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Eco-friendly management of Alternaria fruit rot of tomato *(Lycopersicon esculentum* Mill.) caused by *Alternaria tomato*

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Tomato fruits are affected by many fungal pathogens, but Alternaria fruit rot of tomato caused by *Alternaria tomato* (Cooke) G. F. Weber was most destructive disease leading heavy losses in field as well as in the market. The fruit rot caused by *A. tomato*, which adversely affect the fruit quality, quantity and ultimately reduce the market value. The overdose of chemicals resulted resistance development in pathogens and have adverse effect on the consumer health. There is need to search alternative approaches to chemical control which have minimal deleterious effects and ecofriendly in nature.An antagonist, phytoextract and cow urine were used for ecofriendly management of the Alternaria fruit rot of tomato *in vitro* as well as*in vivo*by pre and post inoculation method. The*Trichoderma viride*waseffective antagonist in inhibiting the mycelial growth of *A. tomato in vitro* by dual culture method with (85.02%) per cent growth inhibition as well as it was found most effective in reducing Alternaria fruit rot severity as both pre (10.63 %) and post -inoculation (13.67 %) treatments. The garlic clove extract (10%) recorded lowest mycelia growth (17.00 mm) with highest percent mycelia growth inhibition. The similar results were also recorded in reducing Alternaria fruit rot severity in pre (8.36 %) and post -inoculation (10.46 %) methods.

Key words: *Alternaria tomato,* antagonist, fruit rot, phytoextract

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crop which have high nutritive value of vitamin A, B, C, E and protein, carbohydrates, fiber, fat, biotin, etc. The food losses in industrialized countries are as high as in developing countries, but in later more than 40 per cent of the food losses occur at postharvest and processing levels, while in former, more than 40 per cent of the food losses occur at retail and consumer levels (Gustavsson *et al*, 2011).

The harvested tomato fruits always succumb to the infection by various pathogens causing fruit rots. Post-harvest diseases of tomato caused by fungi are responsible for causing losses to the tune of 40 per cent of their market value. Tomato fruits are highly perishable in nature and very difficult to store for longer period therefore, it needs immediate marketing and utilization.The injudicious application of chemicals resulted health hazards problems to the users. The search for alternative approaches which have minimal deleterious effects, more ecofriendly and will contribute to the goal of sustainability in agriculture is needed (Jain and Sharma, 2006;Waghunde*et al*. 2016). The present investigation was done to reduce Alternaria fruit rots of tomato bythe utilization of an antagonist, phytoextract and cow urine under south Gujarat condition.

MATERIALS AND METHODS

The antagonists, phytoextracts and cow urine were

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evaluated to know antifungal property against Alternaria fruit rot of tomato *in vitro* and *in vivo*.

Antagonist

In vitro

An antagonistic effect of different antagonists*i.e*. *Trichoderma viride*, *T. harzianum*, *T.fasciculatum*, *Pseudomonas fluorescens* and *Bacillus subtilis*were tested by dual culture technique against *A. tomato*.Seven days old culture of the antagonist and the pathogen were used for the dual culture method (Dennis and Webster, 1971). Then mycelial disc (5 mm) cut from the both seven days old antagonist as well as *A. tomato*Petri plates and placed at 70 mm apart from each other and in case of bacterial antagonist half portion of plates streaked and 5 mm diameter mycelial disc placed against it. In control, only test pathogen was kept in the Petri plate and each treatment repeated four times.

In vivo

In pre-inoculation experiment, the healthy semi-ripe fruits were pricked at stem-end and inoculated with different antagonists separatelyfor 3 min. and air dried for 15 min. Fruits were placed separately in sterilized, loosely tied polythene bag along with a piece of sterilized moist absorbent cotton swab. The cotton swab was placed inside the bag to create humidity and mouth of the bag was loosely tied with rubber band. After 12 hrs, the fruits were inoculated at the same site with spore suspension (10^6 spores/ml) of seven days old culture of *A. tomato* while in post- inoculation method, the procedure described earlier was followed except that the fruits was first inoculated with pathogen and then with antagonists.The bagged fruits were kept at 25 ± 1^0C in BOD incubator and fruits were observed on 4^{th} and 8^{th} days after inoculationwith the help of assessment keyto record disease severity.Each treatment was repeated thrice.

