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Madhushri S Kerakalamatti
Department of Plant Pathology
College of Horticulture Bagalkot, Karnataka, India

RK Mesta
Department of Plant Pathology
College of Horticulture Bagalkot, Karnataka, India

MS Lokesh
Department of Plant Pathology
College of Horticulture
Munirabad, Karnataka, India

Kiran kumar KC
Department of Plant Pathology
College of Horticulture Bagalkot, Karnataka, India

Rudresh DL
Department of Agricultural Microbiology College of Horticulture Bagalkot, Karnataka, India

Raghavendra G
Department of Biotechnology
College of Horticulture Bagalkot, Karnataka, India

Correspondence
Madhushri S Kerakalamatti
Department of Plant Pathology
College of Horticulture Bagalkot, Karnataka, India

In vitro evaluation of non-systemic and systemic fungicides against wilt of pomegranate caused by *Ceratocystis fimbriata*

Madhushri S Kerakalamatti, RK Mesta, MS Lokesh, Kiran kumar KC, Rudresh DL and Raghavendra G

Abstract
Pomegranate (*Punica granatum* L.) is one of the important fruit crops which is nowadays is highly threatened by the wilt caused by *Ceratocystis fimbriata*. Six non systemic and seven systemic fungicides were evaluated *in vitro* against *Ceratocystis fimbriata.* Among non systemic fungicides, mancozeb 63% + carbendazim 12% was found to be most effective and significantly superior over all other treatments with respect to per cent inhibition of mycelial growth. Among systemic fungicides, the mycelial growth of the fungus was inhibited 100 per cent by hexaconazole and propiconazole at 0.025% concentrations. While all other systemic fungicides inhibited the mycelia growth completely at 0.1 per cent concentration.

Keywords: pomegranate, *Ceratocystis fimbriata*, non systemic fungicides, systemic fungicides

Introduction
Pomegranate (*Punica granatum* L.) is a popular fruit also called as "Fruit of Paradise" is a deciduous shrub or a small tree belongs to the family Lythraceae, having 2n=16 number of chromosome and it is native to Iran. Successful cultivation of pomegranate in recent years has met with different traumas of pest and diseases. Among them wilt caused by *Ceratocystis fimbriata* Ell. & Halst is a major threat. It was first noticed in two areas of the Vijayapur district in 1990. This was once deemed as a minor disease, but now has become a serious threat for pomegranate production resulting in severe yield losses (Somasekhara and Wali, 1999) [7]. The disease was prevalent in parts of Maharashtra, Karnataka, Andhra Pradesh, Gujarat and Tamil Nadu states (Jadhav and Sharma, 2009) [3]. Use of fungicide is an important component of management of plant disease caused by fungi. Hence, the present study was taken on testing of different non systemic and systemic fungicides against *Ceratocystis fimbriata* under *in vitro* condition.

Material and Methods
The efficacy of six non systemic and seven systemic fungicides were tested against *Ceratocystis fimbriata* at 0.025, 0.05 and 0.1 per cent concentrations by following "Poison food technique". The pathogen *C. fimbriata* was grown on PDA medium in Petri plates for fifteen days prior to setting the experiment. Fungicide suspension was prepared in PDA by adding required quantity of fungicide to obtain the desired concentration on the basis of active ingredient. Twenty ml of poisoned medium was poured in each of the sterilized Petri plates. Mycelial disc of 0.5 cm was taken from the periphery of ten days old culture, placed in the center and incubated at 25 ± 1^0C till growth of the fungus touched the periphery in control plate. Suitable checks were also maintained without addition of any fungicide. Three replications were maintained for each treatment. The diameter of the colony was measured in two directions and average was worked out. The per cent inhibition of growth was worked out. The per cent inhibition of growth was calculated by using the formula given by Vincent (1947) [11].

