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Biological Management of Post Harvest Diseases 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. Garlic bulb extract and neem leaf extract both at 10 % recorded highest inhibition of mycelial growth (82.17 %) and spore germination (81.10 %) of Botryodiplodia theobromae repectively. Among the bioagents tested highest inhibition of mycelial growth of B. theobromae was observed in T.viride and T. virens (isolate 2). Among the biocontrol agents and plant extracts given as postharvest treatments to fruits, all the treatments were effective and significantly reduced PDI of stem end rot of citrus compared to control. B. subtilis (15.23) and garlic bulb extract (17.70) were most effective as postharvest treatments and were on par with each other in reducing the disease on fruits however chemical control Benomyl 0.1 % to be best among the postharvest treatments.

Keywords: *Citrus postharvest pathogens – Stem end rot - Botryodiplodia theobromae - Botanicals – Biocontrol agents.*

INTRODUCTION

Fruits are living entities and are highly perishable commodities that are affected by a number of factors leading to the postharvest spoilage and hence postharvest losses are very predominant. 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 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.

Postharvest diseases are traditionally managed by synthetic chemical fungicides. However, when harvested fruits are treated with fungicides to manage postharvest diseases, there is greater likelihood of direct human exposure to them. Apart from this, development of resistance in pathogens to fungicides applied for controlling the postharvest diseases has been reported (Spotts and Cervantes, 1986; Spalding, 1982).

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There is an urgent necessity to develop new and effective methods of controlling postharvest diseases that are perceived as safe by the public and pose negligible risk to human health and environment. Biological control is one such method.

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 inermiid* 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 against postharvest pathogens

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 calculatedby using the formula given by Vincent (1927).

C) *In vivo* evaluation of bioagents and botanicals against stem end rot of citrus.

In vivo studies were carried out against stem end rot of citrus by imposing various bioagents and botanicals by following pre inoculation method given by Bhuvaneswari (1999). Apparently, healthy and uninjured fruits were washed in 1:1000 mercuric chloride for 30 seconds followed by rinsing twice in distilled water and allowed to dry.Small wounds were made by pinching sterile paper pins at the stem end of the fruits. Cotton swabs dipped in suspensions of the bioagents and botanicals and swabbed over the wounded surface of the fruit followed by inoculation of the pathogen keeping the cotton swab dipped in spore suspension of the pathogen. The time interval between the postharvest treatments' application and inoculation was 12 h. Fruits were provided with sufficient relative humidity by placing cotton swabs dipped in water along with them. Observations were taken on eighth day after inoculation by following 0-5 scale given by Prasanna Kumar (2001).

Grade	Per cent disease on the fruit surface
0	No disease
1	01 – 5 %
2	5.1 – 10%
3	.10.1 – 25 %
4	25.1 – 50 %
5	50.1 – 5 %

Per cent Disease Index (PDI) was calculated by following the formula given by Wheeler (1969).

$$PDI = \frac{\text{Sum of individual disease rating}}{\text{No. of samples}} \times \frac{100}{\text{Maximum disease grade}}$$

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).

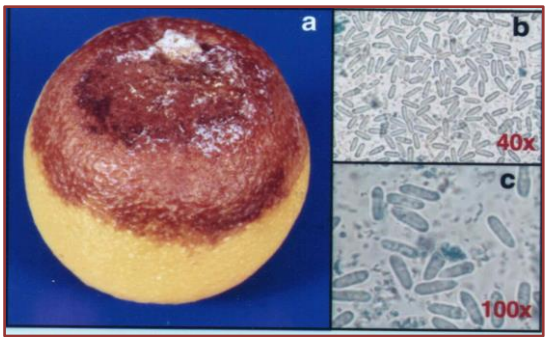

Plate 1: a : Anthracnose of citrus
b & c : Spores of *Colletotrichum gloeosporioides*