Phytoextracts and Cow urine

Total eight phytoextracts along with the cow urine were tested to know antifungal activity against *A. tomato*. The different phytoextractsi.e. garlic, tulsi, cinnamon, piper, lemon grass, ardusi, neem and aloe veraalong with cow urine having medicinal value were tested *in vitro* by Poisoned food technique (Nene and Thapliyal, 1979) against *A.tomato*. All the phytoextracts were tested at 10 per cent concentration. Fresh and healthy 100 g. plant parts of each species was thoroughly washed with tap water and then with distilled sterilized water. They were macerated separately in grinder by adding 100 ml. ethanol.The mixture was filtered through two fold sterilized muslin cloth and the filtrate was centrifuged at 5000 rpm for 10min. and the clear supernatant extract was collected in sterilized conical flasks. After evaporating the ethanol from extract, the clear extract was collected and diluted with 100 ml. distilled sterile water to make volume 1:1 (W/V). This was considered as 100 per cent concentration for the study to test the efficacy of plant extracts (Sinha and Saxena, 1989). Each phytoextract (10 %) was mixed thoroughly in sterilized 100 ml. PDA medium filled in 250 ml. flask under aseptic condition. The PDA medium was supplemented with streptomycin sulphate @ 50 ppm to prevent bacterial contamination. The control was maintained separately for each experiment without inoculating pathogen in Petri plate. Each treatment was repeated thrice. The severity of fruit rots was recorded on $4^{th}$and 8^{th}day after inoculation with the help of assessment key. The rest of procedure was same followed antagonist treatment.

$$\text{Disease severity (\%)} = \frac{\text{Area of infected fruits}}{\text{Total area of fruit tissue}} \times 100$$

RESULTS AND DISCUSSION

Antagonist

A total of six antagonists viz., *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens*Waghai and Navsari isolate, *Bacillus subtilis* and *T. fasciculatum* were evaluated *in vitro* against *Alternaria tomato* by dual culture method. The observations on mycelium growth and per cent growth inhibition (PGI) were recorded after eight days of incubation and the results presented in Table 1.All the antagonists significantly inhibited the mycelial growth of *A. tomato* over the control. The significantly lowest mycelial growth (11.33 mm) with highest per cent growth inhibition was observed in *T. viride* (85.02 %) followed by *T. fasciculatum* (13.33 mm & 82.37 PGI), *P. fluorescens* Navsari isolate (17.33 mm & 77.09 PGI) and *T. harzianum* (20.00 mm & 73.56 PGI) after 8^{th}day of incubation, while, *P. fluorescens*Waghai isolate showed minimum mycelial growth inhibition (46.37 mm & 45.15 PGI) as depicted in Table 1.

Table 1: Effect of antagonists on severity of Alternaria fruit rot of tomato*in vitro* and*in vivo*

Antagonists	Mycelial growth (mm)	Per cent Growth Inhibition	Alternaria Rot Severity (%)			
			Pre -Inoculation		Post -Inoculation	
			4 th day	8th day	4 th day	8th day
T. viride	11.33	85.02	5.00 (12.90)	10.63 (19.02)	7.33 (15.70)	13.67 (21.67)
T. harzianum	20.00	73.56	6.87 (15.18)	13.00 (21.11)	8.80 (17.24)	14.93 (22.71)
P. fluorescens Waghai isolate	37.33	50.66	7.90 (16.31)	14.93 (22.71)	10.67 (19.05)	16.67 (24.07)
P. fluorescens Navsari isolate	17.33	77.09	6.40 (14.63)	15.53 (23.20)	8.23 (16.66)	17.80 (24.93)
Bacillus subtilis	33.00	56.38	8.67 (17.10)	19.03 (25.85)	10.93 (19.29)	22.33 (28.18)
T. fasciculatum	13.33	82.37	5.20 (13.16)	9.53 (17.97)	7.37 (15.73)	11.07 (19.41)
Control	75.67	0.00	19.23 (25.99)	43.47 (41.22)	20.53 (26.92)	44.07 (41.57)
S. Em +		0.94	0.33	0.32	0.47	0.31
C. D. at 5%		2.86	1.01	0.97	1.43	0.94
C.V. %		5.50	3.50	2.28	3.15	2.89

