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IN *VITRO* EVALUATION OF PLANT EXTRACTS AND FUNGICIDES ON *Ceratocystis fimbriata* (ELLIS & HALST.), INCITANT OF POMEGRANATE WILT

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ABSTRACT
Wilt incited by *Ceratocystis fimbriata* is a major disease of pomegranate causing huge losses. Therefore, efforts were made to evaluate the efficacy of six plant extracts and eleven fungicides under *in vitro* conditions against test fungus. Among plant extracts, *Nerium indicum* was most effective giving 40.98 per cent mean growth inhibition of test fungus, followed by *Adhatoda vasica* (19.63%). Amongst systemic fungicides, carbendazim (Bavistin 50 WP), propiconazole (Tilt 25% EC) and hexaconazole (Contaf 5% EC) gave cent per cent mean growth inhibition of test fungus followed by fosetyl-Al (Aliette 80% WP) (58.39%). Non-systemic fungicides, mancozeb (Dithane M-45 75% WP) and propineb (Antracol 70% WP) were most effective with cent per cent mean growth inhibition of test fungus followed by copper oxy chloride (Blitox 50 WP) (75.67%) and captan (captan 50 WP) (55.55%). The combination of fungicides carbendazim 12% + mancozeb 63% (Companion 75 WP) gave absolute (100%) inhibition of test fungus followed by captan 70% + hexaconazole 5% WP (Taqat) and metiram + pyraclostrobin (Cabrio Top 60 WG) with mean per cent inhibition of 62.59 per cent and 45.67 per cent respectively. The plant extracts and fungicides varied in their inhibitory effect at different levels of concentrations.

INTRODUCTION

Pomegranate is an important fruit having 52 per cent of its total fruit weight as edible portion (Dutta Ray *et al*., 2014). Unfortunately, the fruit crop is influenced by a number of diseases out of which wilt caused by *Ceratocystis fimbriata* is most important and has been characterised as a significant biotic restriction of pomegranate in India (Somasekhara, 1999; Khosla *et al*., 2011) and in other pomegranate-cultivating countries such as Pakistan (Fateh *et al*., 2006) and China (Xu *et al*., 2011). Karnataka, Maharashtra, Tamil Nadu, Gujarat and Andhra Pradesh are regarded as hot spots for this disease in India (Somasekhara *et al*., 2000). Initial disease symptoms associated with wilt of pomegranate appear on the plants as yellowing followed by drooping of the leaves on one branch resulting in senescence which further spreads to the entire plant. The fungus has been recorded to thrive best at a temperature of 30ºC and at a pH of 7.5 (Sharma and Khosla, 2020). *Ceratocystis fimbriata* is the incitant of cankers and wilts on numerous woody plants as it is an extensively dispersed fungus (Jason *et al*., 2005).

Therefore, there is a need of the present work to elucidate the measures for management of this disease. Since diseases of plants are managed mostly by making use of chemical pesticides only (Garkoti *et al*., 2013) as a result, fungicides are employed and have become an essential method for suppressing growth and parasitic spores because of their abstemiously minimal application effort, high usefulness and expediency (Sharma, 2019). The suppressive effect of aqueous extracts of plants known for their antimicrobial activity against *Ceratocystis fimbriata* has also been recorded by Sonyal (2015), Vijaya *et al*. (2007) and Khosla (2013). Investigations have been carried out to manage wilt of pomegranate (*Ceratocystis fimbriata*) using systemic (Somu *et al*., 2018; Kerakalamatti *et al*., 2018; Vijaya *et al*., 2006; Somasekhara, 2009; Sharma *et al*., 2010; Sonyal, 2010; Chaudhari *et al*., 2016; Khan *et al*., 2017; Raja *et al*., 2017 and Ismail *et al*., 2018), non-systemic (Somu *et al*., 2018; Kerakalamatti *et al*., 2018; Vijaya, *et al*., 2006; Somasekhara, 2009; Sharma et al., 2010; Sonyal, 2010; Chaudhari *et al*., 2016; Raja *et al*., 2017 and Khan *et al*., 2017) and combination (Sonyal, 2010; Kerakalamatti *et al*., 2018 and Somu, 2017) of fungicides. However, fungicides are used in excess to manage this disease. Therefore, the present studies were directed with the sole aim of assessing the efficacy of plant extracts in addition to fungicides against *Ceratocystis fimbriata* after meticulous *in vitro* evaluation so that plant extracts are also incorporated as a part of control measures.

