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Kubde AV
Ph.D. Scholar, Department of Plant Pathology and Agril. Microbiology, PGI, MPKV, Rahuri, Maharashtra, India

Raghuwanshi KS
Rice Pathologist, Agricultural Research Station, Lonawala, MPKV, Rahuri, Maharashtra, India

Surnar ST
Ph.D. Scholar, Department of Plant Pathology and Agril. Microbiology, PGI, MPKV, Rahuri, Maharashtra, India

Anarse JB
PG Student, VNMKV, Parbhani, Maharashtra, India

Corresponding Author:
Kubde AV
Ph.D. Scholar, Department of Plant Pathology and Agril. Microbiology, PGI, MPKV, Rahuri, Maharashtra, India

In vitro bio-efficacy of fungicides against *Fusarium oxysporum* causing wilt in pomegranate

Kubde AV, Raghuwanshi KS, Surnar ST and Anarse JB

Abstract
Pomegranate wilt caused by *Fusarium oxysporum* gained momentum now days. Losses incurred by wilt disease increasing day by day which results into huge economic loss of pomegranate growing farmers of Western Maharashtra region. *In vitro* experiment was conducted to find out effective fungicides for the management of pomegranate wilt. Different eleven fungicides were tested against *Fusarium oxysporum* in laboratory conditions. Among the tested fungicides propiconazole 25% EC, hexaconazole 5% EC, tricyclazole 75% WP and carbendazim 50% WP significantly inhibited mycelium growth of test pathogen (100%) and noted to be at par with each other. Next best fungicide was difenconazole 25% EC (81.00%) followed by, chlorothalonil 75% WP (78.30%), bordeaux mixture 1% (75.53 %), copper oxychloride 50% WP (59.03 %), metalaxyl 35% WS (58.57%), fosetyl-al 80% WP (53.43%) and least growth inhibition recorded by captan 50% WP (45.67%).

Keywords: Pomegranate wilt, *Fusarium oxysporum*, fungicides, tricyclazole, carbendazim

Introduction
Pomegranate (*Punica granatum* L.) is a small genus of fruit bearing shrubs or trees. Earlier pomegranate placed in monogeneric family *Punicaceae* but now it is shifted to family *Lythraceae* by the Angiosperm Phylogeny Group (Anonymous, 2016) [1] on the basis of recent phylogenetic studies (Graham *et al*., 2005) [5]. Including *Punica* there are 32 genera and about 620 species of flowering plants in this family. *Punica* is a very small genus having only two species named as *Punica protopunica* and *Punica granatum*. *Punica granatum* is a well-known species among these two. On the basis of colour of ovary *Punica granatum* classified in to two species i.e. *Chlorocarpa* and *Porphyrocarpa*. The chromosome number varies among different cultivars of pomegranate from 2n=16 or 2n=18 (Levin, 2006).
Pomegranate is counter to be indigenous to Iran, where it was first cultivated in about 2000 BC (Stover and Mercure, 2007) [18] and is also recognized to be native to Turkey (Ercisli *et al*., 2007) [4]. It is extensively cultivated in India, Tunisia, Spain, Turkey, China, Japan, Greece, Armenia, France, Italy, Morocco, Egypt, Iran, Afghanistan, Arabia and Baluchistan.
Fruit of pomegranate is very much liked for its cool and refreshing juice. Arils of fruit are consumed as it is and different types of juice, concentrates, jelly and syrup are made by processing industries. Anardana is made by using seeds and fleshy portions of sour pomegranate which is used as a seasoner and for adding sourness in curries. Pomegranate has an easy fermentation property so that wine can be prepared by using fruits. Some carbohydrates and minerals such as Calcium, Iron and Sulphur present in pomegranate at good extent. Organic acids such as Vitamin C and citric acid are predominantly present in pomegranate (Malhotra *et al*., 1983) [9]. Glucose (5.46%) and (6.14%) are the main sugars with no sucrose in fruits. Many pharmaceutical and therapeutic properties found in pomegranate fruits. Sweet varieties are purgative and for curing inflammation of stomach and heartache sour type varieties are good. In India, there is a common proverb 'Ek Anar Sau Bimar' meaning one fruit cures hundred diseases. For curing bronchitis the flower buds are very useful in Ayurveda. The bark of the stem, root and rind of the fruit is used for slimming, control of dysentery, diarrhea and killing tape worms.
Pomegranate is regarded as the "Fruit of Paradise". It is one of the most adaptable sub-tropical minor fruit crops and its cultivation is increasing very rapidly. In India, it is regarded as a "Vital Cash Crop", grown in an area of 2.75 lakh ha with a production of 3.26 million MT with an average productivity of 11.84 MT/ha (Indiastat, 2019-20, 3rd Advance Estimates). Among the different pomegranate growing states.

