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Research Article

In Vitro Evaluation of Botanicals and Biocontrol Agents against *Alternaria alternata*, Causing Black Core Rot of Citrus

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ABSTRACT

Postharvest pathogens were isolated from diseased citrus fruits. Different post harvest diseases observed during the present investigations were anthracnose, black core rot, black mould rot, fusarium rot, stem end rot and sour rot. In vitro evaluation of botanicals against Alternaria alternata revealed that 10 % chromolaena leaf extract recorded maximum (69.51 %) mycelial growth inhibition where as 10% garlic bulb extract recorded highest spore germination inhibition. Among all the biocontrol agents tested T. harzianum (75.21 %) and T. virens (isolate 2) (74.45 %) were most effective in inhibiting the mycelial growth of A. alternata.

Keywords: *Biological control, Citrus black core rot of citrus, Alternaria alternata, T. harzianum*

INTRODUCTION

Postharvest losses of perishable crops in developing countries have been estimated in the range of 5-50 per cent or more of the harvest (Salunke and Desai, 1984). Postharvest losses in mango (17-36%), banana (12-14 %), oranges (8.3-30.7%), grapes (23-30 %) have been reported from India (Madan and Ullasa, 1993).

Postharvest losses of citrus fruits in India are in the range of 25-30% as against 5-10% in other developed countries like Brazil, USA, Australia, Spain, Italy and Israel (Sonkar *et al.*, 2008). Nanda *et al* (2012) also reported postharvest losses of citrus fruits at national level to be 6.4% at various stages like sorting/grading, transportation, storage at wholesaler and retailer levels. One of the major cause of postharvest losses is due to diseases caused by post harvest pathogens.

Development of resistance in pathogens to fungicides applied for controlling the postharvest diseases has been reported (Spotts and Cervantes, 1986, Spalding, 1982). Consumers are becoming highly conscious of the fungicide residues in fruits. There is an urgent need to develop novel and alternative postharvest disease management strategies. Non chemical management using botanicals and biocontrol agents provide an opportunity for addressing the fungicide residue problems in the management of postharvest diseases. The present investigations are carried out to find out the various postharvest pathogens prevailing in Dharwad and in vitro antagonistic studies were made using botanicals and biocontrol agents against *Alterrnaria alternata* isolated from citrus fruits.

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MATERIALS AND METHODS

Citrus fruits infected by different postharvest pathogens, showing typical symptoms were collected from Dharwad market and from citrus orchards. Fungi were isolated by following standard tissue isolation method. Pathogenicity of the organisms was proved by proving Koch's postulates.

A) *In vitro* evaluation of botanicals:

Antagonistic activity of the below mentioned botanicals was tested *in vitro.*

Sl. No.	Scientific name	Vernacular name	Family	Part used
1	*Allium sativum* L.	Garlic	Amaryllidaceae	Bulb
2	*Azardirachta indica* Juss.	Neem	Meliaceae	Leaves
3	*Clerodendron inermii* Gaertn.	Kashmir bouquet	Verbenaceae	Leaves
4	*Chromolaena odoratum* L.	Communist weed	Compositae	Leaves
5	*Lantana camara* L.	Lantana	Verbenaceae	Leaves
6	*Ocimum sanctum* L.	Tulsi	Lamiaceae	Leaves
7	*Parthenium hysterophorous* L.	Congress grass	Compositae	Leaves
8	*Tridax procumbens* L.	Tridax	Compositae	Leaves and flowers

Preparation of stock solution of Botanicals:

Fresh leaves/bulb of each botanicals plant was collected and washed first in tap water and then in distilled water. Then, 100 g of fresh sample was crushed in a mixer grinder by adding 100 ml sterile distilled water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Final filtrate thus obtained was used as stock solution.

i) Mycelial growth inhibition:

Antifungal activity of botanicals was tested using the poisoned food technique as suggested by Nene and Thapliyal (1982). Stock solutions of 5 ml and 10 ml were mixed 95 and 90 ml of sterilized molten PDA medium respectively to get 5 and 10 per cent concentrations. Twenty ml of the poisoned medium was poured into each of the 90 mm sterilized petriplates. Each plate was seeded with 0.5 cm mycelial discs taken from the periphery of eight day old fungal culture and Per cent inhibition of mycelial growth over control was calculated when the growth of the fungus is full in control plate by using the formula given by Vincent (1927).

