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Etiology and Management of Cercospora Fruit and Leaf Spot Disease of Pomegranate through Fungicides and Systemic acquired Resistance Inducers

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ABSTRACT: Cercospora leaf and fruit spot is a serious disease of pomegranate plant during warm and humid weather condition resulting deterioration of fruit quality and lesser fruit yield to growers. Its manifestation includes dark reddish brown to almost black with diffused yellow halo, circular to angular (1-4mm) spots on leaves and 1-12mm conspicuous dark brown, circular to unequal irregular blotches on fruits which lower the yield by interfering with photosynthetic activity and reduces the market value of the pomegranate fruit. The pathogen *Cercospora punicae* was identified to be associated with the disease and isolated on PDA (Potato Dextrose Agar) medium. The rapid growth of the fungus was observed on GPLDA (Green Pomegranate Leaf Decoction Agar) medium, 90 mm mycelial growth within 18 days of incubation at temperature 25°C and pH 6.0. This was later on used as a specific medium for *in-vitro* studies. The surface of the colonies in contact with the medium was olivaceous in colour, the exposed surface was smoky and mycelium was densely compacted except at the exposed surface. The present investigations on management included evaluation of various systemic, non systemic fungicides and Systemic acquired resistance (SAR) inducers against the disease. Propiconazole, difenoconazole and Tebuconazole completetly inhibited the mycelial growth of *C. punicae* at 50, 100 and 150 ppm under *in- vitro* condition. Four foliar sprays at 15 days intervals of propiconazole resulted in 82.92 and 83.96 percent disease control on leaves and fruit respectively. Its application increased fruit yield and reduces the losses up to 39 percent. Dipotassium hydrogen phosphate anhydrous was found most effective and reduced the disease incidence to 52.69 per cent over control amongst of six SAR inducers evaluated in pot culture conditions.

Keywords: *Cercospora punicae*, etiology, management, fungicides, SAR, chemicals.

INTRODUCTION

The Cercospora leaf and fruit spot disease of pomegranate plant considered minor importance disease, yet the changing climatic scenario resulted in erratic rainfall as a result the incidence and severity of disease is increasing every year. *Cercospora punicae* P. Henn was first recorded associated with the diseases in Japan by Hennings in 1906 (Chupp, 1954) and was reported from India by various researchers (Agarwal and Hasija 1964; Thirumalachar and Chupp 1948).

The Cercospora leaf and fruit spot disease caused by *Cercospora punicae* was most prominent among various spot pathogens in Solan, Shimla and Sirmaur district during year 2016-17. Khosla and Bhardwaj (2013) recorded 1.1 to 17.31 per cent incidence of fruit and leaf spot disease (*Cercospora punicae* and *Alternaria* sp.) in pomegranate growing area of Himachal Pradesh. The disease appears every year during rainy season in wild pomegranate, thereby forcing the growers to harvest the immature fruits resulting in production of poor quality "Anardana" after drying, fetching less price in market, affecting income and livelihood of resource striven farmers of Himachal Pradesh. Symptoms of the disease tend to be circular to angular, dark reddish brown to almost black with diffused yellow halo on leaves and prominent dark brown, circular blotches which initially appear unequal sizes on fruit (Fig. 1). In extreme infection, interfering with growth as a result of reduced production of photosynthates resulting in less production of fruit. The fungus survives as tiny black fungal tissue known as stromata in old affected leaves and fruits in the soil.

Spores also survive in infected debris for at least one season (Wolf, 1927).

Fungicides can provides successful management of ongoing leaf spot pathogen infection in field through directly killing of spore and inhibiting the metabolic activities diseases. Triazole group of fungicides inhibit ergo-sterol biosynthesis of plant pathogenic fungus which prevent cell wall formation and reduces the colonization of the pathogen. Induced resistance is a host response, systemic acquired resistance (SAR) can be induced by treatment with a variety of agents, including necrotizing pathogens and certain chemicals such as Potassium oxalate ($K_2C_2O_4$), ß-Amino -butyric acid (BABA) $C_4H_9NO_2$, Salicylic acid ($C_7H_6O_3$) and Di Potassium hydrogen phosphate (K_2HPO_4) (Walters *et al.*, 2013). Induction of resistance can lead to the direct activation of defenses resulting in stronger elicitation of defenses and following pathogen attack. (Faize and Faize 2018). The information on the use of latest fungicides especially EBIs and systemic resistance inducers has not been experimented so far in case of pomegranate. Therefore, in the present study fungicides were evaluated for best for management of the disease in field condition to reduce losses of the pomegranate farmer, systemic acquired resistance (SAR) inducers play vital role in diseases management, activates the plant defense mechanisms and it can be alternative to fungicide for eco-friendly management of the disease in future.

