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Management of post-harvest stem-end rot of kinnow (*Citrus deliciosa* Ten.) fruits through chemicals

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Abstract

The stem-end rot of Kinnow (*Citrus deliciosa* Ten.) caused by *Botryodiplodia theobromae* is a major limiting factor in storage life of Kinnow fruits, responsible solely for 20 per cent of the total fruit loss occurred every year due to post-harvest rotting in Sriganganagar belt of Rajasthan. Nine fungicides were tested against the *B. theobromae* as well as incited rot. Prochloraz and carbendazim proved most effective against the pathogen, inhibited cent per cent mycelial growth and provided complete protection from the rot incidence in both pre- and post-inoculation treatments at 1000 ppm concentration. The mancozeb in association with carbendazim (saaf) and mancozeb alone were the next in order of efficacy where, 100 and 91.24 per cent growth inhibition, respectively was observed. These fungicides potencially reduced the incidence to the extent of 87.84 and 81.41 per cent, respectively as compared to control when applied as pre-inoculation treatment at 1000 ppm concentration. The fruit rot incidence correlated positively with incubation period, where the incidence at 8th day, after inoculation (DAI) was significantly higher than incidence occurred at 4th DAI.

Key words: *Kinnow, Botryodiplodia theobromae, post-harvest, stem-end rot, fungicides.*

Introduction

The major part of citrus produce in India is being marketed as fresh fruit and less than 1 per cent is being exported to our neighbouring countries and processed for juice and other products. The major limiting factor in successful trade of citrus in domestic and export market has been the considerable decay and quality deterioration of fruits incurred due to plant pathogens during storage and transit to distant markets (Singh, 2002). The situation is quite alarming especially in the conditions prevailing in India and other South-East Asian countries, where scientific post-harvest management of the pathogens during harvesting, handling, transit and storage are neglected to save the spoilage of citrus fruits. The conservative estimation on post-harvest losses (spoilage) of Kinnow fruits has been foccussed around 25-30 per cent (Singh *et al.*, 2002). Most of the post-harvest losses eventually result from invasion and spoilage of fruit by microorganisms. Fungi are usually the primary agents in the spoilage of fresh fruits.

The Kinnow fruits are attacked by a variety of microorganisms that causes post-harvest rotting. *Botryodiplodia theobromae* is one of the most destructive causal agent of post-harvest rotting of Kinnow fruits, causes stem-end rot, sharing 20 per cent of the total fruit loss (24%) occurred every year due to post-harvest rotting in Sriganganagar belt of Rajasthan. Post-harvest rotting of fruits can be suppressed by low temperature storage, a low oxygen atmosphere and treatment with growth regulators that delay tissue senescence. However, these beneficial practices may not be adequately protect fruits from microbial attack, especially during prolonged storage or movement of fruits through marketing channels. This is particularly true for mandarins which are injured by the near freezing temperature, the temperature required to inhibit pathogenic fungi for an extended period. In these situations, an antimicrobial chemical treatment may be the only means of extending the post-harvest life of a perishable crop (Bondad, 1974). Fungicides are primary antimicrobial chemical compounds to control the post-harvest rotting of fruits.

The present study was undertaken with a view to *in vitro* evaluate the fungicides of different groups including some newly developed for their fungitoxicity against *B. theobromae* Pat. (ID No. 6422.06 and ITCC NO. 6170) and further explored the efficacy to manage the incited post-harvest stem-end rot incidence in Kinnow fruits.

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Materials and methods:

Nine fungicides viz., Carbendazim (Bavistin), Fosetyl-Al (Aliette), Prochloraz, Thiophanate methyl (Roko), 12% Carbendazim + 63% Mancozeb (Saaf), Captan (Captra), Copper oxychloride (Blitox-50), Mancozeb (Indofil M-45) and Propineb (Antracol) were selected to evaluate their fungitoxicity against the mycelial growth of the *B. theobromae* and further use of the most effective fungitoxicants to manage the incited stem-end rot incidence in Kinnow fruits

(1) *In vitro* evaluation of fungicides against the mycelial growth of the *B. theobromae*:

The effect of fungicides at different concentrations (25, 50, 100, 250, 500 & 1000 ppm of a.i.) was tested against the *B. theobromae*, using "Poisoned Food Technique" (Nene and Thapliyal, 1997). The principle involved in this technique is to 'poison' the nutrient medium with a fungitoxicant and then allow a test fungus to grow on such a medium.

