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***In vitro* bio-efficacy of phyto extracts against *Alternaria solani* and *Colletotrichum capsici*, causing tomato fruit rots**

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Abstract

The present study was conducted on developing environmentally safe, long-lasting and effectively biocontrol methods to test the efficacy of plant extracts *in vitro* against *A. solani* and *C. capsici*, causing tomato fruit rot. For eco-friendly, cost effective and non- phytotoxic management the phytoextracts (each @ 10% & 20%) tested *in vitro*. Among the nine phyto extracts *Lawsonia inermis* resulted with highest mycelial growth inhibition (80.21%) followed by *Allium sativum* (70.87%), *Eucalyptus globulus* (68.36%). Whereas, least inhibition of test fungus was recorded in *Aloe barbadensis* (54.56%). However, in *C. capsici*, *A. sativum* resulted with highest mycelial growth inhibition (97.31%) followed *L. inermis* (65.28%), *Zingiber officinale* and *E. globulus* (61.21%). Whereas, least inhibition of test fungus was recorded in *A. barbadensis* (48.99%).

Keywords: *Alternaria solani*, *Colletotrichum capsici*, Phytoextracts, Inhibition

Introduction

Tomato (*Lycopersicon esculentum*) an important fruit vegetable and high value crop cultivated in the protected condition. Tomato fruits also give abundant and well-balanced nutrition, consisting of minerals (potassium, magnesium, calcium, iron and zinc), vitamins (A, B1, B2, C and E), dietary fibers (pectin) and citric acid. In addition has the red pigment (lycopene) which contains high antioxidant potential and ability to neutralize harmful oxygen radicals that probably cause cancer, aging and arteriosclerosis, coronary heart disease and hypertension. Thus, tomato contributes to enjoyable diet and good health all over the world (Beckles, 2012) [3].

In India, area, production and productivity of tomato during 2018-19 were 781 Lakh ha, 19007 Lakh metric tons and 24.33 Lakh metric tons per ha, respectively. Whereas, in Maharashtra were 40.34 Lakh ha, 805.90 Lakh metric tons and 20.01 Lakh metric tons per ha, respectively (Anonymous, 2019) [1].

The major fungi causing tomato fruit rots/ diseases are grey mold (*Botrytis cinerea*), Rhizopus rot (*Rhizopus stolonifer*), anthracnose (*Colletotrichum capsici*, *C. coccodes*, *C. phomoides*, *C. spp.*), early blight (*Alternaria solani*), phoma rot (*Phoma destructiva*), fusarium rot (*F. solani*, *F. oxysporum* f. sp. *lycopersici*), *Aspergillus* rot (*A. flavus*, *A. niger*) etc. in addition, the fungi causing tomato fruit spoilage are *Penicillium* spp., *Cladosporium* spp., *Geotrichum candidum* and *Rhizoctonia solani*. The cumulative attack of these fungal fruit rots results into about 10-30 per cent qualitative losses in tomato, under field, storage and transit conditions. (Malik *et al.*, 2018; Zakawa *et al.*, 2019) [6, 13].

Owing to residual toxicity of over used persistent pesticides and their undesirable effects, it is imperative to search for viable, sustainable, cost-effective and eco-friendly strategies of integrated management of tomato fungal fruit rots. Organic / biological practices (phytoextracts) have been reported to be one of the most potential means to manage several plant diseases, as well as these biofungicides/biologicals are emerging as one of the potential components of integrated disease management strategies (Bankole *et al.*, 2018; Dar *et al.*, 2019) [2, 4].

Materials and Methods

The experiment was conducted during winter, 2020 at Department of Plant Pathology, College of Agriculture, Latur, during present investigations on *in vitro* evaluation of phytoextracts against *A. solani* and *C. capsici*, tomato anthracnose.

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Solvent extracts of locally available higher plant species were separately evaluated *in vitro* (each @ 10 and 20%) against the test fungi (*A. solani*, *C. capsici*), by applying Poisoned food technique (Nene and Thapliyal, 1993)^[7] and using PDA as a basal culture medium. Plant parts (leaves, bulbs, cloves and rhizomes etc.) of the selected plant species were washed thoroughly with distilled water and chopped into small bits with sterilized sharp knife. These were separately macerated and homogenized with pestle and mortar, in equal quantity of distilled water (1:1 w/v). These homogenates obtained were strained separately through double layered muslin cloth and the extract / filtrates obtained were further filtered through Whatman's No. 1 filter paper, using glass volumetric flask (100 ml capacity) and funnel. The clear supernatants obtained constituted the phytoextracts of 100% concentration. An appropriate quantity of these phytoextracts (100%) was