MATERIAL AND METHODS

Periodic surveys for leaf and fruit spot disease were conducted for two consecutive year *i.e*. 2016 and 2017 both in wild and cultivated pomegranate habitats in Solan, Sirmaur and Shimla districts of Himachal Pradesh during July to October. The most prominent spot were taken for isolation which yielded *Cercospora punicae* and further studies its etiology. Infected leaves and fruits showing typical symptoms of this disease were selected for isolation using tissue isolation technique on PDA medium by following the incubation of 18 days at 25±1 ºC. Identification was done as per morphological characters given in Illustrated Genera of Imperfect Fungi and as described by Wolf (1927). The identity of the culture was also got confirmed from NRC, Pomegranate Sholapur, Maharashtra. The growth was very slow on the PDA and found very quickly on GPLDA. Therefore, five different concentrations (5; 10; 15; 20; 25%) of Green Pomegranate Leaf Extract in Potato Dextrose Agar were evaluated for standardization of optimum concentration of leaf extract for getting maximum growth of *Cercospora punicae* in minimum possible time. For recording disease incidence/ severity on leaves 5-7 leaves were plucked from each plant at random from N-S and E-W directions. The disease severity was recorded by using 0-5 and 0-6 scale on leaf and fruit. Per cent disease incidence and severity on leaves and fruits was calculated by following formulae (Mckinney, 1923).

$$\text{Disease incidence (\%)} = \frac{\text{Number of leaves/fruits infected}}{\text{Total number of leaves/fruits observed}} \times 100$$

$$\text{Disease severity (\%)} = \frac{\text{Sum of individual disease ratings}}{\text{Sum of all disease ratings}} \times \frac{100}{\text{Maximum disease grade}}$$

Eight systemic and non systemic fungicides were tested under *in vitro* to study the inhibitory effect of fungicides on the mycelial growth of *C. punicae* by following poisoned food technique as described by Falck (1907). Growth inhibition (%) in each treatment was calculated as described by Vincent (1927)

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

Where,

I = Per cent mycelial inhibition

C = Diametric mycelial growth in control (mm)

T = Diametric mycelial growth in treatment (mm)

Field experiment was conducted on six year old pomegranate plants during 2016 and 2017 at the Model Farm of the University. The pomegranate variety Kandhari Kabuli plants planted at 4×2m spacing, with plant architecture trained to multi-stem were selected for laying out experiment. Systemic and non systemic fungicides were evaluated at the experimental farm where disease outbreak was very high during previous years. The fungicidal solution spray was started with the first initiation of disease symptom and four sprays were given at fortnightly intervals in July-August month. The control plants were sprayed with water to create similar microclimate for the occurrence and progress of the disease. In randomized block design, each treatment was replicated thrice (RBD). The observations were recorded on disease incidence and severity as per the procedure described in Table 2. The data on number of fruits and the yield were recorded at harvesting in September 2016 and 2017.

To study the efficacy of systemic acquired resistance (SAR) or abiotic resistance inducers an experiment was conducted in pots. Inoculation was performed according to Callahan *et al.* (1999). Mycelial suspension was sprayed on both sides of pomegranate leaves on the plants growing in pots (Fig. 2). The observations on appearance of leaf spots were recorded and re-isolation as per procedure described for isolation was taken from the infected tissue and culture obtained was compared with the original. SAR inducer foliar spray was applied on plants grown in pots after emergence of symptoms and repeated once after first 15 days of spraying. To maintain high humidity, the plants were covered with polythene sheet and sprayed with water. In addition, separate control plants were

maintained with only water spray. The observations were taken for the appearance and development of symptoms and rated after one month after inoculation based on scale given by Raju *et al*. (2011). The data obtained from laboratory and field experiments were subjected to statistical analysis. The differences exhibited by treatments in various experiments were tested for their significance using standard statistical procedures as described by Gomez and Gomez (1984). The critical difference (CD) was calculated in each experiment to establish the least significant difference amongst the treatments.