The Potato Dextrose Agar (PDA) medium was sterilized in flasks. Appropriate amount of the fungicides were separately mixed with molten PDA to get final concentrations of 25, 50, 100, 250, 500 & 1000 ppm of the fungicides. The medium was then poured aseptically into sterile Petri plates. After solidifying the medium, mycelial discs (5 mm dia.) of 7 days old actively growing culture of the *B. theobromae* were placed in the center of the individual plate. Suitable control was maintained where the culture disc was grown under the same conditions on PDA without fungicides. Each treatment was replicated thrice. After inoculation, Petri plates were incubated at 25 ± 1°C in BOD incubator. Observations were taken when the control plates were fully covered with mycelial growth of the *B. theobromae*. The per cent inhibition in mycelial growth of the *B. theobromae* as compared to control was calculated as described by Bliss (1934). The data were statistically analysed using a factorial randomized block design.

(2) Management of the stem-end rot of Kinnow fruits through chemicals:

The fruits were surface sterilized [dipping in 0.1 per cent mercuric chloride ($HgCl_2$) solution for 1 minute followed by three washing with sterile distilled water] and pricked through 'pin prick method' (Tomkins and Trout, 1931) upto the depth of 2 mm, making 5 wounds/fruit.

These fruits were separately inoculated by dipping them in spore suspension (10^6 spores ml^{-1}) of the pathogen (*B. theobromae*) for 2 minutes. Each fungicide was dissolved in sterile distilled water so as to get desired concentration (500 & 1000 ppm of a.i.) and used for dip treatment (Thompson, 1996) as pre- as well as post-inoculation treatments. In case of pre-inoculation treatment, the fruits were first dipped in the test fungicide for 5 minutes, air dried for 15 minutes and then inoculated, while in the post-inoculation treatment, the fruits were first inoculated with fungal spores and then treated with fungicide. Parallel control with fruits dipped in sterile distilled water was processed simultaneously. The interval between inoculation of fungal spores and treatment with fungicides or vice-versa was of 12 hr. Each treatment was replicated thrice, having seven fruits in each replication. The experimental design was a factorial randomized block design.

The inoculated fruits were enclosed separately in pre-sterilized perforated polythene bags partially sealed with paper pins and incubated at 25 ± 1°C and 90-100 per cent RH. The number of wounds showed rotting were recorded on 4th and 8th DAI. The per cent rot incidence was calculated as follows:

$$\text{Rot incidence (\%)} = \frac{\text{Number of rotted wounds}}{\text{Total number of wounds observed}} \times 100$$

The rot reduction index (RRI) was calculated as described by Gutter (1969).

Results and discussion:

(1) *In vitro* effect of fungicides on mycelial growth of the *B. theobromae*:

All fungicides significantly inhibited the mycelial growth of *B. theobromae* as compared to control even at low concentrations (25 ppm) except copper oxychloride and fosetyl-Al where, no growth inhibition was observed below 100 ppm concentration. Prochloraz, carbendazim and a combination of carbendazim 12% + mancozeb 63% (saaf) proved most effective, inhibited 100 per cent growth even at 25 ppm concentration. Mancozeb, thiophanate methyl and propineb were found next in order of efficacy against the *B. theobromae*, exhibited 91.24, 84.48 and 80.62 per cent growth inhibition, respectively. The least inhibition (21.17%) was observed in the case of copper oxychloride. The effectiveness of fungicides increased with higher concentrations. At 25, 50, 100, 250, 500 and 1000 ppm concentrations, the growth inhibition was observed as 52.45, 57.20, 62.26, 69.43, 79.07 and 82.50 per cent, respectively which differed significantly to each other (Table 1, Fig 1a &b).

