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Evaluation of different fungicides against pomegranate wilt complex caused by *Ceratocystis fimbriata* and *Fusarium oxysporum*

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Abstract
Non-systemic, systemic and combi product fungicides were tested against *Ceratocystis fimbriata* and *Fusarium oxysporum* under *in vitro* conditions at different concentrations. Among the contact fungicides evaluated against *C. fimbriata*, mancozeb was found to be best in inhibiting the mycelial growth of pathogen. Against *F. oxysporum*, chlorothalonil was recorded as best treatment followed by captan. Among the systemic fungicides, tebuconazole, propiconazole, hexaconazole, thiophanate methyl and carbendazim showed cent percent mycelial inhibition against *C. fimbriata*. In case of *F. oxysporum* tebuconazole showed 100 percent mycelial inhibition. Out of six combi-product fungicides, carbendazium 12% + mancozeb 63% was found to be the most effective and significantly superior to all other treatments. Against *F. oxysporum* tebuconazole 50% + trifloxystrobin 25% showed maximum inhibition of mycelial growth. Based on *in vitro* evaluation among different fungicides by considering both the pathogens, mancozeb @0.2%; tebuconazole and probiconazole @ 0.1%; carbendazium 12% + mancozeb 63%, and captan 70% + hexaconazole 5% @ 0.2% was selected for further evaluation.

Keywords: Pomegranate wilt, fungicides, evaluation, *in vitro*

Introduction
Pomegranate wilt complex is one of the important diseases of pomegranate adversely affecting crop cultivation in all major growing regions of India. The disease found prevalent in many parts of Maharashtra, Karnataka, Andhra Pradesh, Gujarat and Tamil Nadu (Jadhav and Sharma, 2009; Saranya and Yashoda, 2019) [1, 2]. In recent years, pomegranate cultivation has been hampered due to severe infestation of pest and diseases. Some of the important diseases and pests are bacterial blight, wilt, anthracnose, shot hole borer, thrips, and fruit borer. Wilt caused by *Ceratocystis fimbriata* Ell. & Halst and *Fusarium oxysporum* has emerged as a second most important disease of pomegranate (Sonyal *et al.* 2016) [3]. The use of chemicals has become an inevitable method in the management of plant disease in the absence of resistant varieties for various soil-borne diseases. There is a necessity for the evaluation of fungicides in vitro. Since there is manufacturing of new molecules in order to provide useful and preliminary information regarding their efficacy against pathogens within a shorter period of time, in vitro evaluation is essential, which serves as a guide for testing fungicides under field conditions. Therefore, in vitro screening of different contact, systemic and combi-products against *C. fimbriata* and *F. oxysporum* was carried out.

Materials and Methods
Pathogen: The wilt causing pathogens from infected pomegranate plant was isolated using standard isolation procedures (Saranya and Yashoda, 2019) [2] and deposited in NCBI Gene Bank with accession number MN596926 and MN596927 respectively (Saranya and Yashoda, 2022) [4]. These pathogens were stored in plant pathology laboratory, UAS Dharwad. Further it was used in all the studies.

***In-vitro* evaluation of different fungicides:** Non-systemic, systemic fungicides and combi products were tested against *C. fimbriata* and *F. oxysporum* under *in vitro* conditions. The non-systemic fungicides were evaluated at 0.05, 0.1 and 0.2 percent concentrations against *C. fimbriata* and *F. oxysporum*.

The systemic fungicides were evaluated at 0.025, 0.05 and 0.1 percent concentrations against *C. fimbriata* whereas 0.05, 0.1 and 0.2 percent concentrations were used against *F. oxysporum*. The combi products were evaluated at 0.025, 0.5 and 0.1 percent against *C. fimbriata* whereas 0.025, 0.5, 0.1 and 0.2 percent concentrations were used against *F. oxysporum*. The details of evaluated fungicides are given here under.