RESULTS AND DISCUSSION

The disease symptoms were recorded both on leaves and fruits and the leaf spots were sub circular to irregular, 1-4 mm dia. at first brown and grey to pale tan and eventually brown to dark brown at the margin with diffused yellow halo (Fig. 1). These lesions coalesced less frequently. The early infected leaves turned pale green and dropped off prematurely forming a layer of dropped leaves underneath the tree on the ground. During periods of high relative humidity the lower surface of lesions is covered with dense aggregates of conidiophores and conidia which in mass appear brown. The conidial fructifications appear on the upper surface less commonly and less in abundance.

The affected fruits develop small irregular black spots, which later coalesce into large spots measuring 1–12 mm dia. These are circular in outline but due to unequal radial growth soon become irregular in shape becoming unequal irregular blotches covered a considerable proportion of the surface of the fruit which turned light to dark brown in colour (Fig. 1). Similar kind of symptoms on leaves had been observed by (Chupp, 1954) who reported that such leaf spots were circular to somewhat angular, dark reddish brown to almost black with a diffused yellow halo and size varied from 0.5 to 5mm in dia.

Fungus grew very slow on Potato Dextrose Agar where as it produced uniform dense colonies on Green Pomegranate Leaf Decoction Agar Medium. Out of five different media evaluated maximum radial growth of *C. punicae* was recorded on Green Pomegranate Leaf Decoction Agar media 5% (57.50 mm), followed by V8 Juice Agar (21.50 mm) and Oat Meal Agar (18.87 mm). The surface of the colonies in contact with the medium was olivaceous in colour and the exposed surface was smoky (Fig. 1). The mycelium was densely compacted except at the exposed surface. Hyphae branched, 2–3 µm wide, septate, constricted at the septa, distance between septa 6–10 µm. The hypha of the fungus was light brown in colour, septate and unbranched under compound microscope at 40X. The size of hyphae 2–9 µm wide, septate, constricted at the septa, distance between septa 5–26 µm, brownish or sub hyaline, wall 0.3–1 µm wide and smooth. Conidia were not formed in culture.

The conidiophores developed in stromata which were in dense fascicles, septate and medium dark in colour. Bakhshi *et al.* (2014) observed culture surface of *C. punicae* folded, erumpent with moderate aerial mycelium and irregular lobate margins and colour dark olivaceous grey on the surface, dark iron-grey underneath, which corraborate with the present findings and culture behavior observed.

In the present investigation, systemic and non systemic fungicides were tested at three concentrations under *in vitro* conditions for their efficacy against *Cercospora punicae* and inhibition of mycelial growth was recorded. Amongst the five systemic fungicides tested treatment with propiconazole, difenoconazole and tebuconazole recorded complete inhibition of *Cercospora punicae* at all the three concentrations (50, 100 and 150 ppm) followed by 93.39 per cent mean inhibition in treatment with hexaconazole (Fig. 3). The least mean inhibition of mycelial growth among the systemic fungicides was observed in treatment with carbendazim (81.97%) at 100 ppm concentration as illustrated in Table 1. Among the non-systemic fungicides maximum mean mycelial growth inhibition (91.17 per cent) was recorded in treatment with Bordeaux mixture at all the three concentrations (250, 500, and 1000ppm) tested, followed by 86.23 per cent inhibition in captan (Fig. 4). Similarly, efficacy of systemic and non systemic fungicide has also been reported by various workers and found effective against different *Cercospora* spp under *in vitro* condition (Khan *et al.,* 2014; Secor *et al.,* 2010; Dam and Sreedhar 2019).

The data on management of disease during 2016 and 2017 were recorded on disease incidence on fruits, disease severity on the leaves, yield of disease free fruits on per plant basis. The data obtained during both the years were subjected to pooled analysis (Table 2) and revealed that all the treatments significantly reduced the leaf and fruit spot incidence as compared to control. The data indicated that overall minimum average disease incidence on fruits (3.22%) and disease severity on leaves (3.23%) of Cercospora leaf and fruit spot was observed in plant treated with propiconazole (0.05%) with maximum control of disease severity (83.96%) on leaves and disease incidence (82.92%) on fruits. It was followed by treatment with tebuconazole (0.05%) with 77.21 per cent and 81.57 per cent disease control on fruits and leaves, respectively. Overall minimum average disease control of on fruits (52.96%) and leaves (49.70%) was observed on the plant treated with captan. The maximum fruit yield (12.83 kg/ plant) for both the years was recorded in plants treated with propiconazole (0.05%) followed by tebuconazole (0.05%) with 12.33 kg fruit yield per plant, respectively which resulted in 39.30 per cent increase in yield in case of plant treated with propiconazole and 33.87 per cent increase in yield in case of tebuconazole over control.

