Biological Forum – An International Journal **15(3): 553-563(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Pomegranate Cercospora Leaf Spot as an Emerging Problem, Tackle through Biological, Botanical and Fungicidal Approach, both *in-vitro* and Field Condition

Shilpa[1], Somasekhara Y.M.[2], Ravichandra[3]*, Mahesh M.[4] and Shalini M.[5]

[1]Department of Plant Pathology, College of Agriculture, UAS, GKVK, Bangalore (Karnataka), India.
[2]Professor Department of Plant Pathology, UAS, GKVK, Bangalore (Karnataka), India.
[3]Department of Plant Pathology, College of Agriculture, KSNUAHS, Shivamogga (Karnataka), India.
[4]Assistant Professor Department of Plant Pathology, UAS, GKVK, Bangalore (Karnataka), India.
[5]Assistant Professor (Horticulture), UAS, GKVK, Bangalore (Karnataka), India.

(Corresponding author: Ravichandra)*
(Received: 28 January 2023; Revised: 18 February 2023; Accepted: 24 February 2023; Published: 22 March 2023)
(Published by Research Trend)

ABSTRACT: Pomegranate plant affected by many diseases and pests which hinders the production of quality of fruits and reduces the yield. The diseases caused by *Cercospora* sp. in different crops are considered to be of minor importance, yet the changing climatic scenario resulted in erratic rainfall with the resume that the incidence and severity of Cercospora leaf and fruit spots is increasing every year. Recently, pomegranate plants were heavily infected with cercospora leaf spot disease with symptoms like brown to dark brown spot with faint halo symptoms on leaves, but in case of flowers and fruits initially circular spot later become irregular as they grow, due to this there is qualitative and quantitative loss observed (loss about 4-17 %: Anon., 2020). The disease was managed by use of latest fungicides, botanicals and bio-agents under lab condition. *In vitro* evaluation of systemic fungicides indicated that carbendazim (92.31%), in contact fungicides, captan (76.15%) and in case of combi-product fungicides, Carbendazim 12% + Mancozeb 63% WP found most effective against *Cercospora punicae.* total nine botanicals were tested against *C. punicae,* among those Simarouba leaf extract showed maximum per cent mycelial inhibition of *C. punicae.* Among different fungal bio-agents like, *Trichoderma viridae-3* (71.11%) whereas in bacterial bio-agents *Pseudomonas fluorescence* Dharwad (47.96%) isolate showed maximum mycelial growth inhibition of *Cercospora punicae* in lab conditions.

Keywords: Leaf spot, Cercospora, pomegranate, mycelial inhibition, fungicides, bio-agents, plant extract, maximum, growth.

INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the family *Punicaceae,* subfamily *Punicoideae*, having chromosome number 2n = 16 or 18. The plant is native to Iran and which is growing up to 5-10 m in height, bearing multiple spiny branches and is extremely long-lived. The productivity of pomegranate crop was declining now days because of occurrence many pest and diseases. Pomegranate started suffering from major diseases such as *Cercospora punicae, Xanthomonas axonopodis* pv *punicae, Alternaria alternata* and *Colletotrichum gloeosporioides* causes leaf and fruit spots. Similarly, fruit rots are caused by *Coniellagranati, Phomopsis aucubicola* and *Phytophthora* sp. Though the diseases caused by *Cercospora* sp. in different crops are considered to be of minor importance, yet the changing climatic scenario resulted in erratic rainfall which leading to gradual increase in the incidence and severity of cercospora leaf spots every year. In pomegranate, particularly in wild habitats, cercospora leaf spot appeared in epidemic form during August 2015, there by resulting in early leaf fall and forcing the growers to harvest "Daru" at premature stage (Sharma, 2018).

