoculation over control (85.11 %). While the other three spp. *i.e T. fasciculatum* (32.63 %), *T. atroviridae* (41.04 %), *T. virens* (42.83 %) were found least effective against the rot.

Post-inoculation

The culture filtrate of *Trichoderma asperellum* and *T. viride* were found significantly very effective showing lowest Rhizopus fruit rot severity followed by *T. harzianum* (23.78 %) on 5th day after inoculation over control (85.14 %). *Trichoderma fasciculatum* (39.78 %) gave mediocre effect, while *T. virens* (57.80 %) and *T. atroviridae* (66.99 %) were found least effective against the rot. Further, it was observed that among two treatments of inoculation, pre-inoculation treatment was found promising in managing the Rhizopus rot severity over post- inoculation treatment.

The results of present investigations corroborate with the results obtained by Kataty and Emam (2012) [6]. They found that, culture filtrate of *T. harzianum* (T3 & T24) at different conc. (25, 50 & 100 %) inhibited post-harvest pathogenic fungi (*Rhizopus* sp., *Geotricum candidum*, *Penicillium steckii*, *Fusarium* sp., and *Aspergillus* sp.).

Batta (2007) [3] evaluated efficacy of invert emulsion formulation of *T. harzianum* against post-harvest diseases caused by *R. stolonifer*, *B. cinera* and *P. expansum*. The results revealed significant reduction in the mean lesion diameters of *R. stolonifer* on apple, pear, peach and strawberry in comparison with the control treatment. On wounded apple fruit, he observed reduced mean lesion diameter from 73.2 mm in the control to 9.7 mm in treated fruits.

Nirupama and Singh (2011) [2, 10] screened culture filtrate of ten isolates of *Trichoderma* sp. to evaluate the production of volatile and non-volatile inhibitors against the *Fusarium oxysporum* f. sp. *lycopersici*, causing wilt in tomato. Culture filtrate of *T. viride* (TV19) at 15 percent concentration gave highest inhibition in pathogen growth followed by *T. harzianum* (TH7). The similar trend of results in growth inhibition of the pathogen was observed in volatile metabolites of 15-days old cultures of TV19 and TH7.

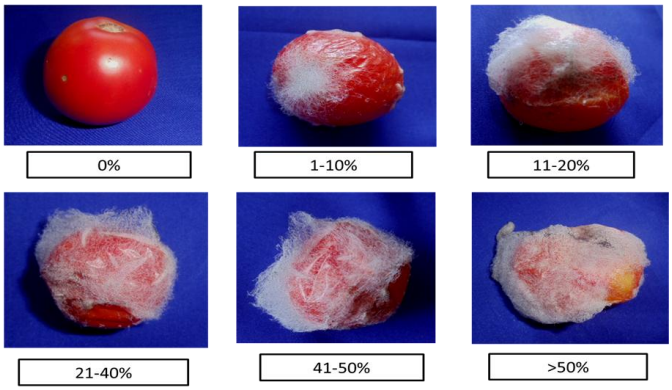

Plate 1: Assessment key used to record the severity of Rhizopus fruit rot of tomato

Table 1: Effect of antagonists on mycelial growth and per cent growth inhibition of *Rhizopus oryzae in vitro* and *in vivo*.

Sr.No.	Antagonists	Rhizopus rot severity (%)			
		In vitro		*In vivo*	
		Mycelial Growth (mm) 7 DAI*	Per cent Growth Inhibition (PGI)	Pre- Inoculation (5th DAI)	Post – Inoculation (5th DAI)
1	*Trichoderma viride*	27.13	69.86	0.45 (0.01)	0.45 (0.01)
2	*Trichoderma harzianum*	29.88	66.80	0.45 (0.01)	23.78 (16.26)
3	*Trichoderma virens*	33.75	62.50	61.49 (77.22)	57.78 (71.57)
4	*Trichoderma asperellum*	22.75	74.72	0.45 (0.01)	0.45 (0.01)
5	*Trichoderma fasciculatum*	32.25	64.16	4.77 (0.69)	32.63 (29.07)
6	*Trichoderma atroviride*	33.53	62.74	35.85 (34.30)	67.49 (85.34)
7	Control	90.00	--	85.11 (98.27)	85.11 (99.27)
S.Em.±		1.27	--	1.38	0.70
C.D. at 5%		3.72	--	4.06	2.06
C.V. (%)		6.58	--	9.92	3.67

*DAI= Days after inoculation

Figures in the parentheses are retransformed values of arc sine transformation

Table 2: Effect of culture filtrate of bio-agents on per cent growth inhibition of Rhizopus rot of tomato *in vitro* and *in vivo.*