MATERIALS AND METHODS

Preparation of plant extracts

To prepare extract of each botanical, 500 grams of fresh healthy plant leaves of locally available plant spp *viz,* Basooti

(*Adhatoda vasica*), Drake (*Melia azadrach*), Eucalyptus (*Eucalyptus terepicornis*), Nerium (*Nerium indicum*), Vitex (*Vitex negundo*) and Walnut (*Juglans regia*) were collected and rinsed with double distilled water, air dried and crushed in a belligerent blender by making use of double distilled water in a proportion of 1:1 (weight by volume) to make the extract solution of each botanical. The extract of botanicals was filtered using 2 layered muslin cloth followed by centrifugation of extract solution for 15 minutes at 5000 rotations per minute. Supernatant thus collected after centrifugation was used as 100 per cent stock solution of respective botanical (Khosla, 2013).

Evaluation of fungicides and plant extracts

Mycelial growth inhibition of *Ceratocystis fimbriata* by plant extracts and fungicides under *in vitro* conditions was evaluated by screening six aqueous extracts of plant spp *viz*, Basooti (*Adhatoda vasica*), Drake (*Melia azadrach*), *Eucalyptus* (*Eucalyptus terepicornis*), Nerium (*Nerium indicum*), Vitex (*Vitex negundo*) and Walnut (*Juglans regia*) at 10, 20 and 30 per cent concentrations. Four systemic fungicides viz, carbendazim (Bavistin 50 WP), propiconazole (Tilt 25% EC), fosetyl-Al (Aliette 80% WP) and hexaconazole (Contaf 5% EC) were evaluated at concentrations of 25, 50 and 100 ppm. Four non-systemic fungicides *viz*, mancozeb (Dithane M-45 75% WP), captan (captan 50 WP), propineb (Antracol 70% WP) and copper oxy chloride (Blitox 50 WP) were tested at concentrations of 250, 500 and 1000 ppm. Three combinations of fungicides *viz*, metiram + pyraclostrobin (Cabrio Top 60 WG), carbendazim 12% + mancozeb 63% WP (Companion 75 WP) and captan 70% + hexaconazole 5% WP (Taqat 75 WP) were screened at concentrations of 25, 50 and 100 ppm for their fungicidal effectiveness against *Ceratocystis fimbriata* by the method of growth inhibition technique (Nene and Thapliyal, 1993).

Potato dextrose agar medium supplemented aseptically with test concentrations of different plant extracts and fungicides was poured in sterilized Petri plates and inoculated with mycelial disc (5mm) from seven-day-old culture of *Ceratocystis fimbriata* and incubated at 25 ± 2^{o}C until colony growth touched the side walls of the control plate. In every treatment, three replications were maintained. Appropriate control plates were maintained where culture discs were placed without addition of fungicide/plant extracts in potato dextrose agar medium. Mean colony diameter was registered in each treatment by measuring the colony's diameter by taking two diametric measurements at right angles to each other (perpendicularly) and averages were worked out. Per cent inhibition of mycelial growth of Ceratocystis fimbriata in comparison to control was evaluated by using formula as follows (Vincent, 1947).

$$I = \frac{(C - T)}{C} X100$$

Where, I = (%) Mycelial growth inhibition, C = Mycelial growth (mm) in control and T = Mycelial growth (mm) in treatment.

RESULTS AND DISCUSSION

In vitro* evaluation of plant extracts and fungicides against *Ceratocystis fimbriata

The mycelial growth inhibition of *Ceratocystis fimbriata* instigating wilt of pomegranate was evaluated at different concentrations of plant extracts, systemic fungicides, non-systemic fungicides and combination of fungicides under in vitro conditions and recorded in Table 1-4. The inspection of results revealed that none of the six plant extracts evaluated *in vitro* at 10, 20 or 30 per cent concentration resulted in absolute (100%) inhibition of mycelial growth of *Ceratocystis fimbriata* but some of them provided considerable mycelial growth inhibition. (Table 1). Extract of *Nerium indicum* at 30 per cent concentration was found to be most effective and showed a mean inhibition rate of 40.98 per cent. However, the efficacy of most plant extracts evaluated against *Ceratocystis fimbriata* was intermediatory and gave mean growth inhibition of test

Table 1: Per cent inhibition of mycelial growth of *Ceratocystis fimbriata* by plant extracts