*Values in parenthesis are arcsine transformed

Table 2: Efficacy of phytoextract against *Alternaria tomato* causing fruit rot of tomato *in vitro* and *in vivo*

Phytoextract (10%)	Mycelial growth (mm)	Per cent Growth Inhibition	Alternaria Rot Severity (%)			
			Pre-Inoculation		Post-Inoculation	
			4th day	8th day	4th day	8th day
Garlic	17.00	78.01	2.90 (9.79)	8.37 (16.79)	3.43 (10.66)	10.47 (18.85)
Tulsi	23.67	69.32	3.70 (11.08)	9.63 (18.05)	3.97 (11.47)	12.73 (20.88)
Cinnamon	52.00	32.75	5.73 (13.84)	21.50 (27.60)	5.93 (14.08)	23.00 (28.63)
Piper	42.33	45.25	4.67 (12.46)	18.60 (25.53)	4.90 (12.77)	20.57 (26.95)
Lemon grass	30.33	60.77	4.83 (12.68)	17.77 (24.91)	5.60 (13.66)	19.50 (26.18)
Ardusi	40.33	47.84	4.17 (11.76)	16.93 (24.28)	5.47 (13.50)	19.0 3(25.84)
Neem	25.33	67.24	3.57 (10.87)	15.50 (23.16)	4.83 (12.69)	18.57 (25.50)
Aloevera	32.67	57.75	4.53 (12.28)	16.70 (24.10)	5.23 (13.21)	19.13 (25.91)
Cow urine	33.00	57.32	3.93 (11.43)	15.93 (23.50)	4.73 (12.55)	18.10 (25.15)
Control	77.33	0.00	13.30 (21.37)	39.90 (39.15)	14.39 (22.27)	44.07 (41.57)
S. Em+		1.20	0.24	0.51	0.34	0.55
C. D. at 5%		3.54	0.73	1.51	1.02	1.63
C.V. %		5.57	3.39	3.60	4.40	3.61

*Values in parenthesis are arcsine transformed

Similar results were reported by Chohan*et al*. (2015) and Babu *et al*. (2000) where they reported significantly highest mycelial growth inhibition of *A. tomato* and *A. solani* by *T. viride* and *T. harzianum*, respectively. Significantly highest per cent growth inhibition of *A. alternata* after 7th day of inoculation was found in *T. viride* (65.35 %) followed by *T. harzianum* (53.86 %) as reported by Panchal,(2008).

In vivo (pre-inoculation &post-inoculation)

All the antagonists were found significantly superior in reducing the Alternaria fruit rot severity after 4th and 8th day of inoculation in pre-and post-inoculation treatments. *T. viride* was found significantly most efficient in reducing the Alternaria fruit rot severity by 5.00 and 10.63 per cent on 4thand 8thday of inoculation followed by *T. fasciculatum*at 5.20 and 9.53 per cent.*B. subtilis* and *P. fluorescens*Waghai isolate was found least effective in reducing the fruit rot severity (8.67 & 19.03 %) and (7.90 & 14.93 %) (Table 1).

An antagonist i.e.*T. viride* was found significantly most efficient in reducing the Alternaria fruit rot severity by 7.33 and 13.67 per cent on 4th and 8th day of inoculation followed by *T. fasciculatum* 7.37 and 11.07 per cent, respectively, while, *B. subtilis* and *P. fluorescens*Waghai isolate were found least effective in reducing the fruit rot severityas presented in Table 1. Similar result to the present investigation was reported by Panchal (2008), where *T. viride* and *T. harzianum*were revealed as most potent antagonists in controlling the rots caused by *A. alternata* in tomato fruits. The Rathod, 2004 also found *T. viride* and *T. harzianum*as most potent antagonist in controlling the Alternaria fruit rot of aonla caused by *A. alternata*.