separately mixed with autoclaved and cooled (400 C) PDA medium in conical flasks (250 ml) to make the PDA medium of 10 and 20 per cent concentration. This PDA medium amended separately with phytoextract was then poured (20 ml/plate) into sterile glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. Three PDA plates per test phytoextract per test pathogen were maintained and replicated thrice. Upon solidification of the PDA, all these treatment plates were aseptically inoculated by placing in the centre a 5 mm mycelial disc of the test fungus, obtained from a week old actively growing pure culture of the test fungus. Plain PDA plates without any phytoextract, inoculated with mycelial disc of the test fungus were maintained as untreated control. All these plates were incubated at 27±2 °C temperature for a week or until the untreated control plates were fully covered with mycelial growth of the test fungus.

Table 1: List of botanicals / phytoextracts used

Tr. No.	Treatments	Plant parts used	Tr. No.	Treatments	Plant parts used
T ₁	<i>Eucalyptus globulus</i> (Nilgiri)	Leaves	T ₆	<i>Pongamia pinnata</i> (Karanj)	Leaves
T ₂	<i>Allium cepa</i> (Onion)	Bulb	T ₇	<i>Zingiber officinale</i> (Ginger)	Rhizome
T ₃	<i>Allium sativum</i> L. (Garlic)	Clove	T ₈	<i>Aloe barbadensis</i> (Aloe-vera)	Gel
T ₄	<i>Azadirachta indica</i> (Neem)	Leaves	T ₉	<i>Lawsonia inermis</i> (mehandi)	Leaves
T ₅	<i>Lantana camara</i> L. (Ghaneri)	Leaves	T ₁₀	Control (untreated)	-----

Observations on radial mycelial growth / colony diameter (mm) were recorded at an interval of 24 hrs and continued upto seven days after incubation or till the untreated PDA plates were covered fully with mycelial growth of the test fungus. Based on cumulative data, per cent mycelial growth inhibition of the test fungus with the test phytoextracts, over untreated control was calculated by applying the following formula (Vincent, 1927)^[11].

$$\text{Growth Inhibition (\%)} = \frac{C-T}{C} \times 100$$

Where

C = Growth (mm) of the test fungus in untreated control plate

T = Growth (mm) of the test fungus in treated plates

Results and Discussion

In vitro efficacy of phytoextracts against *A. solani*, causing tomato fruit rot

Solvent extracts (leaf / rhizome / bulb) of nine plant species were evaluated *in vitro* (each @ 10 and 20%) against *A. solani* and the results obtained on mycelial growth and its inhibition are presented (Table 2, Plate 1).

Table 2: *In vitro* efficacy of phytoextracts against *A. solani*, causing tomato fruit rot

Tr. No.	Treatments	Col. Dia.*(mm)		Av. (mm)	% Inhibition *		Av. (%)
		10%	20%		10	20	
T ₁	<i>Eucalyptus globulus</i>	29.83	27.13	28.48	66.86 (54.85)	69.86 (56.70)	68.36 (55.77)
T ₂	<i>Allium cepa</i>	32.17	31.13	31.65	64.26 (53.29)	65.41 (53.98)	64.84 (53.63)
T ₃	<i>Allium sativum</i>	30.17	22.27	26.22	66.48 (54.62)	75.26 (60.17)	70.87 (57.34)
T ₄	<i>Azadirachta indica</i>	30.50	27.30	28.90	66.11 (54.40)	69.67 (56.58)	67.89 (55.48)
T ₅	<i>Lantana camara</i>	41.33	35.23	38.28	54.08 (47.34)	60.86 (51.27)	57.47 (49.30)
T ₆	<i>Pongamia pinnata</i>	36.67	30.13	33.40	59.26 (50.34)	66.52 (54.65)	62.89 (52.47)
T ₇	<i>Zingiber officinale</i>	32.83	27.17	30.00	63.52 (52.84)	69.81 (56.67)	66.67 (54.73)
T ₈	<i>Aloe barbadensis</i>	43.67	38.13	40.90	51.48 (45.85)	57.63 (49.39)	54.56 (47.61)
T ₉	<i>Lawsonia inermis</i>	20.33	15.30	17.81	77.41 (61.62)	83.00 (65.65)	80.21 (63.58)
T ₁₀	Control (untreated)	90.00	90.00	90	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E. ±	0.820	0.700	-	0.820	0.776	-
	C.D.(P= 0.01)	2.456	2.100	-	2.456	2.322	-

*: Mean of three replications, Dia.: Diameter, Av.: Average, Figures in parentheses are arcsine transformed values.