Sr. No.	Plant Extract	Diametric Growth (mm) Concentration (%)			Mean	Inhibition of mycelial growth (%) Concentration (%)			Mean
		10	20	30		10	20	30	
1	Basooti (*Adhatoda vasica*)	74.66	73.33	69	72.33[b]	17.03 (24.36)	18.51 (25.47)	23.33 (28.86)	19.63[b] (26.23)
2	Drake (*Melia azadrach*)	82.66	81.66	79.33	81.22[e]	8.14 (16.57)	9.25 (17.7)	11.85 (20.12)	9.75[e] (18.13)
3	Eucalyptus (*Eucalyptus terepicornis*)	77.66	75.66	73.66	75.66[d]	13.7 (21.71)	15.92 (23.5)	18.14 (25.2)	15.92[d] (23.47)
4	Nerium (*Nerium indicum*)	56.33	52.66	50.33	53.11[a]	37.4 (37.69)	41.48 (40.07)	44.07 (41.58)	40.98[a] (39.78)
5	Vitex (*Vitex negundo*)	78.66	75.33	72.66	75.55[d]	12.59 (20.77)	16.29 (23.79)	19.25 (26.01)	16.04[d] (23.52)
6	Walnut (*Juglans regia*)	78.66	75.33	68.66	74.22[c]	12.59 (20.77)	16.29 (23.79)	23.7 (29.11)	17.53[c] (24.56)
Mean		74.77	72.33	68.94		16.91 (23.64)	19.63 (25.72)	23.39 (28.48)	
Effect					$C.D._{0.05}$				$C.D._{0.05}$
Plant Extract					0.626				0.52
Concentration					0.443				0.37
Plant Extract x Concentration					1.085				0.91

*Figures represented by different alphabets differ significantly., **Angular transformed values under parantheses

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

Sr. No.	Fungicide		Diametric Growth (mm)				Per cent inhibition of mycelial growth			
	Trade Name	Common Name	Concentration				Concentration			
			25 ppm	50 ppm	100 ppm	Mean	25 ppm	50 ppm	100 ppm	Mean
1	Bavistin	carbendazim 50% WP	0	0	0	0[a]	100 (84.99)	100 (84.99)	100 (84.99)	100[a] (84.99
2	Tilt	propiconazole 25% EC	0	0	0	0[a]	100 (84.99)	100 (84.99)	100 (84.99)	100[a] (84.99)
3	Aliette	fosetyl-Al 80% WP	39	39	34.33	37.44[b]	56.66 (48.81)	56.66 (48.81)	61.85 (51.83)	58.39[b] (49.82)
4	Contaf	hexaconazole 5% EC	0	0	0	0[a]	100 (84.99)	100 (84.99)	100 (84.99)	100[a] (84.99)
Mean			9.75	9.75	8.58		88.6 (75.95)	88.6 (75.95)	89.9 (76.7)	
Effect						C.D.$_{0.05}$				C.D.$_{0.05}$
Fungicide						0.652				0.72
Concentration						0.565				0.62
Fungicide x Concentration						1.13				1.25

*Figures represented by different alphabets differ significantly;**Angular transformed values under parantheses

Table 3: Per cent inhibition of mycelial growth of *Ceratocystis fimbriata* by non-systemic fungicides

Sr. No.	Fungicide		Diametric Growth (mm)			Mean	Per cent inhibition of mycelial growth			Mean
	Trade Name	Common Name	Concentration				Concentration			
			250 ppm	500 ppm	1000 ppm		250 ppm	500 ppm	1000 ppm	
1	Dithane M-45	mancozeb 75% WP	0	0	0	0.00[a]	100 (84.99)	100 (84.99)	100 (84.99)	100[a] (84.99)
2	Captan	captan 50% WP	42.66	38.66	38.66	40.00[c]	52.59 (46.46)	57.03 (49.02)	57.03 (49.02)	55.55[c] (48.17)
3	Antracol	propineb 70% WP	0	0	0	0.00[a]	100 (84.99)	100 (84.99)	100 (84.99)	100[a] (84.99)
4	Blitox	Copper oxy chloride 50% WP	33.33	25.66	6.66	21.88[b]	62.96 (52.49)	71.48 (57.7)	92.59 (74.21)	75.67[b] (61.46)
Mean2			19	16.08	11.33		78.51 (67.23)	81.75 (69.18)	87.03 (73.3)	
Effect						C.D.$_{0.05}$				C.D.$_{0.05}$
Fungicide						0.489				0.54
Concentration						0.424				0.47
Fungicide x Concentration						0.848				0.94

*Figures represented by different alphabets differ significantly;**Angular transformed values under parantheses

Table 4: Per cent inhibition of mycelial growth of *Ceratocystis fimbriata* by combination of fungicides