Maharashtra is the largest producer occupying 2/3rd of total area in the country followed by Karnataka, Andhra Pradesh, Gujrat and Rajastan. Maharashtra has an allocation of tropical pomegranate in an area of 1.48 lakh hectares with 1.79 million MT productions and 12.10 MT/ha productivity (Indiastat, 2017-18).

The successful cultivation of pomegranate in recent years has encounter with different problems such as pest and diseases. Bacterial leaf and fruit spot, anthracnose, wilt complex, leaf and fruit spot, shot hole borer and nematodes are the major diseases and pests in pomegranate. Among the diseases wilt complex caused by *Fusarium oxysporum*, *Ceratocystis fimbriata* Ell. and Halst., *Rhizoctonia bataticola* is a major threat. At present the crop is severely affected by wilt pathogen and day by day the wilting severity is increasing at faster rate. Pomegranate wilt complex disease is prevalence in different states of India in different proportionate up to 64 per cent. In Maharashtra state wilt disease incidence ranged from 5 to 49.2 per cent (Sharma *et al*., 2010; Tirmali *et al*., 2018) [14, 19] whereas, in Karnataka state disease incidence ranged from 0.1 to 61.11 per cent (Sharma *et al*., 2010; Benagi *et al*., 2011; Raja and Amaresh, 2017; Somu *et al*., 2018; Madhushri *et al*., 2019; Shruthi *et al*., 2019) [14, 2, 13, 17, 8, 16] however, in Himachal Pradesh disease incidence ranged from 1.03 to 20.06 per cent (Khosla and Bhardwaj, 2013; Sharma, 2019) [6, 15] whereas, 8.69 per cent in Andhra Pradesh (Sharma *et al*., 2010) [14]. Due to this wilt complex disease substantial loss is occurred every year. Taking into considerations of economic losses due to disease, *in vitro* efficacy of different fungicides was tested so that best one used in field for disease management.

Materials and methods

Isolation and purification of pathogen

The tissue isolation method was followed to isolate the pathogen responsible for wilt of pomegranate. Infected plants parts were washed in running tap water to remove soil adhered to the infected parts. The roots and collar region portion of wilted plants of pomegranate were cut in to suitable small pieces. The internal discolored root bits of 0.5 to 1 cm were excised. These pieces were then disinfected by 0.1 per cent mercury chloride solution for two minutes followed by three washings of sterilized water to remove the trace of mercury chloride. Each bit was blot dried and three such bits were then plated aseptically on previously sterilized PDA Petri plate. These plates were then kept in inverted position and incubated at the room temperature (28 ± 1^0C). They were kept under observation for the growth of associated fungus. When the growth of fungus was noticed, they were transfer to PDA slants to obtained pure cultures by purification.

In vitro evaluation of fungicides

Eleven fungicides with three concentration *viz*., 500, 1000 and 2000 ppm were evaluated against pomegranate wilt pathogen under laboratory condition by food poison technique (Nene and Thapliyal, 1993) [11]. The fungicides suspension was made by adding required quantity of fungicides to the melted PDA medium to obtain the desired concentration. 20 ml of poisoned medium was poured in each sterilized Petri plates. Mycelial disc of a 5 mm size from actively growing zone of seven days old culture was cut by a sterile cork borer and one such disc was placed at the centre of each agar plate. Control treatment was maintained without adding any fungicide to the medium. Three replications were maintained for each concentration. Then such plates were incubated at room temperature and radial growth was measured when fungus attained maximum growth in control plates. Per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947) [21].

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

Whereas,
I = Percent inhibition
C = Radial growth of test fungus in control plate
T = Radial growth of test fungus in treated plate