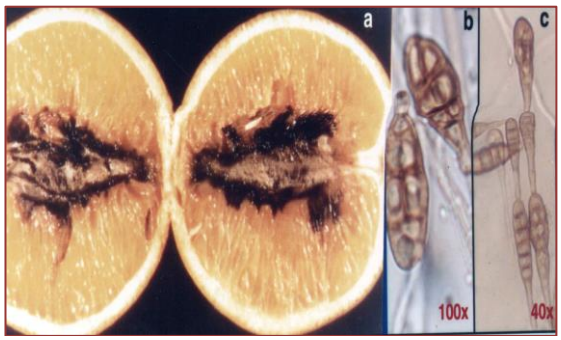

Plate 2: a : Black core rot of citrus
b : Spores of *Alternaria citri*
c : Spores of *Alternaria alternata*

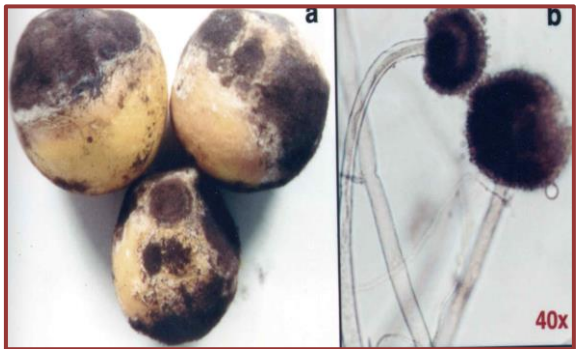

Plate 3: a : Black mould rot of citrus
b : Conidial heads of *Aspergillus citrus*

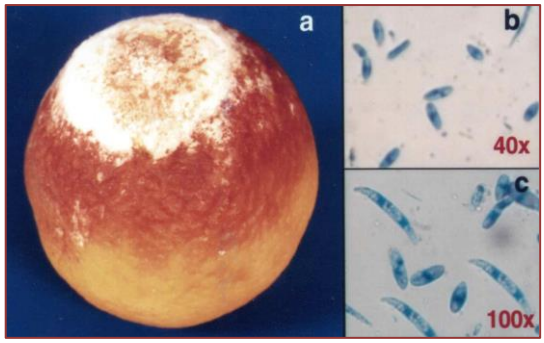

Plate 4: a : Fusarium rot of citrus
b & c : Micro and Macroconidia of *Fusarium sps.*

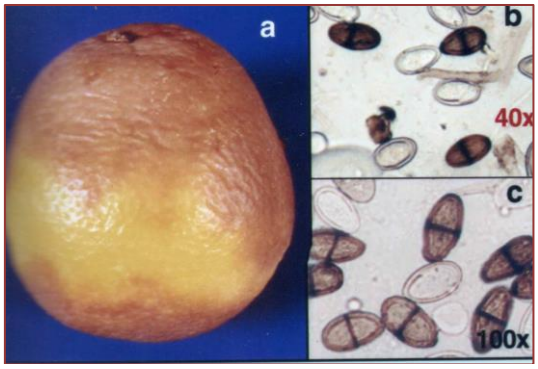

Plate 5: a : Stem end rot of citrus
b & c : Conidia of *Botryodiplodia theobromae*

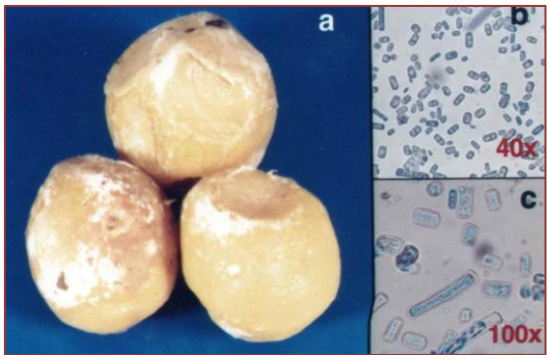

Plate 6: a : Sour rot of citrus
b & c : Spores of *Geotrichum candidum* (Arthospores)

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

i) Mycelial growth inhibition:

Highest mean (5 and 10 per cent together) mycelial inhibition (78.95 %) of mycelial growth of *B. theobromae* was observed in garlic bulb extract, followed by tulsi leaf extract (49.93 %) which was on par with neem leaf extract (47.57 %). Least inhibition was observed in tridax leaf extract and parthenium leaf extract, both inhibiting 1.62 per cent. Among the plant extracts tested at two concentrations highest inhibition (82.17 %) was in 10 % garlic bulb extract, followed by 75.73 % in 5 % garlic bulb extract. Least inhibition (0.00 %) was observed in 5 % tridax leaf extract and 5 % parthenium leaf extract.