ii) Spore germination inhibition:

Effect of botanicals on spore germination of the test fungi was assessed by per cent inhibition of conidial germination. A single drop of the conidial suspension of the test organisms was added to the well of a series of cleaned cavity slides, to which a single drop of different botanicals (double the required concentrations) was also added to get the required concentrations of 5 and 10 per cent. The wells were immediately covered by using coverslips on the cavity slides and the periphery was smeared with Vaseline. Control was maintained with distilled water. The cavity slides were kept in the petriplates lined with moist blotting paper and were incubated at room temperature. Observations were made from ten microscopic fields from each slide. Per cent germination was calculated from the number of total conidia and germinated conidia in each microscopic field. Further, the percent inhibition of spore germination was calculated by using the formula given by Vincent (1927) for each botanical.

B) *In vitro* evaluation of biocontrol agents

From the actively growing cultures of both fungal bioagents and test pathogens, 0.5 cm fungal disc were transferred aseptically to petriplates containing PDA, simultaneously by leaving sufficient space in between two discs. In case of bacterial biocontrol agents, mycelial

discs of the test fungus was kept at opposite ends and bacterium was streaked at the center. A pathogen disc alone placed at the center of the petriplate served as control. Colony diameter of both the test fungus and bioagents were measured when control plate is fully covered and per cent inhibition was calculated by using the formula given by Vincent (1927).

RESULTS AND DISCUSSION

Postharvest diseases observed during the present investigation are anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Plate 1), black core rot caused by *Alternaria alternata* (Fr.) Keissier and *A. citri* Ell. & Pierce (Plate 2), Black mould rot caused by *Aspergillus niger* v. Teighem (Plate 3), fusarium rot caused by *Fusarium sps*. (Plate 4), stem end rot caused by *Botryodiplodia theobromae* (Plate 5) and sour rot caused by *Geotrichum candidum* Link. (Plate 6).

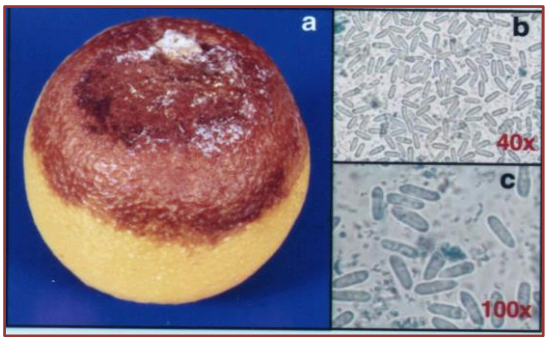

Plate 1: a : Anthracnose of citrus
b & c : Spores of *Colletotrichum gloeosporioides*

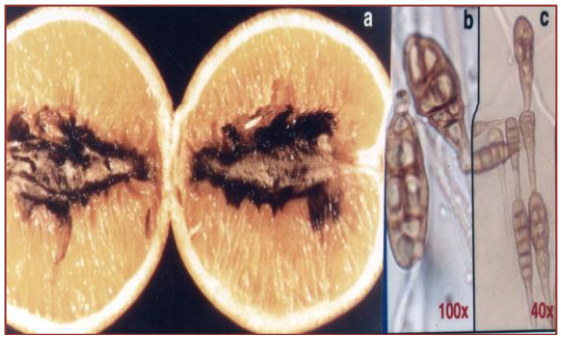

Plate 2: a : Black core rot of citrus
b : Spores of *Alternaria citri*
c : Spores of *Alternaria alternata*

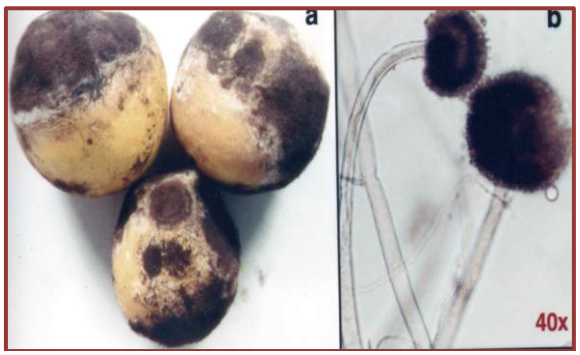

Plate 3: a : Black mould rot of citrus
b : Conidial heads of *Aspergillus citrus*