Phytoextracts

Nine phytoextracts at 10 per cent concentration were tested against mycelial growth of *A. tomato in vitro* by standard poison food technique (Nene and Thapliyal, 1979). The observation on the mycelial growth and PGI were recorded after seven days of incubation and the results are presented in Table 2.

All the phytoextracts screened were found significantly superior in inhibiting the mycelial growth of *A. tomato* over the control. Significantly lowest mycelial growth was recorded in garlic clove extract (17.00 mm) with 78.01 PGI as shown in Table 2. The next best treatment in order of merit were tulsi (23.67 mm) and neem (25.33 mm) with 69.32 and 67.24 PGI, respectively over control. The ardusi (40.33 mm) and cinnamon (52.00 mm) leaf extract found least effective in restricting the mycelial growth of *A. tomato* with 47.84 and 32.75 PGI depicted in Table 2.

Ngoc *et al*. (2013) recorded similar result that garlic bulb at 15 per cent concentration inhibited the 67.41 per cent mycelial growth of *A. solani*in *in vitro* condition. Further,Deshmukh *et al*. (2012)also reported antifungal activity of cow urine against *A. solani* and found that 20 ml of cow urine from indoor feeding cow and 10 ml, 15 ml and 20 ml from outdoor feeding cow effectively controlled the mycelia growth of *A. solani*. The phytoextract of neem and tulsi at 10 per cent concentration were inhibited the mycelial growth ranging from 74.2 to 45 % and 38.2 to 32 %, respectively against *A. solani*in *in vitro* condition (Sadana and Didwania, 2015).

All the nine phytoextracts were found significantly superior in reducing the Alternaria fruit rot severity as compared to control on 4thand 8th day after inoculation in pre and post-inoculation methods at 10 per cent concentration

Pre-inoculation

The presented results revealed that significantly lowest fruit rot severity was observed in fruits treated with garlic cloves extract (2.90 & 8.36 %) followed by tulsi leaf extract (3.70 & 9.64 %) and neem leaf extract (3.57 & 15.50 %) at 4th and 8th day of inoculation, respectively. However, cinnamon leaf extract proved least effective in reducing the Alternaria fruit rot (5.73 & 21.75 %) at 4th and 8th day after inoculationas mentioned in Table 2.

Post-inoculation

The garlic clove extract at 10 per cent proved the best to reduce the severity (3.43 & 10.47 %) of Alternaria fruit rot followed by tulsi leaf extract (3.97 & 12.73 %) and neem leaf extract (4.83 & 18.58 %) at 4th and 8th day after inoculation, respectivelyas revealed in Table 2.The results of present investigations are similar to earlier studies made by Patel (2000) indicating datura extract (1:1) as most effective in reducing Alternaria fruit rot of tomato (*A.*

tomato) in both pre- and post- inoculation treatments. Pre inoculation treatment was found more effective than the post- inoculation treatment. Sandipan *et al*. (2014), revealed that in pre-treatment, after seven days of incubation, the lesion diameter was 24.27, 31.90, 34.03, 39.07 and 43.33 mm in leaf extracts of datura, neem, garlic, nilgiri and lantana, while in case of post-treatment, all the phytoextracts significantly reduced the fruit rot of tomato over control. However, Meena *et al*. (2014) found thatcow urine at 1000 ppm concentration as pre and post-treatment recorded 8 and 10.3 per cent disease incidence, respectively against *A. solani* causing fruit rot in chilli.

Trichoderma viride and *T. fasciculatum* were most potent antagonists for the management of Alternaria fruit rot of tomato *in vitro* and also found effective in pre and post-inoculation treatment for managing fruit rot compared to other tested organism, while garlic clove extracts found best for the management of Alternaria fruit rot *in vitro* and *in vivo*. Whereas, pre-inoculation treatment found more effective as compared topost-inoculation *in vivo*. during the research work.

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