ii) Spore germination inhibition:

Highest mean (5 and 10 per cent together) spore germination inhibition (65.00 %) of *B. theobromae* was observed in neem leaf extract, garlic bulb extract (55.80 %) and tulsi leaf extract (55.68 %) which were on par with each other. Among the different concentrations, 10 % neem leaf extract showed maximum inhibition (81.10 %) which was on par with 10 % garlic bulb extract (77.00%) followed by 10 % tulsi leaf extract (63.95 %). The present results once again reaffirm the various earlier reports. Antifungal activity of garlic bulb extract against *B. theobromae* has been earlier reported by other workers (Shirshikar, 2002; Ahmed and Sultana, 1984). Antifungal activity of tulsi against *B. theobromae* has been reported by various workers (Pathak, 1997; Patil, 1992; Godara and Pathak, 1995).

Table 1: *In vitro* evaluation of botanicals against *Botryodiplodiatheobromae* of Citrus

S. No	Plant extract	Percent inhibition of					
		Mycelial growth			Spore germination		
		5%	10%	Mean	5%	10%	Mean
1	Chromolaena leaf extract	16.55 (4.19)*	19.52 (4.53)	18.03 (4.36)	19.60 (26.27)	26.00 (30.68)	22.80 (28.48)
2	Clerodendron leaf extract	0.00 (1.00)	30.58 (5.62)	15.29 (3.31)	15.80 (23.40)	27.13 (31.33)	21.47 (27.37)
3	Garlic bulb extract	75.73 (8.76)	82.17 (9.12)	78.95 (8.94)	34.00 (35.64)	77.00 (61.30)	55.80 (48.47)
4	Lantana leaf extract	29.93 (5.56)	31.37 (5.69)	30.65 (5.63)	35.42 (36.54)	38.12 (38.10)	36.77 (37.32)
5	Neem leaf extract	38.69 (6.30)	56.45 (7.58)	47.57 (6.95)	48.90 (44.37)	81.10 (64.24)	65.00 (54.30)
6	Parthenium leaf extract	0.00 (1.00)	3.24 (2.06)	1.62 (1.53)	31.50 (34.12)	32.82 (34.98)	32.16 (34.55)
7	Tridax leaf extract	0.00 (1.00)	3.24 (2.06)	1.62 (1.53)	12.96 (21.04)	21.13 (27.34)	17.04 (24.20)
8	Tulsi leaf extract	47.72 (6.98)	52.14 (7.29)	49.93 (7.14)	47.40 (43.32)	63.95 (53.10)	55.68 (48.24)
	Mean	17.90 (4.35)	29.25 (5.50)	23.20 (4.92)	32.35 (34.67)	45.90 (42.63)	39.00 (38.65)

Source	Sem ±	CD at 1 % Level	Sem ±	CD at 1 % Level
Plant extract (P)	0.07	0.26	0.62	2.42
Concentration (C)	0.03	0.13	0.31	1.21
PxC	0.09	0.36	0.88	3.42

*Figures in the parentheses are angular transformed values.