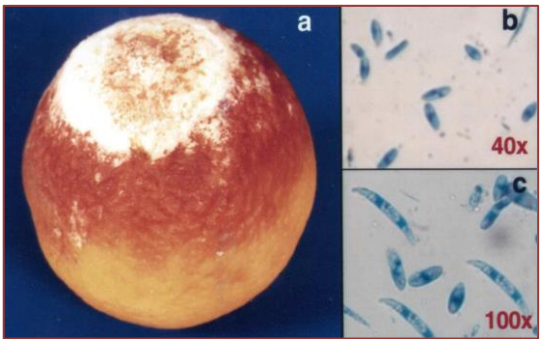

Plate 4: a : Fusarium rot of citrus
b & c : Micro and Macroconidia of *Fusarium sps.*

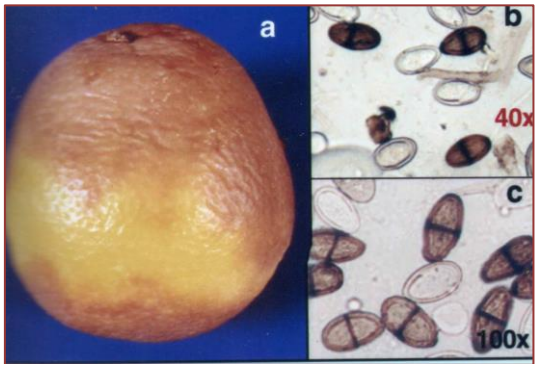

Plate 5: a : Stem end rot of citrus
b & c : Conidia of *Botryodiplodia theobromae*

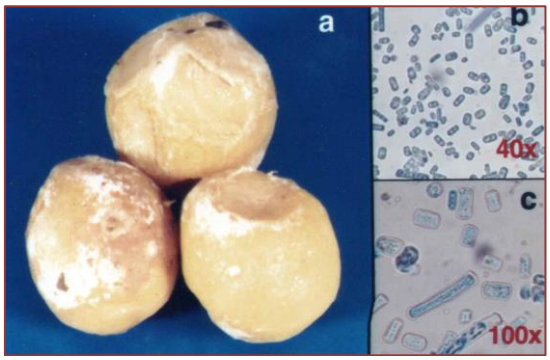

Plate 6: a : Sour rot of citrus
b & c : Spores of *Geotrichum candidum* (Arthospores)

A) *In vitro* evaluation of botanicals against *A. alternata*: (Table 1 & Plate 7)

Among the eight plant extracts tested against *A. alternata*, mean (5 and 10 per cent together) mycelial inhibition is significantly superior in chromolaena leaf extract (62.86 %) over all other plant extracts. Next best was garlic bulb extract (49.68 %), which was on par with neem leaf extract (49.65 %). All the plant extracts at 10 per cent concentration were found to be superior over 5 per cent concentration. Maximum inhibition of 69.51 per cent of mycelial growth was recorded in 10 % chromolaena leaf extract, followed by 56.20 % in 5% chromolaena leaf extract, 55.49 % in 10 % neem leaf extract and 54.01 % in 10% garlic bulb extract. Highest mean (5 and 10 per cent together) spore germination inhibition of *A. alternata*was observed in tulsi leaf extract (62.76 %) and was on par with garlic bulb extract (61.00 %) followed by neem leaf extract (49.89 %) and chromolaena leaf extract

(45.78 %). Among the plant extracts tested at various concentrations, 10 % garlic bulb extract was found to be superior in inhibiting the spore germination (70.89 %), which was on par with 10 % tulsi leaf extract (69.97 %). Antifungal activity of plant extracts against *A. alternata* has been reported earlier by various workers. Antifungal activity of garlic (Mishra and Dixit, 1976; Jitender Singh and Majumdar, 2001; Karade and Sawant, 1999), neem and tulsi (Prasanna Kumar, 2001; Jitender Singh and Majumdar, 2001) were reported earlier against postharvest pathogens.