Sr. No.		Fungicide	Diametric Growth (mm)				Per cent inhibition of mycelial growth			
	Trade Name	Common Name	Concentration			Mean	Concentration			
			25 ppm	50 ppm	100 ppm		25 ppm	50 ppm	100 ppm	Mean
1	Cabrio Top	metiram + pyraclostrobin 60 WG	71.66	58.66	46.33	58.88[c]	31.48 -34.11	45.92 -42.64	59.63 -50.53	45.67[c] -42.43
2	Companion	carbendazim12% + mancozeb 63% WP	0.00	0.00	0.00	0.00[a]	100 -84.99	100 -84.99	100 -84.99	100[a] -84.99
3	Taqat	captan 70% + hexaconazole 5% WP	50.33	40.33	10.33	33.66[b]	44.07 -41.58	55.18 -47.95	88.51 -70.17	62.59[b] -53.23
Mean			40.66	33	18.88		58.26 -53.56	66.78 -58.53	82.46 -68.56	
Effect						C.D.$_{0.05}$				C.D.$_{0.05}$
Fungicide						0.576				0.64
Concentration						0.576				0.64
Fungicide x Concentration						0.998				1.1

*Figures represented by different alphabets differ significantly;**Angular transformed values under parantheses

fungus below 20 per cent. In case of *Adhatoda vasica* and *Juglans regia*, the mean inhibition of mycelium of test fungus was noted to be 19.63 per cent and 17.53 per cent respectively. Vitex negundo showed mean growth inhibition of test fungus as 16.04 per cent followed by *Eucalyptus terepicornis* recording 15.92 per cent mean growth inhibition of *C. fimbriata*. Least mean growth inhibition of pathogen was recorded in case of *Melia azadrach* with mean growth inhibition of 9.75 per cent only. The efficacy of leaf extracts at 30 per cent was significantly superior over 10 and 20 per cent.

A noteworthy inhibition of mycelial development of

Ceratocystis fimbriata from pomegranate (Sonyal, 2015) and of *Ceratocystis paradoxa* leading to sugarcane sett rot (Vijaya *et al*., 2007) has been reported due to the effects of aqueous extracts of garlic and ginger at 30 per cent concentration. However, Sonyal (2015) recorded least growth of *Ceratocystis fimbriata* at all three concentrations (10, 20 and 30%) of Eucalyptus (*E. manniferrae*).

The results of present investigation correspond with Khosla (2013), who confirmed the *in vivo* efficacy of aqueous extracts Basooti (*Adhatoda vasica*) and Drake (*Melia azadrach*) against wilt of pomegranate causing fungi, *Ceratocystis fimbriata*, and reported that when their aqueous extract was used at a concentration of 15 per cent, a survival rate of 55.55 per cent was noted in the field.

Systemic fungicides carbendazim (Bavistin 50 WP), propiconazole (Tilt 25% EC) and hexaconazole (Contaf 5% EC) in present investigation showed absolute inhibition of test fungus at all concentrations (25, 50 and 100 ppm) on which they were evaluated. Similar reports to that of our investigations were also made by Somu *et al*. (2018), who reported absolute (100%) inhibition of *Ceratocystis fimbriata* by carbendazim, propiconazole, hexaconazole at concentrations of 0.1, 0.2 and 0.3 per cent respectively. However, at lower concentrations of 0.025 and 0.05 per cent Kerakalamatti *et al*. (2018) reported that propiconazole and hexaconazole were equally effective. Vijaya *et al*. (2006) reported that carbendazim and propiconazole were most effective in showing absolute (100%) inhibition of the *Ceratocystis paradoxa* at concentrations of 0.05 and 0.1 per cent respectively. Somasekhara (2009) and Sharma *et al*. (2010) reported complete inhibition of mycelial growth of *Ceratocystis fimbriata* due to the action of carbendazim and propiconazole at a concentration of 0.1 per cent. Sonyal (2010) has demonstrated that carbendazim behaved differently at different concentrations and was found effective only at 0.3 per cent concentration however, in present investigations, carbendazim provided absolute inhibition of the mycelial growth of *Ceratocystis fimbriata* at 0.1 per cent concentration subsequently showing its potency. Chaudhari *et al*. (2016) revealed that hexaconazole and tricyclazole completely inhibited colony growth of *Ceratocystis fimbriata* at concentrations of 0.05 per cent, 0.1 per cent and 0.15 per cent respectively.