Table 1: Details of fungicides used in the experiment

Sr. No.	Common name	Chemical name	Trade name
1	Propiconazole 25% EC	1-[{2-(2, 4-Dichlorophenyl)-4-propyl-1, 3dioxolan-2-yl}methyl]-1, 2, 4-triazole	Tilt
2	Hexaconazole 5% EC	2-(2,4-Dichlorophenyl)-1-(1H-1,2,4-triazole-1-yl)hexan-2-ol	Contaf
3	Copper Oxy Chloride 50% WP	dicopper chloride trioxide	Blitox
4	Chlorothalonil 75% WP	2, 4, 5, 6-tetrachloroisophthalonitrile	Kavach
5	Difenconazole 25% EC	1-(2-(2-chloro-4-(4-chlorophenoxy)phenyl) 4methyl-1, 3dioxolan-2-yl)methyl)-1H-1, 2, 4 triazole	Score
6	Metalaxyl 35% WS	methyl-N-(methoxyacetyl)-N-(2, 6-xylyl)-DL-alaninate	Matrix
7	Tricyclazole 75% WP	5-methyl-1, 2, 4-triazolo(3, 4-b) (1, 3) benzothiazole	SIVIC
8	Fosetyl-al 80% WP	Aluminiumtris (ethyl)phosphonate	Allite
9	Carbendazim 50% WP	Methyl1H-benzimidzole-2-ylcarbamate	Bavistin
10	Bordeaux mixture 1%	5kg CuSo4 +5Kg Lime +500 liters water	
11	Captan 50% WP	N-trichloromethylthio-4-cyclohexane-1,2-dicarboximide	Captan

Statistical analysis

The statistically analysis has done as per the methods given by Panse and Sukhatme (1967) [12].

Result and Discussion

In vitro evaluation of fungicides

The eleven fungicides were tested against *Fusarium oxysporum* at different three concentrations *viz*., 500, 1000 and 2000 ppm in the laboratory for testing bio-efficacy against the test pathogen by using poisoned food technique. The results showing difference among the treatments and concentrations were found to be statistically significant are presented in Table 2, Plate 1 and Fig. 1.

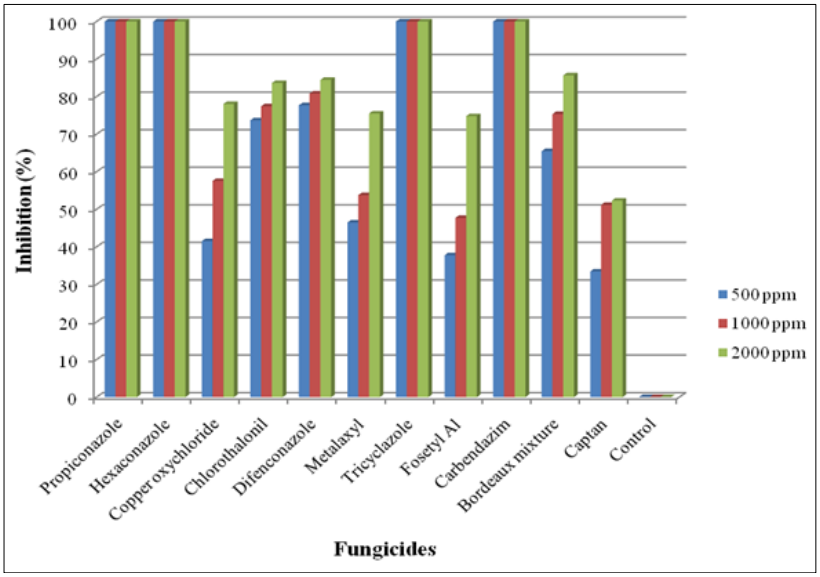

Fig 1: Efficacy of different fungicides on the growth inhibition of *Fusarium oxysporum* under *in vitro* conditions