The Cercospora affected plant shows small, dark brown spots on leaves, flowers and fruits that are initially circular but eventually become irregular as they grow. On the leaves, the lesions are dark, reddish brown to almost black and show a faint halo. The spots on fruit resemble bacterial spots, but they are darker of various sizes without cracks and no stickiness, and the twigs dry out and die. More severe infection causes the leaves to turn yellow and fall prematurely. Spots on leaves and fruits leads to reduced yield and quality of the fruit.

Now it has been considered a major problem because of its occurrence and spreading nature results from heavy losses to pomegranate growers.

Hence, the management of leaf and fruit spot disease of pomegranate is of major concern to the growers, wherever pomegranate is cultivated. Major practices like the use of disease-free planting material, orchard sanitation, pruning of diseased branches and application of fungicides aids in checking the spread of the disease. Mostly the disease is managed by sprays of carbendazim, mancozeb, captan and saaf under field conditions, but the information on the use of the latest fungicides, bio-resources, bio-agents *etc*. is completely lacking for the management of this emerging important

disease. Considering the importance of the fruit crop and disease caused by biotic factors like pathogens which lead to significant yield losses in the state.

Hennings (1906) observed the leaf spot disease caused by *Cercospora punicae* on pomegranate as circular to irregular, amphigenous, grey centre with blackish brown margin.

Gaikwad (2000) evaluated different fungicides combinations for the control of leaf and fruit spot diseases of pomegranate and observed that application of *i.e.* carbendazim (0.1%) + mancozeb (0.2%) or benomyl (0.1%) + mancozeb (0.2%) were effective for controlling leaf and fruit spot.

Praveen *et al.* (2016) evaluated efficacy of different fungicides among those, propiconazole 0.1% and hexaconazole + captan 0.15% have significantly reduced radial growth (0.53 and 0.64 cm, respectively) of *C. canescens* over rest of the chemicals and showed highest per cent inhibition of radial growth over control (89.83% and 87.06%, respectively).

Ram *et al.* (2018) had used different systemic fungicides among those fungicides Roko sprayed plots showed lowest disease severity and higher yield and thousand grain weight followed by Bavistin.

Sharma (2018) recorded *Cercospora punicae* growth by using different contact fungicides among those maximum mean mycelial growth inhibition (91.17 per cent) was recorded in Bordeaux mixture at all the three concentrations (250, 500, and 1000ppm) tested, followed by 86.23 per cent inhibition in Captan.

Kumar (2017) suggested that foliar spray of *Pseudomonas fluorescens* @ 5% led to a significant reduction of *Cercospora* leaf spot disease incidence (20.1%) followed by *Trichoderma viride* @ 5% (22.19%), neem oil @ 5% (23.29%), garlic oil @ 4% (23.70) and onion oil @ 4% (24.4%) compared to untreated control (27.90% and 20.12%).

Vasava and Patel (2020) tested the seven fungal bio-agents against *C. malayensis.* They recorded out of seven antagonists, *T. viride* showed significantly maximum per cent growth inhibition (86.86 per cent) with the lowest fungal colony diameter of pathogen 10.33mm.

Poornima *et al.* (2011) studied field efficacy of botanical extracts, *viz.*, the plant extracts of *Allium sativum, Azadirachta indica, Chromoleana odoratum, Duranta repens, Lantana camera, Ocimum sanctum* against *Cercospora beticola*. The extracts of *Allium sativum* and *Azadirachta indica* at 20% were most effective in reducing the leaf spot disease of palak.

Sharma (2018) studied eight plant extracts among them, Maximum mycelial inhibition of (55.92%) was observed in neem extract (25%) and the least inhibition (5.37%) was recorded in bougainvillea against *Cercospora punicae.*

MATERIAL AND METHODS

A. In vitro evaluation of fungicides against *C. punicae*

The test fungal disc of five mm was taken from actively growing culture and was placed on centre of Petri plate. The control plate was maintained without any fungicides. Each treatment was replicated for three times. The efficacy of different fungicides was expressed as per cent inhibition of mycelial growth over control and calculated by using the formula suggested by Vincent (1947) as follows