List of non-systemic fungicides

Common Name	Trade Name
Captan 50% WP	Captaf 50 WP
Chlorothalonil 75% WP	Kavach 75 WP
Copper hydroxide 77% WP	Kocide 101 77 WP
Copper oxy-chloride 50% WP	Blitox 50 WP
Mancozeb 75% WP	Indofil M-45 75 WP

List of systemic fungicides

Common Name	Trade Name
Azoxystrobin 23% SC	Amistar 23 SC
Carbendazim 50% WP	Bavistin 50 WP
Difenconazole 25% EC	Score 25 EC
Hexaconazole 5% EC	Contaf 5 EC
Propiconazole 25% EC	Tilt 25 EC
Thiophanate methyl 70% WP	Roko 70 WP
Tebuconazole 25% WP	Folicur 25 WP

Combi product fungicides

Common name	Trade name
Captan 70% + Hexaconazole 5% WP	Taqat 75 WP
Carbendazim 12% + Mancozeb 63% WP	SAAF 75 WP
Hexaconazole 4% + Zineb 68% WP	Avtar 72 WP
Tebuconazole 50% + Trifloxystrobin 25% WG	Nativo 75 WG
Tricyclazole 18% + Mancozeb 62% WP	Merger 80 WP
Thiram 37.5% + Carboxin 37.5% WP	Vitavax power 75 WP

Poison food technique was followed to test the efficacy of the above-mentioned fungicides. Pathogens *C. fimbriata* and *F. oxysporum* were grown on OMA and PDA media respectively in Petri plates for fifteen and ten days prior to setting the experiment. Fungicide suspension was prepared in growth media by adding required quantity of fungicide to obtain the desired concentration on the basis of active ingredient and whole product present in the chemical. 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 the culture and placed in the centre and incubated at 25±2ºC 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 percent inhibition of growth was worked out.

The percent inhibition of growth was calculated by using the formula given by Vincent (1947) [5].

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Percent inhibition of mycelial growth

C = Radial growth in Control

T = Radial growth in treatment

Results

Evaluation of contact fungicides against *C. fimbriata* and *F. oxysporum*

The results indicated that there was a significant difference among the contact fungicides in inhibiting the growth of *C. fimbriata* (Table 1). Among the five contact fungicides, mancozeb (100%) was significantly superior over other treatments, followed by captan (92.24%), and the least inhibition was observed in copper oxychloride (74.56%). Among all the treatments, mancozeb showed maximum inhibition of mycelial growth, which was on par with captan at 0.2 percent concentration. In the case of *F. oxysporum*, among the five contact fungicides tested, chlorothalonil (78.49%) was very effective in inhibiting mycelial growth of *F. oxysporum* and was significantly superior to all other treatments, which was followed by captan (74.18%). Among the interactions, maximum inhibition was observed in chlorothalonil, which was significantly superior to all other treatments. At different concentrations evaluated, 0.2 percent (57.49%) was significantly superior to 0.1 and 0.05 percent concentrations (Table 2).

Table 1: *In vitro* evaluation of contact fungicides against *Ceratocystis fimbriata*

Fungicides	Inhibition of mycelial growth (%) Concentration (%)			Mean
	0.05	0.1	0.2	
Copper hydroxide 50% WP	70.02(56.80)*	79.09(62.79)	88.36(70.05)	79.16(63.22)
Copper oxychloride 50%WP	56.80(48.91)	77.12(61.42)	89.74(71.32)	74.56(60.55)
Captan 50% WP	88.56(70.23)	88.17(69.88)	100.00(90.00)	92.24(76.70)
Chlorothalonil 75% WP	57.00(49.03)	83.63(66.13)	93.29(74.99)	77.98(63.38)
Mancozeb 75% WP	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Mean	74.48(62.99)	85.60(70.05)	94.28(79.27)	84.79(70.77)
Source			S.E.M. ±	C.D. @ 1%
Fungicides (F)			0.31	1.17
Concentration (C)			0.19	0.71
F × C			0.53	0.53

*Arcsine transformed value

Table 2: *In vitro* evaluation of contact fungicides against *Fusarium oxysporum*