Sr. No.	Antagonist	Rhizopus Rot Severity (%)			
		In vitro		*In vivo*	
		Mycelial Growth (mm) 7 DAI*	Per cent Growth Inhibition (PGI)	Pre- Inoculation (5th DAI)	Post – Inoculation (5th DAI)
1	*Trichoderma viride*	0.00	100.00	0.45 (0.01)	0.45 (0.01)
2	*Trichoderma harzianum*	25.25	71.19	6.94 (1.46)	23.78 (16.26)
3	*Trichoderma virens*	36.00	60.00	42.83 (46.22)	57.80 (71.60)
4	*Trichoderma asperellum*	0.00	100.00	0.45 (0.01)	0.45 (0.01)
5	*Trichoderma fasciculatum*	32.25	64.16	32.63 (29.07)	39.78 (40.94)
6	*Trichoderma atroviride*	33.53	62.74	41.04 (43.11)	66.99 (85.72)
7	Control	90.00	--	85.11 (99.27)	85.14 (99.28)
S.Em.±		0.86	--	0.66	0.88
C.D. at 5%		2.53	--	1.94	2.66
C.V. (%)		5.55	--	4.55	3.32

*DAI= Days after inoculation

Figures in the parentheses are retransformed values of arc sine transformation

References

1. Asalmol MN, Sen B, Awasthi J. Role of temperature and pH in antagonism of *Aspergillus Niger* and *Trichoderma viride* against *Fusarium solani*. Proc. Indian Phyto-Pathol. Society. 1990, 11-13.
2. Balai LP, Singh RB. Integration management of Alternaria blight of pigeon pea with some fungicides and antagonists in pot condition. The Bios can. 2011; 8(3):881-886.
3. Batta YA. Post-harvest biological control of apple gray mold by *Trichoderma harzianum* Rifai formulated in an invert emulsion. Crop Protection.2004; 23:19-26.
4. Damaram. Investigation on Fusarium Fruit Rot [*Fusarium pallidoroseum* (Cooke) Sacc.] of Tomato (*Lycopersicon esculentum* Mill.) and its Management. M. Sc. Thesis submitted to AAU, Anand. 2012.
5. Dennis C, Webster J. Antagonistic properties of species-groups of *Trichoderma*. III. Hyphal interaction. Trans. Brit. Mycol. Society. 1971; 57(3):363-369.
6. Kataty EM, Emam AS. Control of post-harvest tomato rot by spore suspension and antifungal metabolites of *Trichoderma harzianum*. J. Microbiology, Biotechnology and Food Science. 2012; 1(6):1505-1528.
7. Mokhtar H, Dehimat A. *In vitro* and *in vivo* efficiency *of Trichoderma harzianum* against *Rhizopus* soft rot occurred on tomato fruits (*Lycopersicon esculentum*). Agriculture and Biology Journal of North America. 2014; 5(6):240-244.
8. Naresh. Investigation on Fusarium Fruit Rot [*Fusarium solani* (Mart.) Sacc.] of Papaya (*Carica papaya* L.) and its Management. M. Sc. (Agri.) thesis submitted to Anand Agricultural University, Anand, Gujarat, 2014.
9. Nene YL, Thapliyal PN. Fungicides in plant disease control. Oxford and IBH Publishing Co., New Delhi, 11 Edn; 1979, 7-10.
10. Nirupama T, Singh MS. Evaluation of fungicide tolerance by *Fusarium oxysporum* f. sp. *lycopersici* and antagonist *Trichoderma* isolates. Indian Phytopathlogy. 2011; 64(3):87-101.
11. Okoli CAN, Erinle ID. Factor responsible for market losses of tomato fruits in the Zaria area of Nigeria. J Hort. Science. 1989; 64(1):69-71.
12. Padmodaya B, Reddy HR. Screening of *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *lycopersici* causing wilt in tomato. Indian J. Mycol. Pl. Pathology. 1966; 26(3):266-270.
13. Patil RK. Studies on Rhizopus rot and Botryodiplodia rot of mango fruits. Ph.D thesis submitted to RAU, Bikaner, Rajasthan, 1992.
14. Pratella GC, Mari M. Effectiveness of *Trichoderma*, *Gliocladium* and *Paecilomyces* in post-harvest fruit protection. Post- harvest Biology and Technology. 1993; 3(1):49-56.
15. Rajendiran R, Jegadeeshkumar D, Suresh Kumar BT, Nisha T. *In vitro* assessment of antagonistic activity of *Trichoderma viride* against post-harvest pathogens. Journal of Agricultural Technology. 2010; 6(1):31-35.
16. Ratnam CV, Nema KG. Studies on market diseases of fruits and vegetables. Andhra Agric. Journal. 1967; 14:60-65.

17. Salman MAM. Biological Control of Rhizopus Soft Rot on Apple, Pear and Peach by *Trichoderma harzianum*. Submitted for the M.Sc degree in Environment Sciences at An-Najah National University, Nablus, Palestine, 2005.
18. Sharma RL, Choudhary M. Incidence of postharvest rot of tomato in Himachal Pradesh. J Mycol. Pl. Pathology. 2004; 34(3):906-908.