Table 1: *In vitro* evaluation of botanicals against *Alternaria alternata* of citrus.

S. No	Plant extract	Percent inhibition of					
		Mycelial growth			Spore germination		
		5 %	10 %	Mean	5 %	10 %	Mean
1	Chromolaena leaf extract	56.20 (48.57)*	69.51 (56.50)	62.86 (52.53)	41.33 (39.99)	50.23 (45.13)	45.78 (42.56)
2	Clerodendron leaf extract	27.22 (31.42)	30.52 (33.51)	28.87 (32.47)	38.21 (38.16)	51.12 (45.58)	44.67 (41.87)
3	Garlic bulb extract	45.31 (42.32)	54.01 (47.31)	49.68 (44.82)	51.10 (45.59)	70.89 (57.33)	61.00 (51.46)
4	Lantana leaf extract	22.60 (28.39)	33.12 (35.11)	27.86 (31.75)	30.21 (33.20)	38.69 (38.48)	34.45 (35.89)
5	Neem leaf extract	43.81 (41.45)	55.49 (48.17)	49.65 (44.81)	43.89 (41.48)	55.89 (48.37)	49.89 (44.93)
6	Parthenium leaf extract	27.32 (31.51)	37.00 (37.47)	32.16 (34.49)	31.72 (34.28)	47.15 (43.35)	39.44 (38.82)
7	Tridax leaf extract	25.51 (30.30)	33.71 (35.46)	29.61 (32.88)	33.56 (35.38)	45.67 (42.45)	39.62 (38.92)
8	Tulsi leaf extract	28.60 (32.20)	42.12 (40.46)	35.42 (36.38)	55.54 (48.11)	69.97 (56.75)	62.76 (52.44)
	Mean	34.20 (35.78)	44.36 (41.75)	39.20 (38.76)	40.52 (39.54)	53.80 (47.18)	47.15 (43.36)
	Source	Sem ±		CD at 1 % Level	Sem ±		CD at 1 % Level
	Plant extract (P)	0.37		1.45	0.52		2.03
	Concentration (C)	0.18		0.72	0.26		1.01
	PxC	0.52		2.05	0.74		2.80

*Figures in the parentheses are angular transformed values

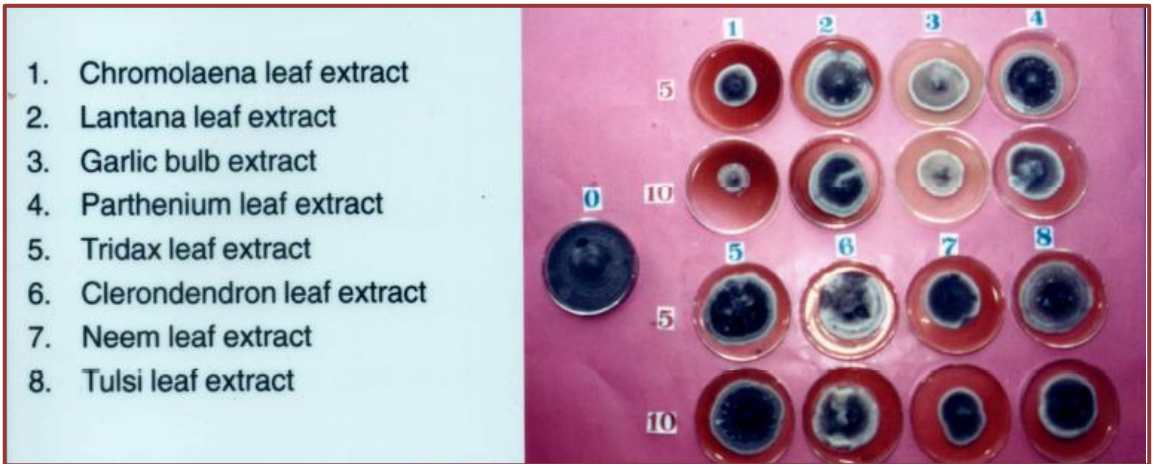

Plate 7: Effect of botanicals on mycelial growth of *Alternaria alternata*

B) *In vitro* evaluation of biocontrol against *A. alternata*: (Table 2 & Plate 8)

The antagonistic activity of six fungal biocontrol agents *viz., Trichoderma viride*, *T. harzianum*, *T. reesei*, *T. virens* (isolate 1), *T. virens* (isolate 2) , *T. peseudokoningi*, two bacterial biocontrol agents *Bacillus subtilis* and *Pseudomonas fluorescens* was a tested against *Alternaria alternata* isolated from citrus fruits.