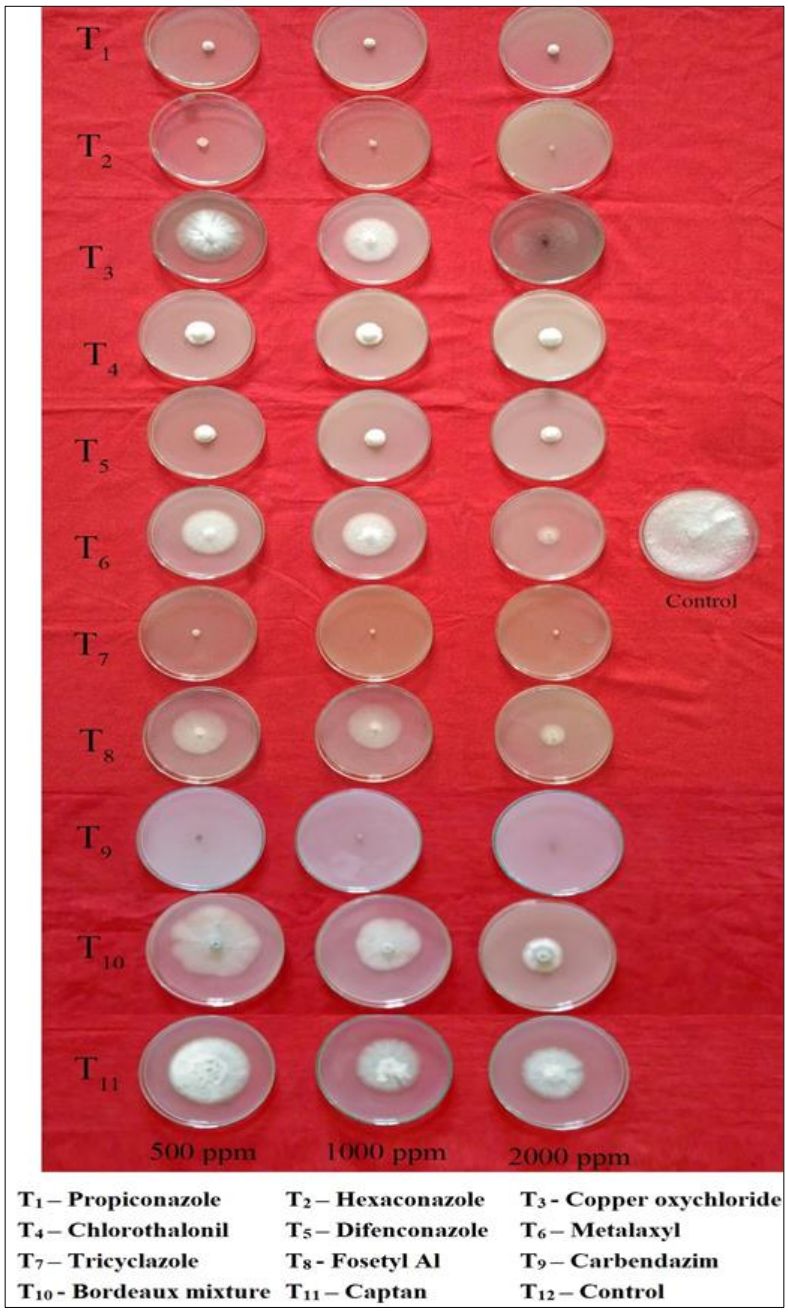

Plate 1: Efficacy of different fungicides on the growth inhibition of Fusarium oxysporum under *in vitro* condition

Table 2: Bio efficacy of different fungicides on the growth inhibition of *Fusarium oxysporum* under *in vitro* conditions

Treat. No.	Fungicides	Colony diameter (mm)* at ppm				Per cent inhibition (%)* at ppm			
		Concentrations			Average	Concentrations			Average
		500	1000	2000	mean (mm)	500	1000	2000	inhibition (%)
T_1	Propiconazole 25% EC	0.00	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_2	Hexaconazole 5% EC	0.00	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_3	Copper oxychloride 50% WP	52.62	38.19	19.78	36.88	41.53 (40.13)	57.57 (49.35)	78.03 (62.03)	59.03 (50.20)
T_4	Chlorothalonil 75% WP	23.67	20.31	14.73	19.53	73.70 (59.15)	77.43 (61.64)	83.63 (66.14)	78.30 (62.24)
T_5	Difenconazole 25% EC	20.07	17.25	13.98	17.1	77.70 (61.82)	80.83 (64.04)	84.47 (66.79)	81.00 (64.16)
T_6	Metalaxyl 35% WS	48.15	41.61	22.05	37.29	46.50 (42.99)	53.77 (47.16)	75.50 (60.33)	58.57 (49.93)
T_7	Tricyclazole 75% WP	0.00	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_8	Fosetyl-al 80% WP	56.01	47.04	22.68	41.91	37.77 (37.92)	47.73 (43.70)	74.80 (59.87)	53.43 (46.97)
T_9	Carbendazim 50% WP	0.00	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_{10}	Bordeaux mixture 1%	31.05	22.17	12.90	22.02	65.50 (54.03)	75.37 (60.24)	85.67 (67.75)	75.53 (60.35)
T_{11}	Captan 50% WP	59.88	43.95	42.90	48.90	33.47 (35.35)	51.17 (45.67)	52.33 (46.34)	45.67 (42.51)
T_{12}	Control	90.00	90.00	90.00	90.00	00.00 (00.00)	0.00 (00.00)	0.00 (00.00)	0.00 (00.00)
S.Em (±)		0.03	0.06	0.06	0.07	0.10	0.17	0.11	0.16
CD at 5%		0.10	0.17	0.17	0.23	0.28	0.50	0.31	0.47

*: Average of three replications
Figures in parentheses are arc sin transformed values

Mycelial growth

The data depicted in Table 2, Plate 1 and Fig. 1 presented that all of the eleven fungicides tested exhibited a wide range of radial mycelial growth of *F. oxysporum* and it was decreased drastically with increase in their concentrations. The fungicides resulted with mycelial growth in the range of 0.00 to 59.88 mm, 0.00 to 43.95 mm and 0.00 to 42.90 mm, @ 500, 1000 and 2000 ppm respectively, as against 90.00 mm in untreated control.