$$I = 100 (C-T) / C$$

Where,
I = Per cent inhibition of mycelium
C = Growth of mycelium in control

T = Growth of mycelium in treatment

Results

The result pertaining to the evaluation of non-systemic fungicides against the *C. fimbriata* are presented in the Table 1, Fig. 1 and Plate 1. There was a significant difference between the non-systemic fungicides with respect to per cent inhibition of mycelial growth. Among them mancozeb 63% + carbendazim 12% was found most effective and significantly superior over all other treatments which inhibited 100 per cent growth of the fungus at all concentrations tested. The next best treatment was mancozeb and captan which inhibited 96.33 and 95.45 per cent growth of the fungus respectively. However, there was least inhibition in chlorothalonil (31.18%) and copper oxy chloride (52.27%) at all concentrations. Among the concentrations, 0.1 per cent (84.35) was found significantly superior over 0.05 (74.54) and 0.025 (56.13) per cent concentration in inhibition of growth of the fungus. Interaction studies showed that mancozeb 63% + carbendazim 12% inhibited the mycelial growth completely (100 per cent) at 0.025% and above concentration. Mancozeb inhibited the mycelia growth completely at 0.05 and 0.1 per cent whereas at 0.025 per cent there was 68.44 per cent inhibition. Captan inhibited the mycelial growth of fungus by 76.77, 78.18 and 83.07 per cent at 0.025, 0.05 and 0.1 per cent concentrations. Least per cent inhibition was found in chlorothalonil (27.64, 32.45, 40.92), copper oxy chloride (25.16, 50.95, 62.40) and propineb (23.49, 52.49, 71.84) at 0.025, 0.05 and 0.1% concentrations respectively.

The result pertaining to the evaluation of systemic fungicides against the *C. fimbriata* are presented in the Table 2, Fig. 2 and Plate 2. The mycelial growth of the fungus was inhibited 100 per cent by hexaconazole and propiconazole at 0.025%, 0.05% and 0.1% concentrations. While all other systemic fungicides inhibited the mycelial growth completely at 0.1 per cent concentration. Least inhibition was found in difenoconazole (66.52 per cent) at 0.025 concentration.

Table 1: *In vitro* evaluation of non-systemic fungicides against the mycelial growth of *Ceratocystis fimbriata* through poison food technique

Sl. No.	Fungicide	Per cent inhibition of mycelial growth (mm)			Mean
		Concentration			
		0.025%	0.05%	0.10%	
1	Captan	94.73(76.77) *	95.75(78.18)	98.52 (83.07)	96.33(79.34)
2	Chlorothalonil	21.59(27.64)	28.78(32.45)	43.18 (40.92)	31.18 (33.67)
3	Copper oxy chloride	18.18(25.16)	60.22(50.95)	78.40 (62.40)	52.27 (46.17)
4	Mancozeb	86.36 (68.44)	100.00(90.05)	100.00 (90.05)	95.45 (82.85)
5	Mancozeb 63%+ Carbendazim 12%	100.00 (90.05)	100.00(90.05)	100.00 (90.05)	100.00 (90.05)
6	Propineb	15.91 (23.49)	62.50(52.49)	85.98 (71.84)	54.79(49.28)
Mean		56.13 (51.92)	74.54(65.70)	84.35(73.06)	71.67 (62.47)
Source				S. *Em*±	CD@1%
Fungicides (F)				1.65	4.75
Concentration (C)				1.17	3.36
Fungicide x Concentration				2.85	8.22

*****Values in parenthesis are arc sine transformed values

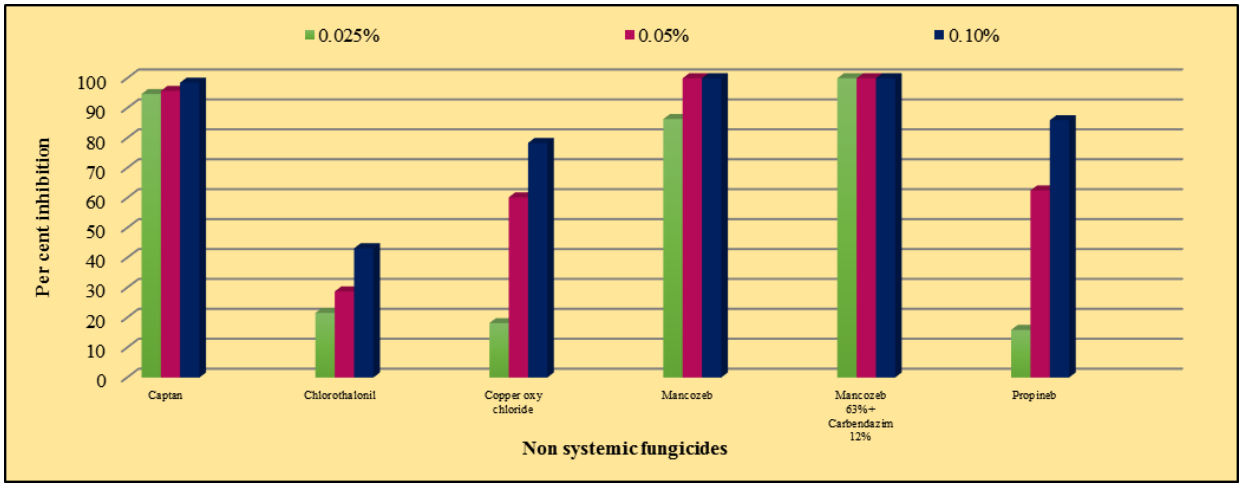

Fig 1: Per cent inhibition of the mycelial growth of *Ceratocystis fimbriata* by non systemic fungicides