Fungicides	Inhibition of mycelial growth (%) Concentration (%)			Mean
	0.05	0.1	0.2	
Captan 50% WP	71.76(57.88)*	74.51(59.65)	76.27(60.83)	74.18(59.46)
Chlorothalonil 75% WP	75.39(60.17)	77.65(61.76)	82.45(65.14)	78.49(62.36)
Copper hydroxide 50% WP	5.69(13.76)	24.71(29.78)	30.98(33.79)	20.46(25.78)
Copper oxychloride 50%WP	27.45(31.58)	30.20(33.13)	36.47(37.13)	31.37(34.01)
Mancozeb 75% WP	34.12(35.72)	38.24(38.17)	61.28(51.43)	44.54(41.77)
Mean	42.88(40.90)	49.06(44.46)	57.49(49.30)	49.81(44.89)
Source			S.Em. ±	C.D. @ 1%
Fungicides (F)			0.40	1.58
Concentration (C)			0.31	1.22
F × C			0.70	2.74

*Arcsine transformed value

Evaluation of systemic fungicides against *C. fimbriata* and *F. oxysporum*

Among the seven systemic fungicides tested against *C. fimbriata*, tebuconazole, propiconazole, hexaconazole, thiophanate methyl, and carbendazim showed 100 percent inhibition over control at all three concentrations (0.025, 0.05, and 0.1%) evaluated (Table 3). The next best was difenconazole with 86.21 percent mycelial inhibition, and the least inhibition was observed in azoxystrobin (25.36%). At different concentrations, 0.1 percent (91.04%) was significantly superior to 0.05 and 0.025 percent. In the case of *F. oxysporum*, among the six systemic fungicides evaluated, tebuconazole was the best, with 100 percent inhibition at all concentrations and significantly superior to all other treatments. This was followed by carbendazim, with an 86.08 percent inhibition of mycelial growth. The least inhibition was observed in thiophanate methyl (61.96%). In interaction, tebuconazole showed cent percent mycelial inhibition, which was followed by carbendazim (89.90%) at 0.2 percent (Table 4).

Table 3: *In vitro* evaluation of systemic fungicides against *Ceratocystis fimbriata*

Fungicide	Inhibition of mycelial growth (%) Concentration (%)			Mean
	0.025	0.05	0.1	
Azoxystrobin 23% SC	3.53(10.83)*	24.71(29.81)	47.84(43.76)	25.36(28.13)
Carbendazim 50% WP	100(90.00)	100(90.00)	100(90.00)	100(90.00)
Difenconazole 25% EC	81.57(64.58)	87.65(69.42)	89.41(71.01)	86.21(68.33)
Hexaconazole 5% EC	100(90.00)	100(90.00)	100(90.00)	100(90.00)
Propiconazole 25% EC	100(90.00)	100(90.00)	100(90.00)	100(90.00)
Tebuconazole 25% EC	100(90.00)	100(90.00)	100(90.00)	100(90.00)
Thiophanate methyl 70% WP	100(90.00)	100(90.00)	100(90.00)	100(90.00)
Mean	83.59(75.06)	87.48(78.46)	91.04(80.68)	87.37(78.07)
Source			S. Em. ±	C. D. @ 1%
Fungicides (F)			0.27	1.02
Concentration (C)			0.16	0.62
F × C			0.47	1.76

*Arcsine transformed value

Table 4: *In vitro* evaluation of systemic fungicides against *Fusarium oxysporum*

Fungicide	Inhibition of mycelial growth (%) Concentration (%)			Mean
	0.05	0.1	0.2	
Carbendazim 50% WP	79.22(62.98)	89.22(70.59)	89.80(71.69)	86.08(68.42)
Difenconazole 25% EC	69.61(56.10)	74.90(59.59)	78.82(62.88)	74.44(59.53)
Hexaconazole 5% EC	63.33(52.47)	82.20(63.07)	83.92(66.16)	76.48(60.57)
Propiconazole 25% EC	72.16(57.99)	81.18(64.87)	84.12(65.82)	79.15(62.89)
Tebuconazole 25% EC	100(90.00)	100(90.00)	100(90.00)	100.00(90.00)
Thiophanate methyl 70% WP	57.06(49.7)	61.96(51.61)	66.86(54.70)	61.96(51.83)
Mean	75.20(60.13)	81.58(64.58)	82.29(65.11)	79.69(63.21)
Source			S. Em. ±	C.D. @ 1%
Fungicides (F)			0.40	1.53
Concentration (C)			0.28	1.08
F × C			0.69	2.66