(2) Efficacy of different fungicides against the stem-end rot incidence in Kinnow fruits:

The results presented in table 2 depicts that the fruits treated with fungicides showed significantly less incidence of the stem-end rot as compared to untreated ones. Among the fungicides tested, prochloraz and carbendazim proved most effective against the rot. The fruits remained completely free from infection upto 8th DAI when treated with prochloraz and carbendazim at 1000 ppm concentration in both, pre- as well as post-inoculation treatments. A combination of carbendazim 12% + mancozeb 63% (saaf)

Table 1. *In vitro* evaluation of different fungicides against the mycelial growth of the *B. theobromae*

	Fungicides / Conc. (ppm) Per cent inhibition (PI)* in mycelial growth						
	25	50	100	250	500	1000	Mean
Copper oxychloride	0.00	0.00	5.55	14.81	47.41	59.26	21.17
	(0.57)	(0.57)	(11.38)	(22.63)	(43.52)	(50.33)	(21.50)
Propineb	52.41	64.26	76.11	90.93	100.00	100.00	80.62
	(46.38)	(53.29)	(60.75)	(72.48)	(90.00)	(90.00)	(68.82)
Thiophanate methyl	63.33	72.22	78.89	92.41	100.00	100.00	84.48
	(52.73)	(58.19)	(62.65)	(74.00)	(90.00)	(90.00)	(71.26)
Captan	37.78	52.78	60.93	72.96	79.26	86.67	65.06
	(37.92)	(46.59)	(51.31)	(58.67)	(62.91)	(68.60)	(54.33)
Mancozeb	70.93	82.78	93.70	100.00	100.00	100.00	91.24
Mancozeb	(57.37)	(65.49)	(75.51)	(90.00)	(90.00)	(90.00)	(77.23)
Carbendazim 12% + Mancozeb	100.00	100.00	100.00	100.00	100.00	100.00	100.00
63% (Saaf)	(90.00)	(90.00)	(90.00)	(90.00)	(90.00)	(90.00)	(90.00)
Carbendazim	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	(90.00)	(90.00)	(90.00)	(90.00)	(90.00)	(90.00)	(90.00)
Fosetyl-Al	0.00	0.00	7.41	23.15	64.07	79.07	28.95
	(0.57)	(0.57)	(15.39)	(28.76)	(53.17)	(62.78)	(26.87)
Prochloraz	00.00	100.00	100.00	100.00	100.00	100.00	100.00
	(90.00)	(90.00)	(90.00)	(90.00)	(90.00)	(90.00)	(90.00)
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(0.57)	(0.57)	(0.57)	(0.57)	(0.57)	(0.57)	(0.57)
Mean	52.45	57.20	62.26	69.43	79.07	82.50	
	(46.61)	(49.53)	(54.26)	(61.71)	(70.02)	(72.23)	

Source:	SEm±	CD at 5%	CV (%)
Fungicides	0.33	0.93	2.37
Concentrations	0.26	0.72	
Fungicides x Concentrations	0.81	2.27	

* Average of three replications

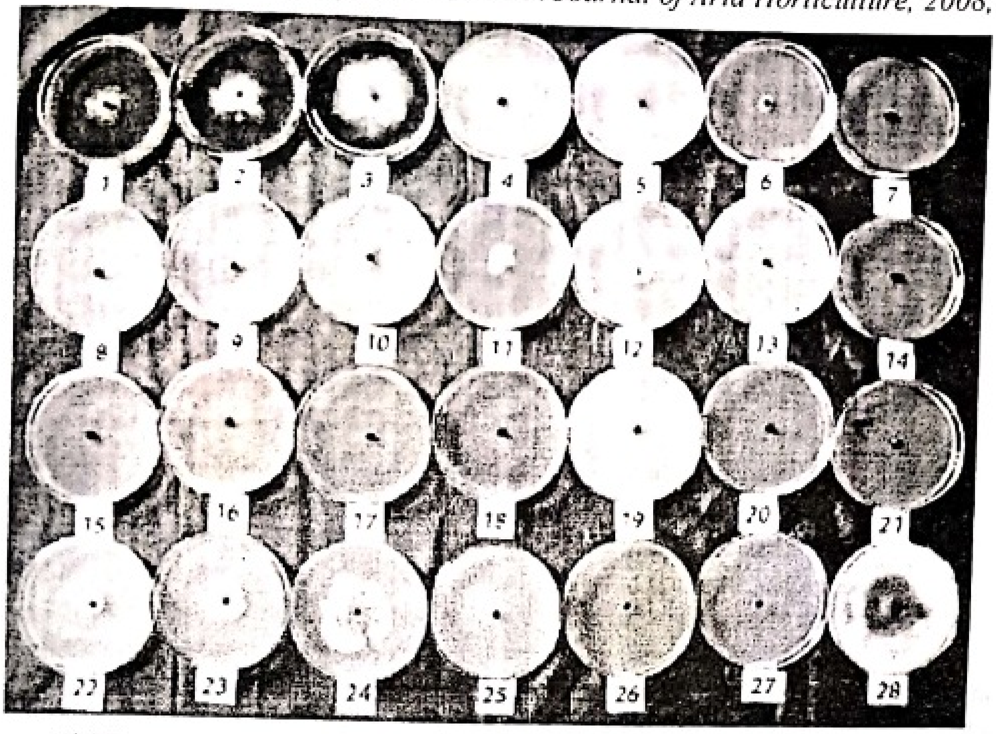
Figures in parenthesis are angular transformed values.