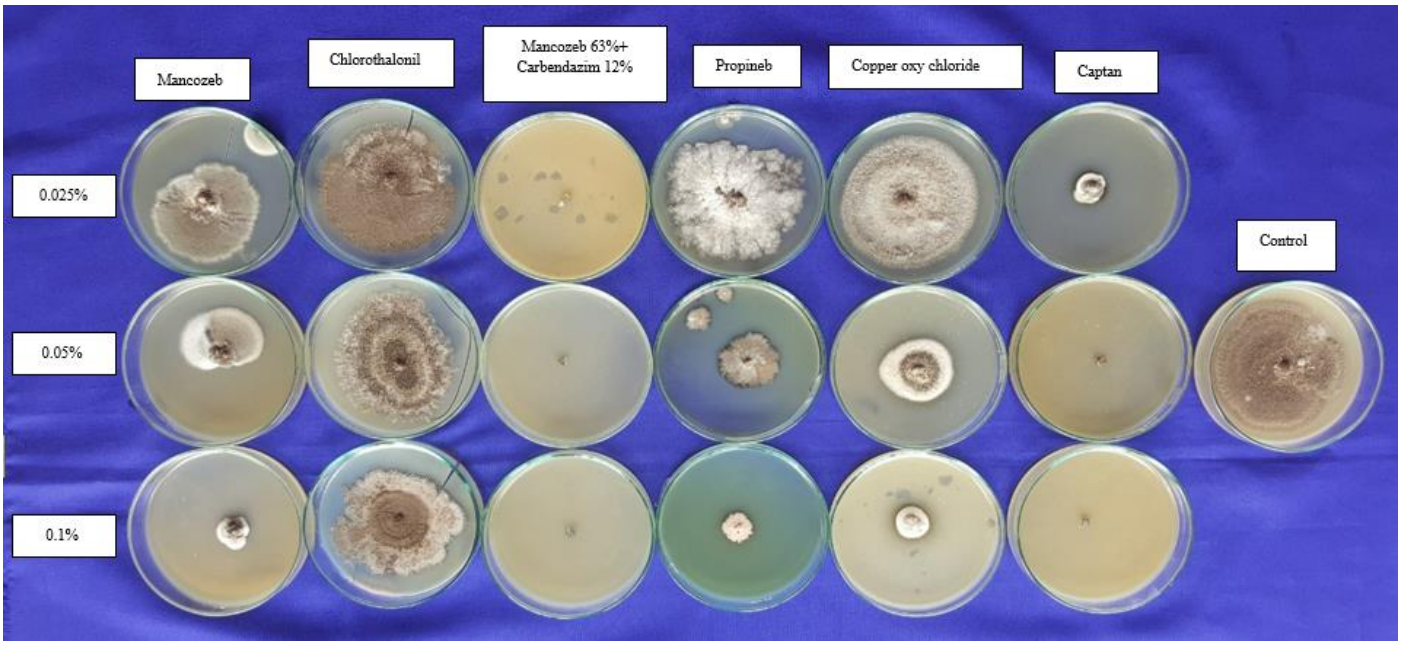

Plate 1: Inhibition of mycelial growth of *Ceratocystis fimbriata* by non systemic fungicides

Table 2: *In vitro* evaluation of systemic fungicides against the mycelial growth of *Ceratocystis fimbriata* through poison food technique

Sl. No.	Fungicide	Per cent inhibition of mycelial growth (mm)			Mean
		Concentration			
		0.025%	0.05%	0.10%	
1.	Carboxin +Thiram	89.40(71.04) *	95.92(78.47)	100.00(90.05)	95.10(79.85)
2.	Difenoconazole	84.07(66.52)	98.88(83.97)	100.00(90.05)	94.31(80.18)
3.	Hexaconazole	100.00(90.05)	100.00(90.05)	100.00(90.05)	100.00(90.05)
4.	Propiconazole	100.00(90.05)	100.00(90.05)	100.00(90.05)	100.00(90.05)
5.	Tebuconazole	98.88(83.97)	98.88(83.97)	100.00(90.05)	99.25(86.00)
6.	Thiophanate methyl	96.66(79.51)	97.77(81.45)	100.00(90.05)	98.14(83.67)
7.	Tricyclazole	97.77(81.45)	98.88(83.97)	100.00(90.05)	98.88(85.16)
Mean		95.26(80.37)	98.62(84.56)	100.00(90.05)	
Source				S. *Em*±	CD @ 1%
Fungicides (F)				0.15	0.43
Concentration (C)				0.10	0.28
Fungicide x Concentration				0.26	0.74