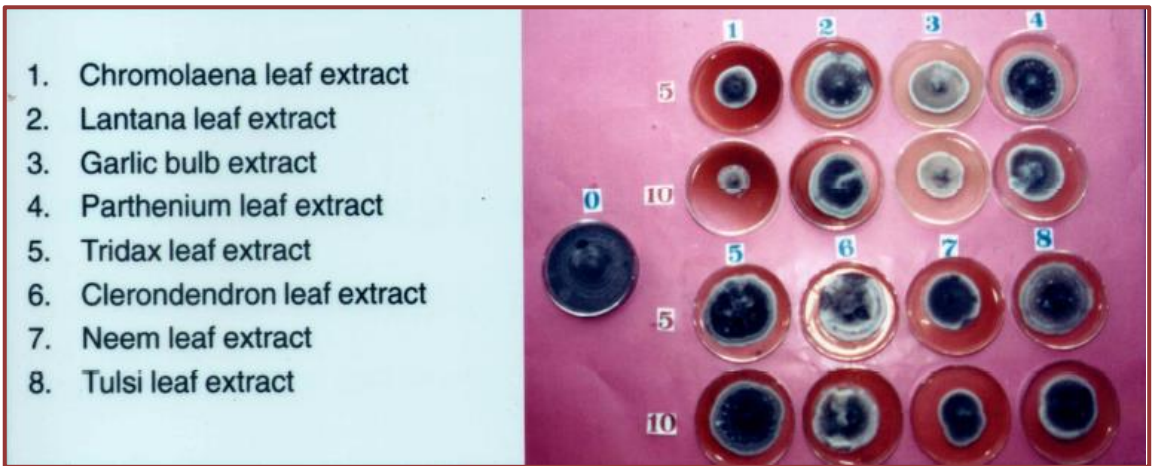

Plate 7: Effect of botanicals on mycelial growth of *Alternaria alternata*

B) *In vitro* evaluation of biocontrol agents 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 stem end rot causing pathogen *Botryodiplodia theobromae* isolated from citrus fruits.

All the antagonistic organisms except *P. fluorescens* significantly inhibited the growth of *B. theobromae.* Highest inhibition (74.34 %) was observed in *T.viride* and *T. virens* (isolate 2) and *T. virens* (isolate 1) (68.38 %) were on par and superior over all other antagonists. *T.harzianum* (61.09 %), *T.*

reesei (56.30 %), *B. subtilis* (55.55 %) and *T. pseudokoningi* (55.40 %) were statistically on par with each other. Least inhibition was observed in *P. fluorescens*. Antagonism of *T. viride* against *B. theobromae* has been reported by Bhuvaneswari (1999) and Shirshikar (2002). Several other workers, Patil (1992), Aurangueren *et al.* (1994), Majumdar and Pathak (1995) have also reported the antagonistic nature of *Trichoderma spp.* against *B. theobromae.*

Table 2: *In vitro* evaluation of biocontrol agents against *Botryodiplodia theobromae* of Citrus

Sl. No.	Biocontrol agent	Per cent inhibition of mycelia growth
1	*Bacillus subtilis*	55.55 (7.52)*
2	*Pseudomonas fluorescens*	0.00 (1.00)
3	*Trichoderma harzianum*	61.09 (7.88)
4	*T. pseudokoningi*	55.40 (7.51)
5	*T. reesei*	56.30 (7.57)
6	*T. virens*(isolate 1)	68.38 (8.33)
7	*T. virens*(isolate 2)	74.34 (8.68)
8	*T. viride*	74.34 (8.68)
	SEm ±	0.09
	CD at 1% level	0.39

*Figures in the parentheses are angular transformed values.