Table 2. Comparative efficacy of different fungicidal compounds on post-harvest rotting due to stem-end rot in Kinnow fruits

Fungicidal compounds	Conc. (ppm)	Per cent rot incidence in pre-inoculation treatment at different intervals (days)				Per cent rot incidence in post-inoculation treatment at different intervals (days)			
		4	8	Mean	RRI	4	8	Mean	RRI
Thiophanate methyl	500	32.39 (34.67)	46.19 (42.81)	39.29 (38.74)	44.25	36.19 (36.98)	50.48 (45.27)	43.34 (41.13)	40.32
Thiophanate methyl	1000	17.62 (24.77)	24.76 (29.82)	21.19 (27.30)	69.93	20.00 (26.55)	31.91 (34.39)	25.96 (30.47)	64.25
Mancozeb	500	18.58 (25.39)	26.67 (31.03)	22.63 (28.21)	67.89	21.43 (27.50)	34.77 (36.09)	28.10 (31.80)	61.31
Mancozeb	1000	10.48 (18.80)	15.72 (23.28)	13.10 (21.04)	81.41	14.76 (22.52)	22.38 (28.17)	18.57 (25.35)	74.43
Carbendazim 12% + Mancozeb 63%	500	12.39 (20.53)	18.58 (25.43)	15.49 (22.98)	78.02	16.67 (24.04)	24.77 (29.47)	20.72 (26.76)	71.47
Carbendazim 12% + Mancozeb 63%	1000	7.14 (15.42)	10.00 (18.39)	8.57 (16.91)	87.84	9.53 (17.93)	15.24 (22.93)	12.39 (20.43)	82.94
Carbendazim	500	5.71 (13.82)	8.10 (16.49)	6.91 (15.16)	90.20	6.66 (14.89)	10.48 (18.85)	8.57 (16.87)	88.20
Carbendazim	1000	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)	100.00	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)	100.00
Prochloraz	500	3.34 (10.42)	5.24 (13.14)	4.29 (11.78)	93.91	4.76 (12.46)	7.62 (15.95)	6.19 (14.21)	91.48
Prochloraz	1000	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)	100.00	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)	100.00
Control	-	57.15 (49.11)	83.81 (66.29)	70.48 (57.70)	0.00	59.05 (50.22)	86.19 (68.54)	72.62 (59.38)	0.00
Mean		14.98 (19.46)	21.73 (24.35)			17.19 (21.29)	25.80 (27.35)		