*Arcsine transformed value

Evaluation of combi-products against *C. fimbriata* and *F. oxysporum*

It is inferred from the result (Table 5) that carbendazium 12% + mancozeb 63% was found to be the most effective and significantly superior to all other treatments, which inhibited 100 percent growth of *C. fimbriata* at all the concentrations.

This was followed by captan 70% + hexaconazole 5% and hexaconazole 4% + zineb 68%, which gave cent percent inhibition of fungus at 0.1 percent concentration with mean inhibition of 94.27 and 91.33 percent, respectively. The least percent inhibition of 77.32 percent was observed for tricyclazole 18% + mancozeb 62%. Among the six combi-products evaluated against *F. oxysporum* (Table 6), tebuconazole 50% + trifloxystrobin 25% showed maximum inhibition of 93.90 percent, which was on par with thiram 37.5% + carboxin 37.5% (90.65%) and carbendazium 12% + mancozeb 63% (90.55%). Tebuconazole 50% + trifloxystrobin 25% WG showed maximum inhibition of 97.15 percent at 0.2 percent concentration, and the same chemical was equally effective at 0.05 and 0.1 percent concentrations (96.75%) and was on par with 0.2 percent concentration. Though tebuconazole 50% + trifloxystrobin 25% showed maximum mycelial inhibition, cent pet cent mycelial inhibition was recorded in thiram 37.5% + carboxin 37.5% at 0.1 and 0.2 percent concentrations; captan 70% + hexaconazole 5%; and carbendazium 12% + mancozeb 63% at 0.1 percent concentration. Least inhibition was recorded in tricyclazole 18% + mancozeb 62% (54.27%).

Table 5: *In vi*tro evaluation of combi-products against *Ceratocystis fimbriata*

Fungicide	Mycelial growth inhibition (%)			Mean
	0.025	0.05	0.1	
Tricyclazole 18% + Mancozeb 62% WP	67.99(55.54) *	77.19(62.02)	86.79(68.69)	77.32(61.56)
Hexaconazole 4% + Zineb 68% WP	90.40(71.95)	83.60(75.34)	100.00(90.00)	91.33(72.87)
Captan 70% + Hexaconazole 5% WP	90.00(71.56)	92.80(74.43)	100.00(90.00)	94.27(76.14)
Tebuconazole 50% + Trifloxystrobin 25% WG	86.79(68.69)	89.60(71.18)	94.80(76.82)	90.40(71.94)
Carbendazium 12% + Mancozeb 63% WP	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Thiram 37.5% + Carboxin 37.5% WP	74.79(59.86)	88.00(69.73)	90.80(72.34)	84.53(66.83)
Mean	85.00(67.21)	88.53(70.20)	95.40(77.62)	89.64(71.22)
Source			S. Em. ±	C.D. @ 1%
Fungicides (F)			0.78	3.01
Concentration (C)			0.55	2.13
F × C			1.36	5.22