The systemic acquired resistance (SAR) or abiotic resistance inducers were evaluated under pot culture conditions. It is evident from the data (Table 3) that all the treatments were effective in reducing the disease incidence as compared to control. However, treatment of Dipotassium hydrogen phosphate was found most effective and reducing the disease incidence to 52.69 per cent over control which was followed by treatment with ß-amino-butyric acid and salicylic acid with 46.87 and 43.77 per cent disease reduction, respectively. Foliar spray of potassium oxalate and calcium carbonate were least effective against the disease with 25.40 and 25.45 per cent reduction in disease incidence over control, respectively. The findings were consistent with the findings of Morsy *et al.* (2022), who discovered that combining salicylic acid treatment with fungicides (methyl benzimidazole carbamate (MBC), quinone outside inhibitor (QoI), and demethylation inhibitor (DMI) resulted in a significant reduction of sugar beet leaf spot disease (Cercospora beticola), When combined with salicylic acid, the efficacy of epoxiconazole (EPO) and propiconazole increased to 77.5-79.1% and 77.0-78.2% which was 67.2-69.1% and 63.4-63.6% when used alone. Carbendazim alone was 47.5-45.1% effective but the efficacy increased to 67.1% when mixed with SA**.**

Table 1: *In vitro* efficacy of various systemic and non-systemic fungicides against *C. punicae.*

Fungicide	**Per cent inhibition of mycelial growth at different concentrations**			
	C_1	**C_2**	**C_3**	**Mean A**
Carbendazim**	69.63 (56.54)	76.29 (60.84)	100.00 (89.39)	**81.97 (68.92)**
Mancozeb*	75 (59.98)	81.75 (68.76)	100.00 (89.39)	**85.58 (71.37)**
Captan*	74.19 (59.45)	84.50 (66.80)	100.00 (89.39)	**86.23 (71.80)**
Bordeaux mixture 1%*	82.96 (65.61)	90.55 (72.10)	100.00 (89.39)	**91.17 (75.70)**
Hexaconazole**	86.48 (68.40)	93.70 (75.48)	100.00 (89.39)	**93.39 (77.76)**
Tebuconazole 50% + Trifloxystrobin 25% **	100.00 (89.39)	100.00 (89.39)	100.00 (89.39)	**100.00 (89.39)**
Difenoconazole**	100.00 (89.39)	100.00 (89.39)	100.00 (89.39)	**100.00 (89.39)**
Propiconazole**	100.00 (89.39)	100.00 (89.39)	100.00 (89.39)	**100.00 (89.39)**
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
Mean B	**76.47 (64.24)**	**80.75 (67.57)**	**88.88 (79.45)**	
		$C.D._{0.05}$	**SE±**	
Fungicide		0.78	**0.27**	
Concentration		0.45	**0.15**	
Fungicide× Concentration		1.35	**0.47**	

Figures in the parentheses are arc sine transformed values

** Systemic fungicide concentrations C_1, C_2, C_3 used were 50, 100 and 150ppm, respectively

*Non systemic fungicide concentrations C_1, C_2, C used were 250,500 and 1000ppm, respectively

Fig. 1. (a, b) Cercospora infested pomegranate fruit and leaf; (c) Pure culture of *C. punicae on GLPDA medium*; (d) Mycelium and stromata initiation in the culture.

Table 2: Field Evaluation of various fungicides against Cercospora leaf and fruit spot of pomegranate during 2016 and 2017.