Effect on mycelial growth

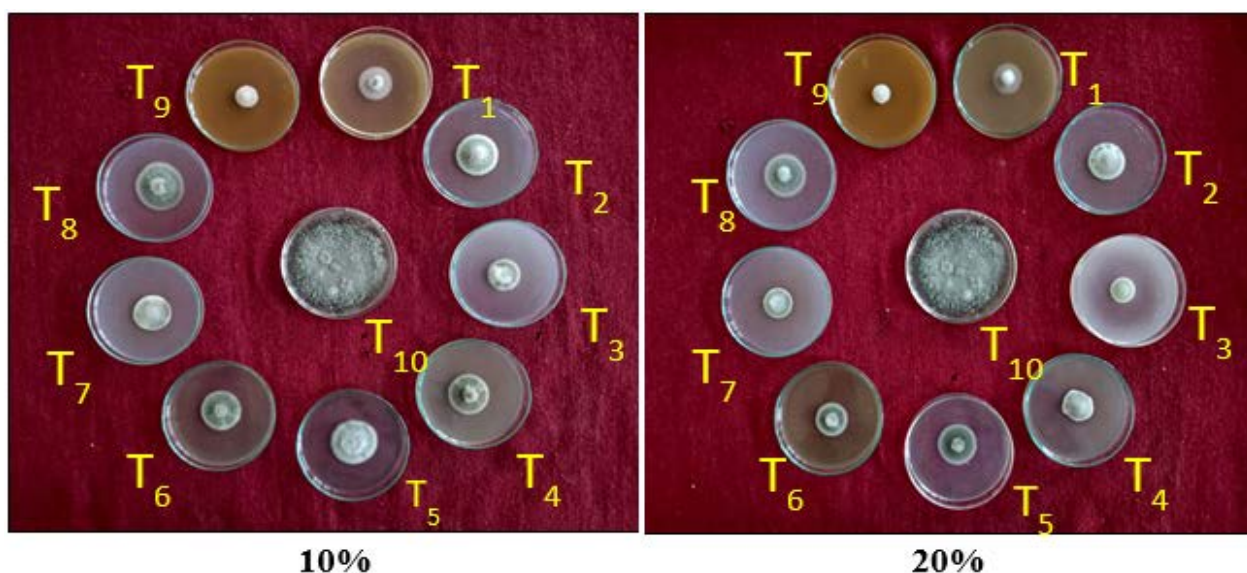
At 10 per cent, radial mycelial growth of *A. solani* ranged from 20.33 to 43.67 mm. However, it was significantly least with *L. inermis* (20.33 mm), followed by *E. globulus* (29.83 mm), *A. sativum* (30.17 mm), *A. indica* (30.50 mm), *A. cepa* (32.17 mm), *Z. officinale* (32.83 mm), which were on par to each other, *P. pinnata* (36.67 mm), *L. camara* (41.33) and *A. barbadensis* (43.67 mm). At 20 per cent, radial mycelial growth of *A. solani* ranged from 15.30 to 38.13 mm. However, it was significantly least with *L. inermis* (15.30 mm), followed by *A. sativum* (22.27 mm), *E. globulus* (27.13 mm), *Z. officinale* (27.17 mm), *A. indica* (27.30 mm), *P. pinnata* (30.13 mm), later three were on par *A. cepa* (31.13 mm), *L. camara* (35.23 mm) and *Aloe barbadensis* (38.13 mm).

Effect on mycelial growth inhibition

At 10 per cent, mycelial growth inhibition of *A. solani* ranged from 51.48 to 77.41 per cent. However, it was significantly highest with *L. inermis* (77.41%), followed by *E. globulus*

(66.86%), *A. sativum* (66.48%), *A. indica* (66.11%), *A. cepa* (64.26%), later four were on par to each other, *Z. officinale* (63.52%), *P. pinnata* (59.26%), *L. camara* (54.08%) and *A. barbadensis* (51.48%). At 20 per cent, mycelial growth inhibition of *A. solani* ranged from 57.63 to 83.00 per cent. However, it was significantly highest with *L. inermis* (83.00%), followed by *A. sativum* (75.26%), *E. globulus* (69.86%), *Z. officinale* (69.81%), *A. indica* (69.67%), later three were on par with each other, *P. pinnata* (66.52%), *A. cepa* (65.41%), both were on par, *L. camara* (60.86%) and *A. barbadensis* (57.63%).