*****Values in parenthesis are arc sine transformed values

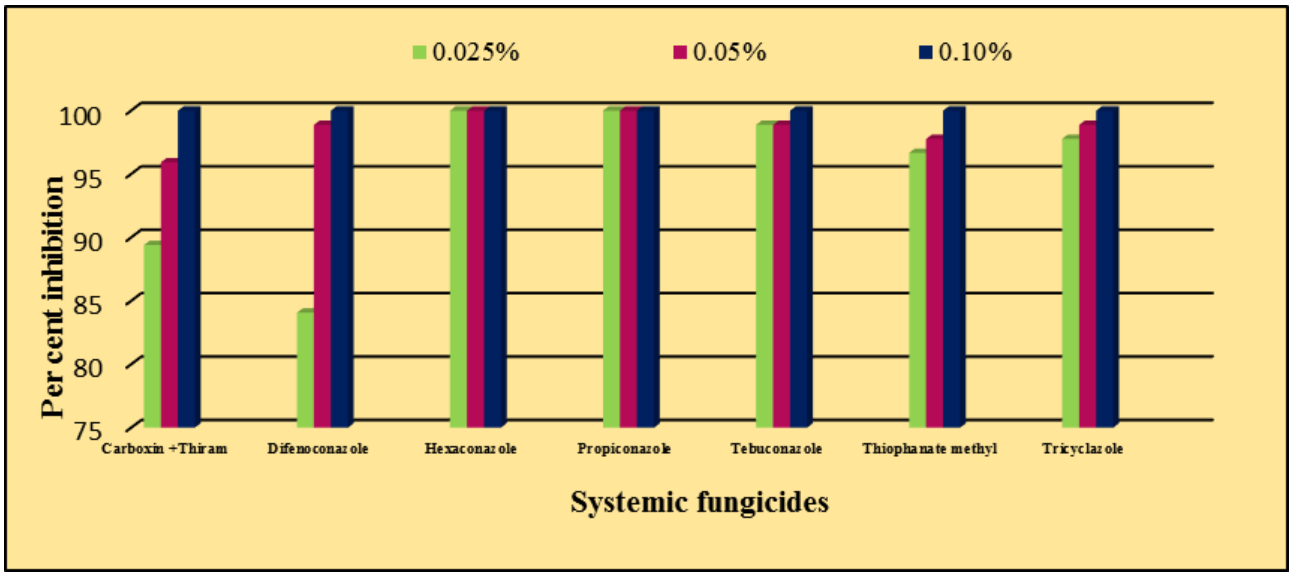

Fig 2: Per cent inhibition of the mycelial growth of *Ceratocystis fimbriata* by systemic fungicides