Fungicide	Disease severity on leaves (%)*			Disease control on leaves (%)**			Disease incidence on fruits (%)*			Disease control on fruits (%)**			Yield/plant (kg)			No of fruits/ plant	Yield / Ha (Q)	Increase in yield (%)
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled			
Mancozeb (0.25%)	7.40 (2.88)	5.55 (2.55)	**6.47 (2.73)**	61.91 (51.95)	73.83 (59.22)	**67.87 (55.49)**	7.52 (2.90)	5.37 (2.50)	**6.44 (2.55)**	66.68 (54.81)	66.81 (55.05)	**66.74 (54.88)**	9.83	9.66	**9.75**	37	121.87	5.86
Carbendazim (0.05%)	7.40 (2.88)	7.40 (2.88)	**7.40 (2.88)**	61.91 (51.95)	65.49 (54.04)	**63.70 (52.99)**	7.52 (2.90)	5.37 (2.50)	**6.44 (2.88)**	66.68 (54.81)	66.81 (55.05)	**66.74 (54.76)**	9.96	10.33	**10.15**	34	126.87	10.20
Difenconazole (0.05%)	3.69 (2.14)	5.55 (2.55)	**4.62 (2.36)**	80.98 (64.42)	73.83 (59.22)	**77.40 (61.64)**	5.37 (2.50)	4.29 (2.27)	**4.83 (2.55)**	76.20 (61.04)	73.46 (59.27)	**74.83 (59.87)**	12.10	12.30	**12.20**	43	152.50	32.46
Tebuconazole 50% + Trifloxystrobin 25% (0.05%)	5.55 (2.55)	6.47 (2.72)	**6.01 (2.64)**	71.45 (57.67)	69.66 (56.63)	**70.55 (57.13)**	6.45 (2.72)	4.29 (2.27)	**5.37 (2.72)**	71.43 (57.66)	73.46 (59.27)	**72.44 (58.38)**	10.50	10.16	**10.33**	38	129.12	12.16
Tebuconazole (0.05%)	3.69 (2.14)	3.69 (2.14)	**3.69 (2.14)**	80.98 (64.42)	82.17 (65.42)	**81.57 (64.91)**	4.29 (2.27)	4.29 (2.27)	**4.29 (2.14)**	80.97 (64.41)	73.46 (59.27)	**77.21 (61.56)**	12.16	12.50	**12.33**	46	154.12	33.87
Propiconazole (0.05%)	3.69 (2.14)	2.77 (1.94)	**3.23 (2.05)**	80.98 (64.42)	86.94 (68.79)	**83.96 (66.45)**	3.22 (2.05)	3.22 (2.05)	**3.22 (1.94)**	85.74 (67.78)	80.11 (63.48)	**82.92 (65.56)**	12.76	12.90	**12.83**	46	160.37	39.30
Captan (0.25%)	10.18 (3.33)	10.18 (3.33)	**10.18 (3.34)**	47.61 (43.61)	51.787 (46.02)	**49.70 (44.80)**	10.74 (3.42)	7.52 (3.42)	**9.13 (3.33)**	52.40 (46.36)	53.53 (47.01)	**52.96 (46.69)**	9.83	9.66	**9.75**	33	121.87	5.86
Bordeaux mixture 1%	5.55 (2.55)	5.55 (2.51)	**5.55 (2.55)**	71.45 (57.67)	73.235 (59.45)	**72.34 (58.38)**	4.29 (2.27)	4.29 (2.27)	**4.29 (2.51)**	80.97 (64.41)	73.46 (59.27)	**77.21 (61.77)**	12.13	12.00	**12.06**	46	150.75	30.94
Control	19.44 (4.52)	21.29 (4.71)	**20.36 (4.62)**	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	22.58 (4.85)	16.19 (4.14)	**19.38 (4.71)**	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**	9.26	9.16	**9.21**	31	115.12	
C.D.$_{0.05}$	0.48	0.50	**0.39**	8.81	8.45	**6.43**	0.49	0.47	**0.50**	6.43	8.91	**5.20**	1.23	1.19	**0.91**			
SE±	0.15	0.16	**0.13**	2.87	2.79	**2.12**	0.16	0.15	**0.16**	2.12	2.94	**1.69**	0.40	0.39	**0.30**			

**Figures in the parentheses are arc sine transformed values;

*Figures in the parentheses are square root transformed values

Table 3: Evaluation of systemic acquired resistance (SAR) inducers against *C. punicae* under pot conditions.