Thus, based on average mycelial growth inhibition, the most potential antifungal phytoextracts found in their order of merit were *L. inermis* > *A. sativum* > *E. globulus* > *A. indica* > *Z. officinale*. Similarly, the phytoextracts viz., *L. inermis*, *A. sativum*, *E. globulus*, *A. indica* and *Z. officinale* were reported as potential antifungal / fungistatic against *Alternaria solani*, earlier by several workers (Singh *et al.*, 2018b; Yadav *et al.*, 2020) [10, 12].



Plant 1: The results obtained on mycelial growth and its inhibition are presented

In vitro efficacy of phytoextracts against *C. capsici*, causing tomato fruit rot

Solvent extracts (leaf / rhizome / bulb) of nine plant species were evaluated *in vitro* (each @ 10 and 20%) against *C. capsici* and the results obtained on mycelial growth and its inhibition are presented (Table 3, Plate 2).

Effect on mycelial growth

At 10 per cent, radial mycelial growth of *C. capsici* ranged from 3.17 to 47.83 mm. However, it was significantly least with *A. sativum* (3.17 mm), followed by *L. inermis* (33.17 mm), *Z. officinale* (37.50 mm), *E. globulus* (37.83 mm), *A. indica* (40.50 mm), *A. cepa* (41.17 mm), *L. camera* (43.17 mm), *P. pinnata* (46.17 mm) and *A. barbadensis* (47.83 mm). At 20 per cent, radial mycelial growth of *C. capsici* ranged from 1.67 to 44.00 mm. However, it was significantly least with *A. sativum* (1.67 mm), followed by *L. inermis* (29.33 mm), *E. globulus* (32.00 mm), *Z. officinale* (32.33 mm), *A. cepa* (36.00 mm), *A. indica* (36.67 mm), *L. camera* (40.33 mm), *P. pinnata* (42.67 mm) and *A. barbadensis* (44.00 mm).