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

Where I = Per cent inhibition of mycelial growth
C = Growth of mycelium in control
T = Growth of mycelium in treatment

B. In vitro evaluation of botanicals against *Cercospora punicae*

Twenty ml of medium was poured into sterilized Petri plates and then fungal disc of five mm was placed at the center of Petri plate and then such plates were incubated at 25 ± 1°C. The control plate was maintained on PDA medium without any plant extract. The radial growth of fungus was recorded in treatment plates when colony growth reached periphery in control plate. The per cent inhibition of mycelial growth of test fungus was calculated by using the formula suggested by Vincent (1947) as follow:

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

Where, I = Per cent inhibition of mycelial growth
C = Growth of mycelium in control
T = Growth of mycelium in treatment

C. In vitro evaluation of bio-agents against *Cercospora punicae*

The observation in treatment plates were recorded when fungal growth reaches periphery of Petri plate in the control plate. The inhibition zone between test organism and antagonistic microorganism was measured and compared with control. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

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

Where, I = Per cent inhibition of mycelial growth
C = Growth of mycelium in control.
T = Growth of mycelium in treatment.

D. In vivo evaluation of fungicides against *C. punicae*

Effective fungicides from *in vitro* experiment was further evaluated under field conditions at pomegranate orchard in in the farmer's field located at Maralawadi and Hiriyur in order to manage the cercospora leaf spot of pomegranate. The experiment included six fungicides along with check with three replications.

Details of experiment

Details	Orchard 1	Orchard 2
Location	Maralawadi village	Hiriyur
Treatment	7	7
Replication	3	3
Number of plants /replication	3	3
Year	2020-2021	2020-2021

Observation on per cent disease incidence was recorded at before spray and 10th, 20th and 30th days after application of fungicides and the data was analysed statistically.

E. Statistical analysis

The data obtained in the present investigation for various parameters were subjected to ANOVA for a completely randomized design for *in vitro* studies and randomized complete block design for *in vivo* studies.

Disease incidence (%) = Number of leaves/fruits infected /total number of leaves/fruits observed × 100

Disease severity (%) = Σ disease ratings/{Total number of ratings × Maximum disease grade} × 100

Observations:

— Disease incidence on leaves/fruits

— Disease severity on leaves/fruits

RESULTS

A. In vitro evaluation of systemic fungicides against C. punicae

Six systemic fungicides were tested against *C. punicae* at four different concentrations 100, 250, 500 and 1000 ppm respectively. The per cent mycelial inhibition of *C. punicae* was calculated and presented in Table 1 and Fig. 1.

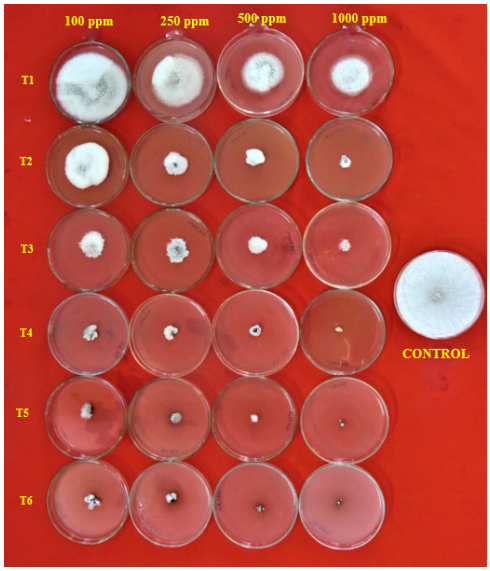
The results presented in table revealed that, statistically differences between systemic fungicides in per cent mycelial inhibition. Carbendazim was significantly most effective and statistically superior over all fungicides which inhibited 92.31 per cent followed by thiophanate methyl which inhibited 90.32 per cent when compared to propiconazole (87.68%) difenconazole (66.29%) and hexaconazole (51.57%). The least inhibition was recorded in dimethomorph (26.99%). The result has been compared with

El-Housni *et al*. (2020) have been used different chemicals extensively in the management of Cercospora leaf spot. Among the treatment thiophanate methyl molecule showed resistance to manage the disease at different degrees in sugarbeet.