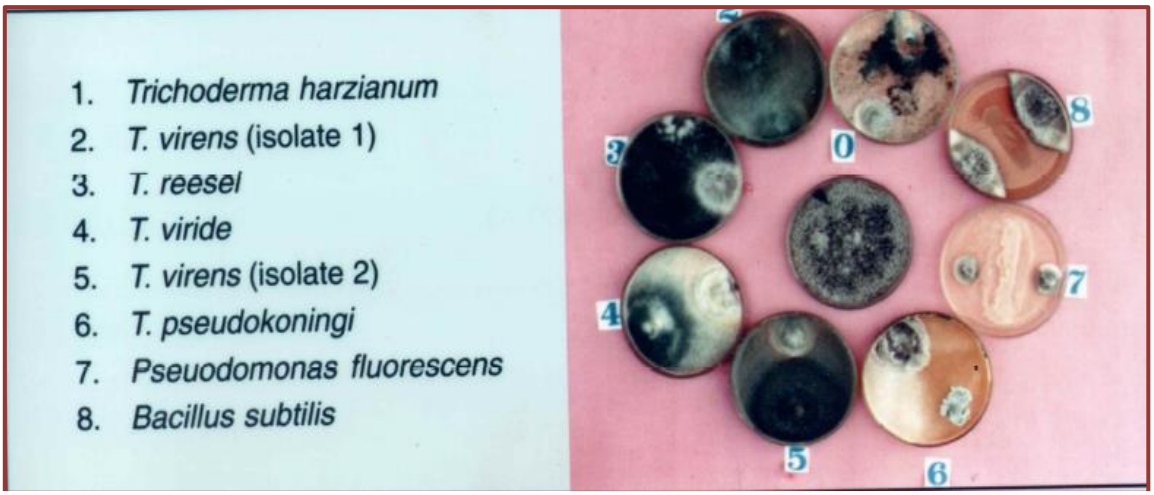

Plate 8: Effect of biocontrol agents on mycelial growth of *Alternaria alternate*

C) *In vivo* evaluation against stemend rot of citrus: (Table 3, Plate 9 & Figure 1)

Among the biocontrol agents and plant extracts given as postharvest treatments, all the treatments were effective and significantly reducedPDI of stem end rot of citrus compared to control. *B. subtilis* (15.23) and garlic bulb extract (17.70) were most effective as postharvest treatments and were on par with each other. Neem leaf extract (27.78), *T. viride* culture filtrate (32.21), *T. viride* spore suspension (37.98) were also effective but chemical Benomyl (0.1%) wasmost effective as with lowest PDI of 8.83 per cent compared to control 69.45. Similar reports of the efficacy of *B. subtilis* in reducing the postharvest diseases of citrus has been well established by the reports of Gutter and Littauer (1953) and

Singh and Deverall (1984). Also, chemical check (Benomyl @ 0.1 %) was found to be superior to other biological treatments. Similar results of superiority of chemical control over plant extracts and biocontrol agents were reported by Shirshikar (2002) and Prasanna Kumar (2001).

From the investigations, it is clear that garlic, neem and tulsi extracts were found to be effective and *B. theobromae*. The antifungal activity of the tulsi is reported to be due to thymol and phenol present in it, which are toxic to many pathogens (Anon., 1975). Patil (1992) reported that, extract of tulsi contains polyamine biosynthesis inhibitors(s), which block the ornithine decarboxylase pathway in *B.theobrornae.* Sharma and Prasad (1980) reported that, allicin (diallyl disulphide), allisatin I, allisatin Il, garlicin, garlic phytoncide were the active principles of *A. sativum.* Antifungal activity of the garlic may be attributed to any of these compounds. Antifungal activity of neem has been reviewed in detail by Parveen and Alam (1993). The antifungal activity of *Trichoderma spp.* can be attributed to the production of antibiotics or competition for substrate or hyperparasitism. One of these mechanisms may play an important role in suppression of pathogen.

Table 3: *In vivo* Evaluation of Bioagents and Botanicals Against Stem End Rot of Citrus

Sl. No.	Treatment	Per cent Disease Index (PDI) at 8 DAI
1	*Bacillus subtilis*(10^7 - 10^8cfu/ml)	15.23 (22.96)*
2	Garlic bulb extract (10%)	17.70 (24.93)
3	Neem leaf extract (10%)	27.78 (31.77)
4	*Trichoderma viride* spore suspension (5 X 10^5 spores/ml)	37.98 (37.93)
5	*T. viride* culture filtrate	32.21 (34.56)
6	Benomyl (0.1%)	8.83 (17.26)
7	Control	69.46 (56.43)
	SEm ±	0.80
	CD at 1% level	3.45

DAI –Days after inoculation.
*Figures in the parentheses are angular transformed values.