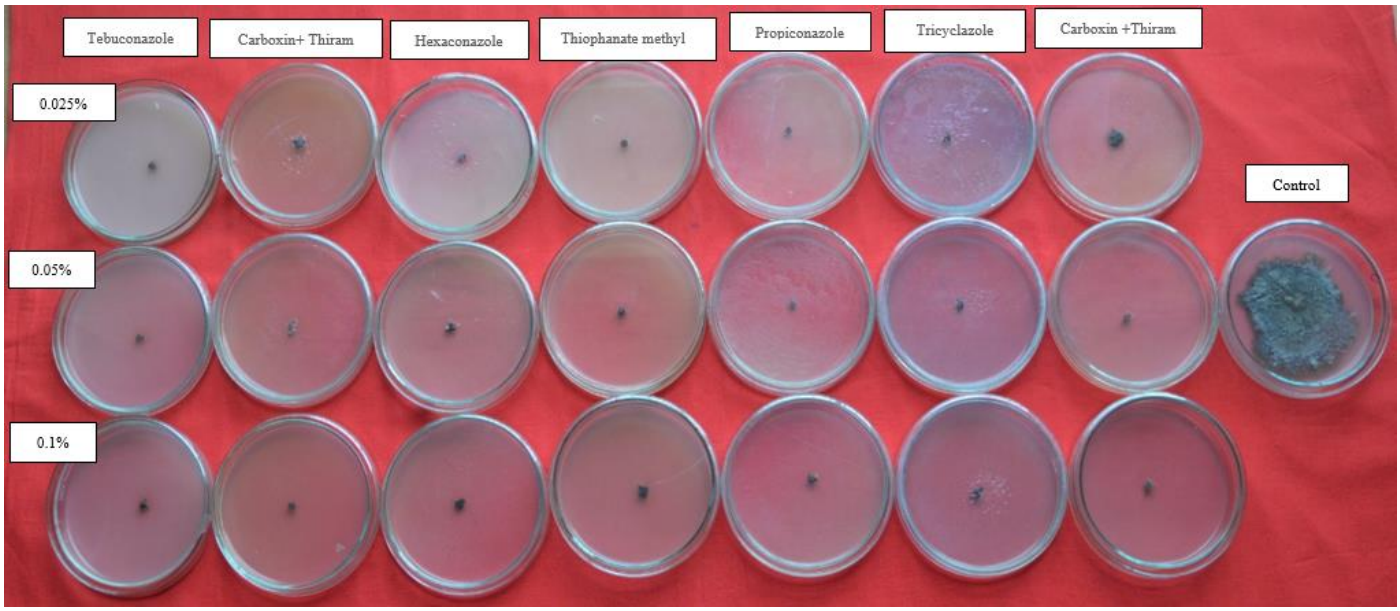

Plate 2: Inhibition of mycelial growth of *Ceratocystis fimbriata* by systemic fungicides

Discussion

Among six non systemic fungicides tested, mancozeb 63% + carbendazim 12% was found to be most effective and significantly superior over all other treatments which inhibited 100 per cent growth of the fungus. The next best treatment was mancozeb and captan which inhibited 95.45 and 96.33 per cent growth of the fungus respectively. Carbendazim is systemic fungicide with protective and curative action. Carbendazim works by inhibiting the development of fungi probably by interfering with spindle formation at mitosis (cell division). It is a broad spectrum systemic protective and curative fungicide. It inhibits the development of germ tubes, formation of appressoria and the growth of mycelia by inhibiting cell division. Mancozeb inactivates the sulfhydryl groups of amino acids and enzymes of fungal cell, resulting in disruption of lipid metabolism, respiration and production of adenosine triphosphate. The captan reacts with sulfhydryl groups and reduces fungal spore germination, growth, and oxygen uptake. Sharma *et al.* (2010) [6] observed complete inhibition of *C. fimbriata* by mancozeb (0.2%) and captan (0.2%). Sonyal (2010) [10] reported that copper oxychloride was more effective than other fungicides at 0.1, 0.2 and 0.3 per cent against *C. fimbriata*. Chaudhari *et al.* (2016) [1] reported that copper oxy chloride and mancozeb were found most effective at 0.2, 0.25 and 0.3 per cent against *C. fimbriata*. Somu (2017) [9] reported that cymoxanil + mancozeb, mancozeb and captan recorded the 100% inhibition of mycelial growth at all tested concentrations *viz.*, 0.10 %, 0.20 % and 0.30 %.

Among seven systemic fungicides tested, the mycelial growth of the fungus was inhibited 100 per cent by hexaconazole and propiconazole at 0.025, 0.05 and 0.1 per cent concentrations. All the systemic fungicides inhibited the mycelia growth completely at 0.1 per cent concentration. In many fungi, ergosterol is essential to the structure of cell wall and its absence causes irrepairable damage to cell wall leading to death of fungal cell. Hexaconazole is ergosterol biosynthesis inhibitor, thereby inhibiting the growth and reproduction of plant fungal pathogens. It is a systemic triazole fungicide, with protective and curative action. Propiconazole also interfere with the biosynthesis of sterols in cell membranes. A similar study was reported for the effectiveness of triazoles, which inhibited the biosynthesis pathway in fungi (Nene and Thapliyal, 1993) [5]. The present findings are supported by earlier workers. Somasekhara (2009) [8], Sharma *et al.* (2010) [6] and Kishore and Bhardwaj (2011) [4] observed complete inhibition of mycelial growth of *C. fimbriata* by carbendazim and propiconazole. Sonyal (2010) [10] observed complete inhibition shown by propiconazole and tricyclazole at all concentrations tested. Imran Khan (2017) [2] reported that among systemic fungicides tested, cent per cent inhibition of mycelial growth was recorded in propiconazole followed by hexaconazole (94.65 per cent).

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