Among all the biocontrol agents tested *T. harzianum* (75.21 %) and *T. virens* (isolate 2) (74.45 %) were on par and most effective in inhibiting the mycelial growth. *T. virens* (isolate 1) with 66.68 % inhibition and *T. reesei* with 64.41 % inhibition were on par with each other, followed by *T. viride* (62.13%) and *T. pseudokoningi* (59.60%). Among the bacterial biocontrol agents, highest inhibition (70.78 %) was observed in *P. fluorescens* while *B. subtilis* could inhibit only up to 43.99 per cent. Antagonistic nature of *Trichoderma spp.* against plant pathogens has been well documented by Cook and Baker (1983). Production of a variety of antagonistic secondary metabolites like siderophores (Fravel,1988) and antibiotics like phenozines, pyroluterin. pyrrolnitrin (Vidyasekharan and Muthamilan, 1995) by *P. fluorescens* was well documented earlier and in the present investigations also are in agreement with them.

Table 2: *In vitro* evaluation of biocontrol agents against *Alternaria alternata* of Citrus

Sl. No.	Biocontrol agent	Per cent inhibition of mycelial growth
1	*Bacillus subtilis*	43.99 (40.51)*
2	*Pseudomonas fluorescens*	70.78 (57.25)
3	*Trichoderma harzianum*	75.21 (60.13)
4	*T. pseudokoningi*	59.60 (50.50)
5	*T. reesei*	64.41 (53.37)
6	*T. virens* (isolate 1)	66.68 (54.74)
7	*T. virens* (isolate 2)	74.45 (59.63)
8	*T. viride*	62.13 (51.57)
	SEm ±	0.43
	CD at 1% level	1.82

*Figures in the parentheses are angular transformed values.

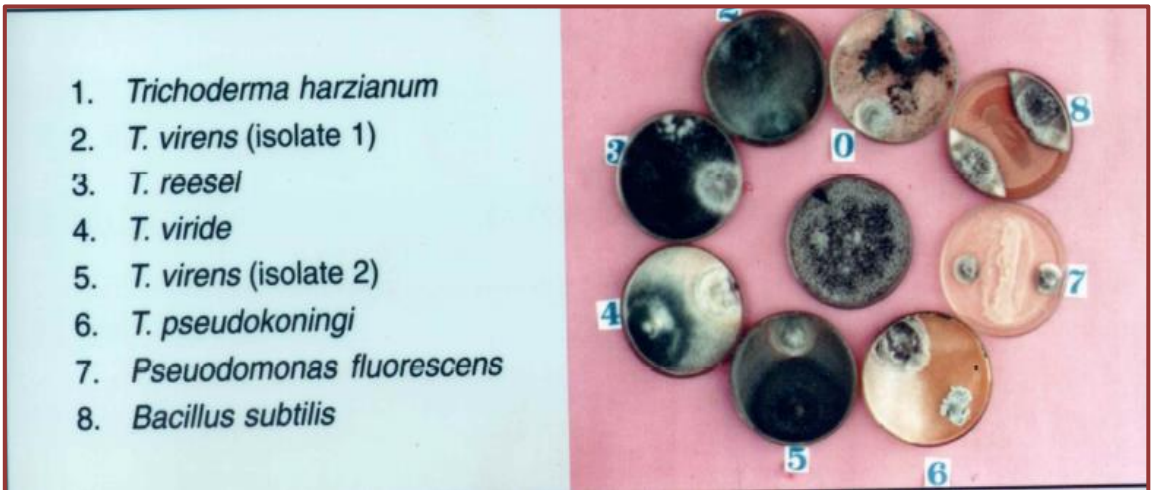

Plate 8: Effect of biocontrol agents on mycelial growth of *Alternaria alternata*

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