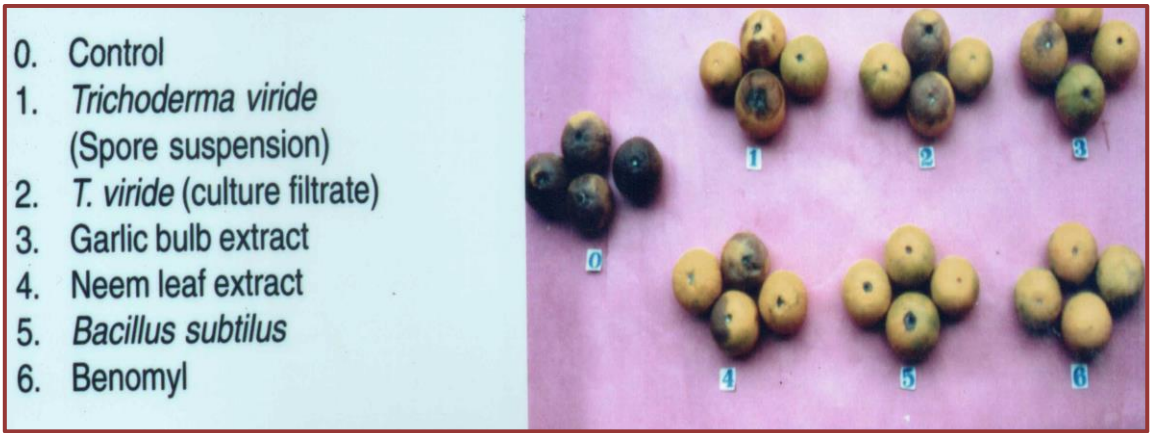

Plate: 9: Effect of postharvest treatments of botanicals and biocontrol agents on stem end rot of citrus

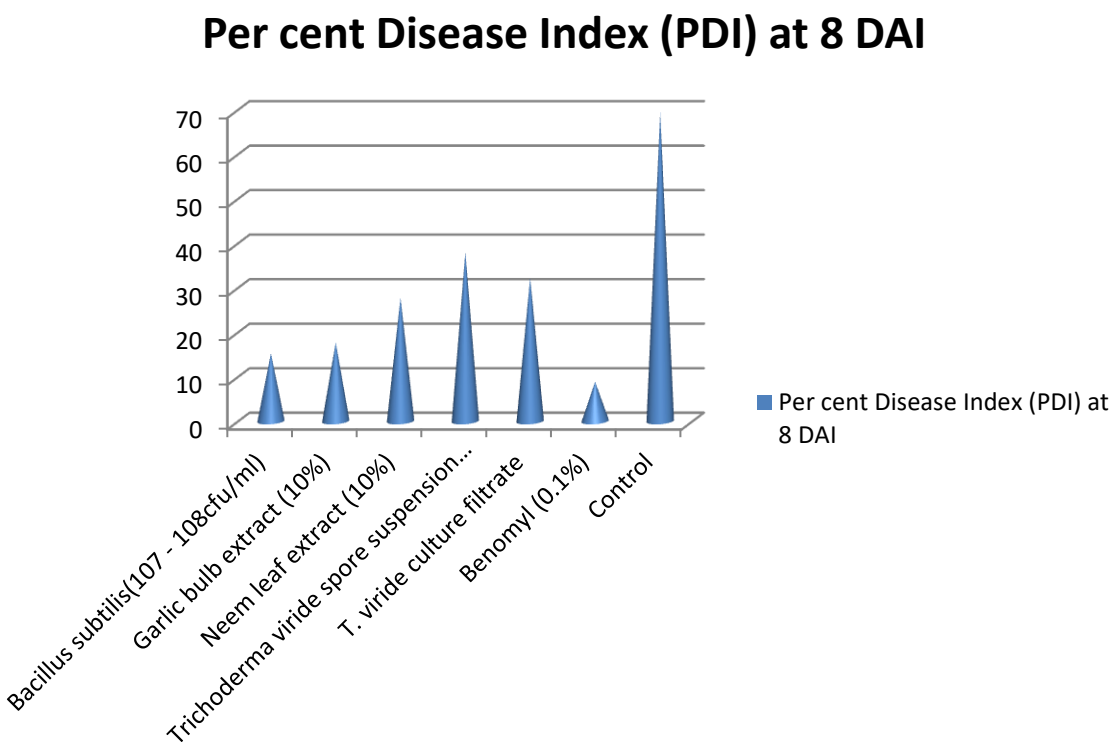

Figure 1. *In vivo* Evaluation of Bioagents and Botanicals Against Stem End Rot of Citrus

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