Sr. No.	SAR inducer	Concentration (ppm) Disease severity on leaves (%)*				Disease control (%)**			
		C1	C2	C3	Mean	C1	C2	C3	Mean
1.	Sodium salicylate	17.58 (4.30)	15.73 (4.08)	14.80 (3.97)	16.04 (4.12)	24.31 (29.52)	28.84 (32.43)	35.67 (36.52)	29.61 (32.82)
2.	Dipotassium hydrogen phosphate	13.88 (3.85)	10.18 (3.33)	8.33 (3.05)	10.79 (3.41)	40.03 (39.22)	54.14 (47.36)	63.90 (53.05)	52.69 (46.54)
3.	Potassium oxalate	18.51 (4.41)	16.66 (4.20)	15.73 (4.08)	16.96 (4.23)	20.18 (26.50)	24.05 (29.01)	31.97 (34.34)	25.40 (29.95)
4.	Calcium carbonate	18.51 (4.41)	16.66 (4.19)	15.73 (4.08)	16.96 (4.23)	20.18 (26.50)	24.67 (29.33)	31.50 (33.94)	25.45 (29.92)
5.	ß-amino-butyric acid	14.80 (3.97)	12.03 (3.60)	9.25 (3.19)	12.03 (3.59)	36.32 (37.04)	44.57 (41.73)	59.73 (50.64)	46.87 (43.13)
6.	Salicylic acid	16.66 (4.20)	12.95 (3.73)	10.18 (3.33)	13.26 (3.75)	28.87 (32.45)	44.89 (42.02)	57.56 (49.37)	43.77 (41.28)
7.	Control	23.21 (4.91)	22.18 (4.80)	23.14 (4.91)	22.84 (4.88)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Mean	17.59 (4.29)	15.20 (3.99)	13.88 (3.80)		24.27 (27.32)	31.59 (31.70)	40.05 (36.84)	
		C.D.$_{0.05}$		**SE±**		**C.D$_{0.05}$**		**SE±**	
	Treatments	1.16		0.40		5.93		1.50	
	Concentrations	0.76		0.33		1.55		0.85	
	Treatment× Concentration	2.01		0.87		10.25		2.26	

(a) Untreated pot plants of pomegranate
(b) Polythene covering of pot plants after spryaing of *C. punicae* conidial suspension
(c) Induction of symptoms of *C. punicae* after 14 days of conidial suspension spray
Fig. 2. Inoculation of *C. punicae* and induction of symptoms.

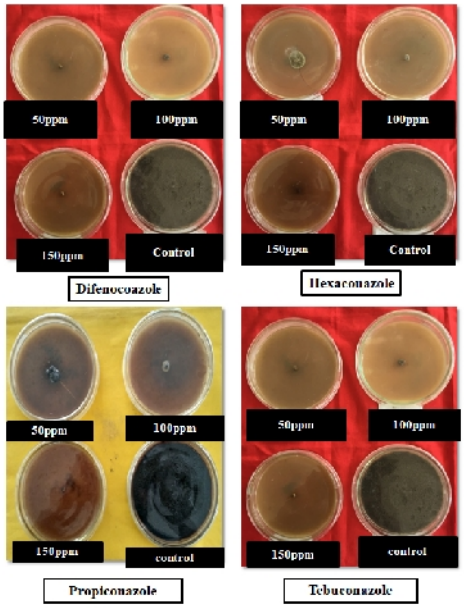

Fig. 3. Growth of *C. punicae* at different concentrations of difenocoazole, hexaconazole, propiconazole and tebuconazole on PDA.

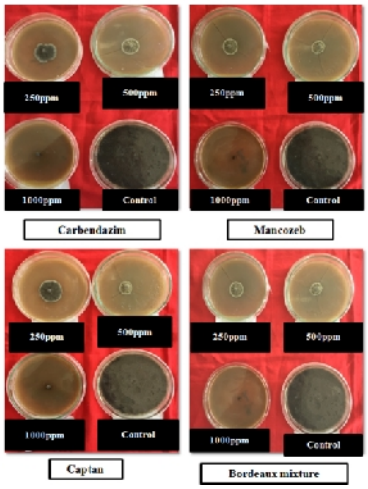

Fig. 4. Growth of *C. punicae* at different concentrations of carbandazim, Mancozeb, Captan and Bordeaux mixture on PDA.

CONCLUSION

The findings of this study showed that the concentrations of the fungicides studied, as well as their interactions, differed significantly. At low concentrations, systemic fungicides were found to be more effective than non-systemic fungicides. SAR inducers have the potential to reduce the use of toxic chemicals in agriculture by directly activating defense mechanisms, resulting in stronger elicitation of defenses and subsequent pathogen attack. SAR inducers have emerged as an alternative, non-conventional, non-biocidal, and eco-friendly approach for plant protection and thus for sustainable agriculture.

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Conflict of Interest. None.

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