*Arcsine transformed value

Table 6: *In vi*tro evaluation of combi-products against *Fusarium oxysporum*

Fungicide	Inhibition of mycelial growth (%)				Mean
	Concentration (%)				
	0.025	0.05	0.1	0.2	
Captan 70% + hexaconazole 5% WP	67.48(55.24)*	79.47(63.06)	82.93(65.60)	100.00(90.00)	82.47(65.25)
Carbendazium 12% + mancozeb 63% WP	75.61(60.41)	95.12(82.50)	91.46(76.03)	100.00(90.00)	90.55(72.10)
Hexaconazole 4% + zineb 68% WP	54.07(47.33)	54.88(47.80)	63.01(52.54)	66.67(54.74)	59.66(50.57)
Tebuconazole 50% + trifloxystrobin 25% WG	84.96(67.18)	96.75(83.93)	96.75(83.93)	97.15(84.34)	93.90(75.70)
Thiram 37.5% + carboxin 37.5% WP	75.20(60.14)	87.40(69.21)	100.00(90.00)	100.00(90.00)	90.65(72.20)
Tricyclazole 18% + mancozeb 62% WP	25.61(30.40)	45.53(42.41)	64.63(53.51)	81.30(64.76)	54.27(47.45)
Mean	63.82(53.02)	76.53(61.02)	83.13(65.75)	90.85(72.39)	78.58(62.43)
Source				S. Em. ±	C.D. (1%)
Fungicides (F)				1.53	5.81
Concentration (C)				1.25	4.74
F × C				3.06	11.62

*Arcsine transformed value

Discussion

Among the contact fungicides tested, mancozeb at all concentrations was found to be significantly superior, showing 100 percent inhibition of the mycelial growth of *C. fimbriata*. Similarly, Sharma *et al.* (2010) [6] and Chaudhari *et al.* (2016) [7] reported that mancozeb at 0.2 percent was highly effective (100%) against *C. fimbriata* under *in vitro*. Mancozeb inactivates the sulphaydryl groups of amino acids by interrupting the enzymatic activities inside the fungal cell, resulting in disruption of lipid metabolism, respiration and the production of adenosine triphosphate. This might be the probable reason for the inhibition of the growth of the test fungus. Against *F. oxysporum*, chlorothalonil showed maximum inhibition, which was followed by captan.

Among the tested systemic fungicides, tebuconazole, propiconazole, hexaconazole, thiophanate methyl and carbendazim recorded the maximum inhibition of mycelial growth (100%) of *C. fimbriata*. The obtained results were in accordance with the findings made by Sharma *et al.* (2010) [6], Kishore and Bhardwaj (2011) [8] and Sonyal (2015) [9] who reported 100 percent inhibition against *C. fimbriata*. Against *F. oxysporum*, tebuconazole proved to be best for *F. oxysporum*, as it showed 100 percent inhibition of mycelial growth at all three concentrations tested. These findings are in conformity with Divya Bharathi (2017) [10], who noticed 100 percent inhibition of *F. solani*. Triazoles are the most potent group of fungicides, having a strong ergosterol synthesis inhibitory action that blocks the cytochrome P-450 dependant enzyme, C-14 alpha de-methylase, needed to convert lanosterol to ergosterol (Nene and Thapliyal, 1993) [11].

Among the different combi-product fungicides evaluated, carbendazim 12% + mancozeb 63% showed cent percent mycelial inhibition of *C. fimbriata* was observed at all three concentrations. Similar findings were recorded by Madhushri *et al.* (2018) [12] who reported that SAAF at all concentrations tested (0.025, 0.05, and 0.1%) showed cent percent inhibition. In the case of *F. oxysporum* tebuconazole 50% + trifloxystrobin 25% recorded maximum mycelial inhibition.

Carbendazim is a systemic fungicide with protective and curative actions. Carbendazim inhibits the development of fungi by interfering with spindle formation at mitosis. It is a broad-spectrum systemic protective and curative fungicide. Which inhibits the germ tubes development and appressoria formation, thereby inhibiting the growth of the mycelia. Mancozeb inactivates the sulfohydryl groups of amino acids and enzymes present in the fungal cell, disrupting the lipid metabolism, respiration and production of adenosine triphosphate. Tebuconazole is a strong dimethyl inhibitor that interferes with the process of building the structure of the fungal cell wall, thereby inhibiting fungal germination. While trifloxystrobin interferes with mitochondrial respiration by blocking electron transfer in the electron transfer chain.

Conclusion

Among all tested fungicides, mancozeb at 0.2 percent, tebuconazole, propiconazole at 0.1 percent, carbendazim 12% + mancozeb 63%, captan 70% + hexaconazole 5% were found effective against both the pathogens at maximum concentration. These fungicides were taken for further *in vivo* experiments.

Declarations

Conflict of Interest

All contributing authors declare no conflicts of interest.

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