Among the fungicides, propiconazole 25% EC , hexaconazole 5% EC, tricyclazole 75% WP and carbendazim 50% WP resulted with no mycelial growth at 500, 1000 and 2000 ppm. The next fungicides with significantly least mycelial growth were difenconazole 25% EC (20.07, 17.25 and 13.98 mm, respectively) followed by, chlorothalonil 75% WP (23.67, 20.31 and 14.73 mm, respectively), bordeaux mixture 1% (31.05, 22.17and 12.90 mm respectively), metalaxyl 35% WS (48.15, 41.61and 22.05 mm respectively), copper oxychloride 50% WP (52.62, 38.19 and 19.78 mm respectively), fosetyl-al 80% WP (56.01, 47.04 and 22.68 mm respectively) and captan 50% WP (59.88, 43.95 and 42.90 mm respectively) respectively @ 500, 1000 and 2000 ppm.

Mycelial growth inhibition as per concentration of fungicide

The data depicted in Table 2, Plate 1 and Fig. 1 presented that all of the fungicides tested (each @ 500, 1000 and 2000 ppm) significantly inhibited mycelial growth of *F. oxysporum*, over untreated control and it was found to increase with increase in concentrations of the fungicides tested. The mycelial growth inhibition resulted with the test fungicides ranged from 33.47 to 100 per cent at 500 ppm, 47.73 to 100 per cent at 1000 ppm and 52.33 to 100 per cent at 2000 ppm concentrations respectively over untreated control.

At 500 ppm concentration, fungicides propiconazole 25% EC, hexaconazole 5% EC, tricyclazole 75% WP and carbendazim 50% WP significantly inhibited mycelium growth of test pathogen (100%) and noted to be at par with each other. Next best fungicide was difenconazole 25% EC (77.70%) followed by, chlorothalonil 75% WP (73.70%), bordeaux mixture 1% (65.50%), metalaxyl 35% WS (46.50%), copper oxychloride 50% WP (41.53%), fosetyl-al 80% WP (37.77%) and least growth inhibition recorded by captan 50% WP (33.47%).

At 1000 ppm concentration, fungicides propiconazole 25% EC, hexaconazole 5% EC, tricyclazole 75% WP and carbendazim 50% WP significantly inhibited mycelium growth of test pathogen (100%) and noted to be at par with each other. Next best fungicide was difenconazole 25% EC (80.83%) followed by, chlorothalonil 75% WP (77.43%), bordeaux mixture 1% (75.37%), copper oxychloride 50% WP (57.57%), metalaxyl 35% WS (53.77%), captan 50% WP (51.17%) and least growth inhibition recorded by fosetyl-al 80% WP (47.73%).

At 2000 ppm concentration, fungicides propiconazole 25% EC, hexaconazole 5% EC, tricyclazole 75% WP and carbendazim 50% WP significantly inhibited mycelium growth of test pathogen (100%) and noted to be at par with each other. Next best fungicide was bordeaux mixture 1% (85.67%) followed by, difenconazole 25% EC (84.47%), chlorothalonil 75% WP (83.63%), copper oxychloride 50% WP (78.03%), metalaxyl 35% WS (75.50%), fosetyl-al 80% WP (74.80%) and least growth inhibition recorded by captan 50% WP (52.33%).

Similar results were reported by Mande (2003) [10] who found that the carbendazim (0.1%) and captan (0.1%) were most effective fungicides against *Fusarium oxysporum* causing pomegranate wilt disease. Choudhari *et al*. (2016) [3] reported that the carbendazim inhibited cent per cent colony growth of *Fusarium oxysporum* causing wilt in pomegranate. Similarly, Varma (2016) [20] tested fungicides *viz.*, difenconazole, propiconazole, azoxystrobin and carbendazim against *Fusarium solani* causing pomegranate wilt and reported that among these fungicides propiconazole proved to be best in inhibiting mycelial growth of test pathogen.

Conclusion

Eleven fungicides tested against pomegranate wilt incited by *Fusarium oxysporum* under *in vitro* conditions. Among these fungicides tested propiconazole 25% EC, hexaconazole 5% EC, tricyclazole 75% WP and carbendazim 50% WP significantly inhibited mycelium growth of test pathogen (100%) and noted to be at par with each other. Next best fungicide was difenconazole 25% EC (81.00%) followed by, chlorothalonil 75% WP (78.30%), bordeaux mixture 1% (75.53%), copper oxychloride 50% WP (59.03%) and metalaxyl 35% WS (58.57%).

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