Experiment conducted by Khan *et al*. (2017) revealed that systemic fungicide hexaconazole gave 94.65 per cent inhibition of mycelial growth of *Ceratocystis fimbriata* and propiconazole gave cent per cent mycelial growth inhibition. Raja *et al*. (2017) reported that cent per cent inhibition of *Ceratocystis fimbriata* was shown by hexaconazole, tebuconazole, carbendazim, propiconazole and thiophanate methyl at all concentrations on which they were evaluated.

Fosetyl-Al (Aliette 80% WP) performed well and recorded mean inhibition of mycelial growth at 58.39 per cent (Table 2). Among the different concentrations of fosetyl-Al (Aliette 80% WP), a concentration of 100 ppm recorded the maximum inhibition rate of 61.85 per cent followed by concentrations of 25 and 50 ppm each showing 56.66 per cent mycelial growth inhibition. Results of present investigation are in concurrence with Ismail *et al*. (2018), who reported that mean per cent mycelial growth inhibition of *Ceratocystis fimbriata* by fosetyl-Al (Aliette 80% WP) to be 69.08 per cent when evaluated at concentrations of 5, 10, 20 and 40 ppm.

Likewise, non-systemic fungicides mancozeb (Dithane M-45 75% WP) and propineb (Antracol 70% WP) showed 100 per cent mycelial growth inhibition at all three concentrations (200, 500 and 1000 ppm) on which they were evaluated and present investigation is in accordance with those reported by Somu *et al*. (2018), who revealed that mancozeb, captan and copper oxy chloride gave cent per cent inhibition of *Ceratocystis fimbriata* at concentrations of 0.1, 0.2 and 0.3 per cent respectively. Similarly, Kerakalamatti *et al*. (2018) reported that at concentrations of 0.025, 0.05 and 0.1 per cent mean inhibition of mycelial growth of *Ceratocystis fimbriata* by captan, copper oxy chloride, mancozeb and propineb was noted to be 96.33, 52.27, 95.45 and 54.79 per cent respectively. Vijaya *et al*. (2006) observed that captan was most effective against *Ceratocystis fimbriata* at concentrations of 0.1 and 0.2 per cent, whereas copper oxy chloride was observed to be least effective against *Ceratocystis paradoxa*. Somasekhara (2009) evaluated various fungicides and reported that *Ceratocystis fimbriata* was completely inhibited by the fungicides mancozeb and ziram. Sharma *et al*. (2010) reported that absolute (100%) inhibition of *Ceratocystis fimbriata* was observed by the action of mancozeb and captan at a concentration of 0.2 per cent, Sonyal (2010) reported that copper oxy chloride and propineb were effective at all three concentrations (0.1, 0.2 and 0.3%) and found them superior to all other non-systemic fungicides tested. Chaudhari *et al*. (2016) reported that copper oxy chloride and mancozeb were most effective at concentrations of 0.2 per cent, 0.25 per cent and 0.3 per cent. Raja *et al*. (2017) revealed mancozeb, zineb, captan and thiram to be most effective against *C. fimbriata* .

Copper oxy chloride (Blitox 50 WP) was noteworthy with mean inhibition to the tune of 75.67 per cent. The least efficacy was noted in case of captan (captan 50 WP) with mean inhibition rate of 55.55 per cent (Table 3). Khan *et al*. (2017) revealed that non-systemic fungicide Thiram (74.35 %) was the most effective against *Ceratocystis fimbriata* and copper oxy chloride was reported to show 70.52 per cent inhibition of mycelial growth of *C. fimbriata*.

The results of combination of fungicides is presented in Table 4 which showed that carbendazim 12% + mancozeb 63% (Companion 75 WP) was most effective in all concentrations (25, 50 and 100 ppm) on which it was screened against test fungus and gave cent per cent inhibition of mycelial growth of *Ceratocystis fimbriata* and the results of our investigation concur with those of Sonyal (2010) who had also reported the efficacy of carbendazim 12% + mancozeb 63% at 0.2 and 0.3 per cent concentration. Similarly, at lower concentrations of 0.025 and 0.05 Kerakalamatti *et al*. (2018) reported that combination of carbendazim 12% + mancozeb 63% was equally effective. captan 70% + hexaconazole 5% WP (Taqat) revealed mean inhibition of 62.59 per cent against test fungus. Least effective combination of fungicides was metiram + pyraclostrobin (Cabrio Top 60 WG) with mean inhibition of 45.67 per cent. Somu (2017) reported that combinations of cymoxanil + mancozeb and mancozeb +

captan recorded absolute (100%) inhibition of mycelial growth of *Ceratocystis fimbriata* at concentrations of 0.1 per cent, 0.2 per cent and 0.3 per cent.

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