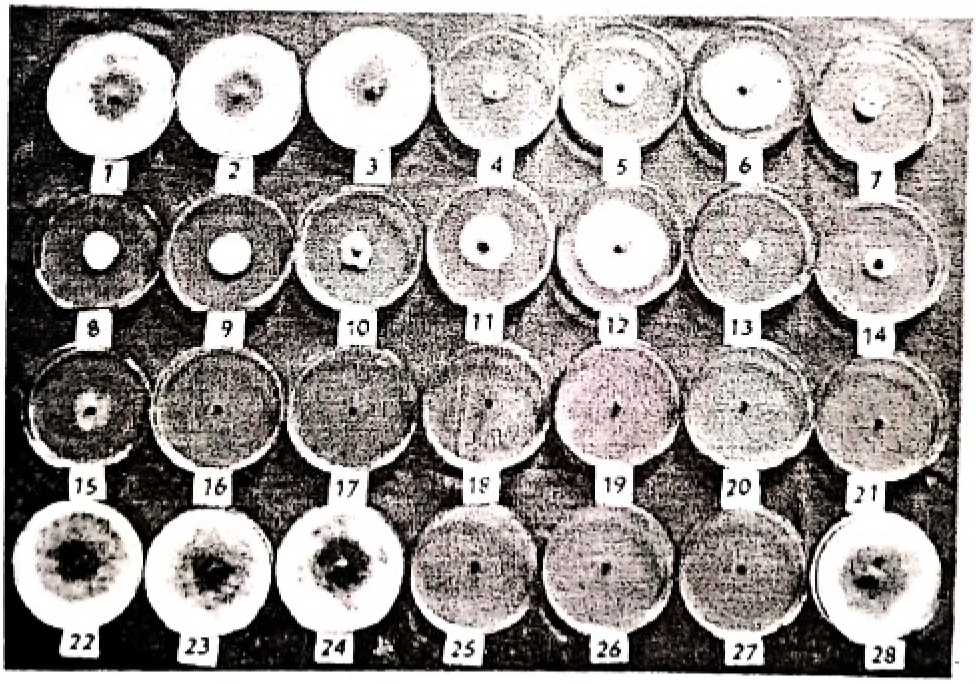
Source:	SEm±	CD at 5%	CV (%)	SEm±	CD at 5%	CV (%)
Fungicidal compounds	0.30	0.87	3.41	0.45	1.28	4.51
Days	0.13	0.37		0.19	0.54	
Fungicidal compounds x Days	0.43	1.23		0.63	1.81	

Figures in parenthesis are angular transformed values



1000	500	250 ppm	Control
1,4,7,10,13, 16,19,22,25	2,5,8,11,14, 17,20,23,26	3,6,9,12,15, 18,21,24,27	28

(a) Higher concentrations



100	50	25 ppm	Control
1,4,7,10,13, 16,19,22,25	2,5,8,11,14, 17,20,23,26	3,6,9,12,15, 18,21,24,27	28

(b) Lower concentrations

1-3. Copper oxychloride 4-6. Propineb 7-9. Thiophanate methyl

10-12. Captan 13-15. Mancozeb 16-18. Saaf

19-21. Carbendazim 22-24. Fosetyl-Al 25-27. Prochloraz 28. Control

Fig 1. Effect of fungicides at different concentrations on mycelial growth of *B. theobromae*

and mancozeb were the next fungicides in order of efficacy, provide 87.84 and 81.41 per cent protection from the incidence, respectively as compared to control when applied as pre-inoculation treatment at 1000 ppm concentration. The least reduction (40.32%) in incidence was noted when the fruit treated with thiophanate methyl as post-inoculation treatment at 500 ppm concentration.

The fruit rot incidence correlated positively with incubation period, where, incidence at 8th DAI was significantly higher to the incidence occurred at 4th DAI. In general, fungicides were more effective when applied as pre-inoculation as compared to post-inoculation treatment, which might be due to lack of time for establishment and multiplication of pathogen.

The present results are in conformity with those of Brown (1983) who reported that prochloraz was effective against the *Botryodiplodia* stem-end rots of citrus fruits. Prochloraz inhibit the growth of pathogenic fungi by interfering at C-14 demethylation step of ergosterol synthesis, the synthesis of which is vital to structural formation of fungal cell membranes. Both preventive and curative action have also been shown. Due to its non-systemic nature, should therefore be less affected by development of fungal resistance, than true systemics (Danderson, 1986).

Naqvi (1993) reported the efficacy of benzimidazole fungicides against the *B. theobromae*. The efficacy of carbendazim in controlling the *Botryodiplodia* rot of citrus fruits had also been proved by Godara (1994). The carbendazim rendered their lethal effect on pathogenic fungi through inhibition of mitosis by interfering with spindle formation at the level of tubulin biosynthesis (Thind, 2007). According to Beerh *et al.* (1976), fungicides might have checked the growth of fungi and resulted into change in biochemical constituents of the fruits. This in turn imparted resistance to the fruits against the penetration and growth of the pathogen resulting into less rotting of fruits.

Similar to present investigation, the effectiveness of mancozeb against the post-harvest rotting of Kinnow fruits has also been established by Singh and Thakur (2005). Mancozeb when applied with combination of carbendazim, showed however, more efficacious against the stem-end rot pathogen as well as rot incidence, which might be due to synergistic action of both fungicides in combination.

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