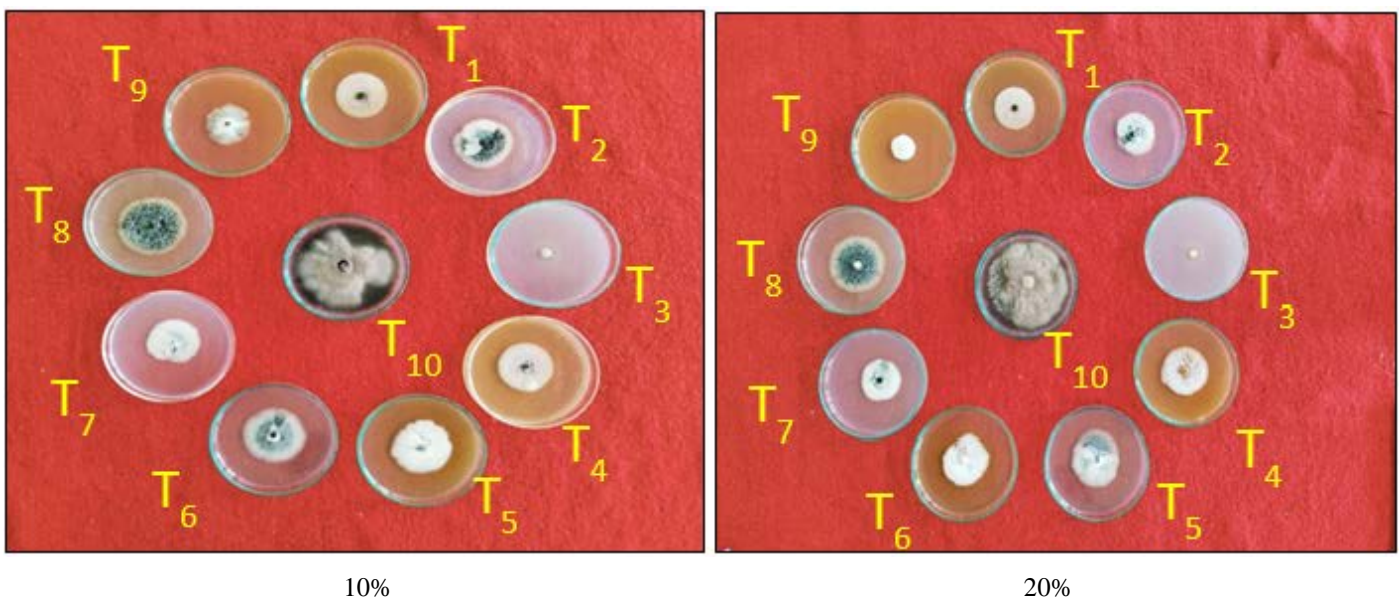
Effect on mycelial growth inhibition

At 10 per cent, mycelial growth inhibition of *C. capsici* ranged from 46.86 to 96.48 per cent. However, it was significantly highest with *A. sativum* (96.48%), followed by *L. inermis* (63.14%), *Z. officinale* (58.33%), *E. globulus* (57.97%), *A. indica* (55.00%), *A. cepa* (54.26%), *L. camera* (52.03%), *P. pinnata* (48.70%) and *A. barbadensis* (46.86%). At 20 per cent, mycelial growth inhibition of *C. capsici* ranged from 51.11 to 98.14 per cent. However, it was significantly highest with *A. sativum* (98.14%), followed by *L. inermis* (67.41%), *E. globulus* (64.44%), *Z. officinale* (64.08%), *A. cepa* (60.00%), *A. indica* (59.26%), *L. camera* (55.19%), *P. pinnata* (52.59%) and *A. barbadensis* (51.11%). Thus, based on average mycelial growth inhibition, the most potential antifungal phytoextracts found in their order of merit were *A. sativum* > *L. inermis* > *Z. officinale* > *E. globulus* > *A. indica* > *A. cepa*. These results of the present study on fungicidal / fungistatic potential of the test phytoextracts viz., *A. sativum*, *L. inermis*, *Z. officinale*, *E. globulus*, *A. indica* and *A. cepa* were reported antifungal / fungistatic against *C. capsici* are in agreement with the findings of several earlier workers (Salam *et al.*, 2018; Jehani *et al.*, 2019).

Table 3: *In vitro* efficacy of phytoextracts against *C. capsici*, causing tomato fruit rot

Tr. No.	Treatments	Col. Dia.* (mm)		Av. (mm)	% Inhibition *		Av. (%)
		10%	20%		10	20	
T ₁	<i>Eucalyptus globulus</i>	37.83	32.00	34.91	57.97 (49.59)	64.44 (53.39)	61.21 (51.47)
T ₂	<i>Allium cepa</i>	41.17	36.00	38.58	54.26 (47.44)	60.00 (50.77)	57.13 (49.10)
T ₃	<i>Allium sativum</i>	3.17	1.67	2.42	96.48 (79.19)	98.14 (82.16)	97.31 (80.56)
T ₄	<i>Azadirachta indica</i>	40.50	36.67	38.58	55.00 (47.87)	59.26 (50.34)	57.13 (49.10)
T ₅	<i>Lantana camara</i>	43.17	40.33	41.75	52.03 (46.16)	55.19 (47.98)	53.61 (47.07)
T ₆	<i>Pongamia pinnata</i>	46.17	42.67	44.42	48.70 (44.26)	52.59 (46.48)	50.65 (45.37)
T ₇	<i>Zingiber officinale</i>	37.50	32.33	34.91	58.33 (49.80)	64.08 (53.18)	61.21 (51.47)
T ₈	<i>Aloe barbadensis</i>	47.83	44.00	45.91	46.86 (43.20)	51.11 (45.64)	48.99 (44.42)
T ₉	<i>Lawsonia inermis</i>	33.17	29.33	31.25	63.14 (52.62)	67.41 (55.19)	65.28 (53.89)
T ₁₀	Control (untreated)	90.00	90.00	90	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E. ±	0.735	0.793	-	0.817	0.882	-
	C.D.(P= 0.01)	2.201	2.376	-	2.445	2.641	-

*: Mean of three replications, Dia.: Diameter, Av.: Average, Figures in parentheses are arcsine transformed values.



Plant 2: Solvent extracts (leaf / rhizome / bulb) of nine plant species were evaluated *in vitro* (each @ 10 and 20%) against *C. capsici* and the results obtained on mycelial growth and its inhibition are presented

Conclusions

In conclusions, the findings of this experiment confirmed that plant extracts can be used as natural fungitoxicant to control the growth of pathogenic fungi (*A. solani* and *C. capsici*) and thus reduce the dependence on the synthetic fungicide. Among the phytoextracts tested *in vitro*, *L. inermis*, *A. sativum*, *A. cepa*, *Z. officinale*, *E. globulus* and *A. indica*, were found efficient with significantly high mycelial growth inhibition of the fungi (*A. solani* and *C. capsici*) causing tomato fruit rots. Therefore, this study suggest that aqueous extracts of these species would be helpful in treating diseases in plants.

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