Table 1: *In vitro* evaluation of systemic fungicides against *Cercospora punicae*.

Sr. No.	Systemic fungicides	Percent inhibition over control				
		Concentration				
		100ppm	250ppm	500ppm	1000ppm	Mean
1.	Dimethomorph	0.00 (0.00)	24.25 (29.50)	28.14 (32.04)	55.55 (48.18)	26.99 (27.43)
2.	Hexaconazole	23.70 (29.13)	44.44 (41.81)	50.74 (45.42)	87.40 (69.21)	51.57 (46.39)
3.	Difenoconazole	40.74 (39.66)	65.92 (54.28)	71.11 (57.48)	87.40 (69.21)	66.29 (55.16)
4.	Propiconazole	76.29 (60.86)	82.22 (65.06)	92.22 (73.80)	100.00 (90.00)	87.68 (72.43)
5.	Thiophanate methyl	77.96 (62.00)	88.88 (70.52)	94.44 (76.36)	100.00 (90.00)	90.32 (74.72)
6.	Carbendazim	85.18 (67.36)	85.92 (67.96)	98.14 (82.17)	100.00 (90.00)	92.31 (76.87)
Mean		50.64 (43.17)	65.27 (54.86)	72.46 (61.21)	88.39 (76.10)	69.19 (58.83)
		Fungicides (F)	Concentration(C)	Interaction (F×C)		
SEm ±		0.54	0.44	1.09		
CD @ 1%		1.55	1.27	3.11		

*Figures in parenthesis are arc sine transformed values.



T1. Dimethomorph; T2. Hexaconazole; T3. Difenconazole; T4. Propiconazole; T5. Thiophanate methyl; T6. Carbendazim

Fig. 1. *In vitro* evaluation of systemic fungicides against *C. punicae*.

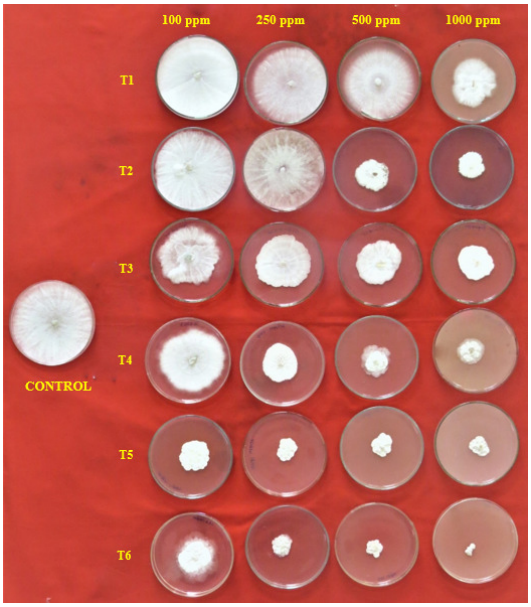
B. In vitro evaluation of non-systemic fungicides against Cercospora punicae

Among non-systemic fungicides, captan was found most effective and statistically superior over the all tested fungicides which inhibited the mycelial growth up to 76.15% which is followed by chlorothalonil (72.63%), zineb (51.29%), propineb (36.99%) and COC (35.55%). whereas least mycelial inhibition was recorded in mancozeb (11.66%) (Table 2 and Fig. 2).

Table 2: Evaluation of non-systemic fungicides *Cercospora punicae.*

Sr. No.	Non-systemic fungicides	Percent inhibition over control				
		Concentration				
		100ppm	250ppm	500ppm	1000ppm	Mean
1.	Mancozeb	0.00 (0.00)	2.59 (9.26)	5.55 (13.63)	38.52 (38.36)	11.66 (15.31)
2.	Copper oxychloride	1.48 (6.98)	2.96 (9.90)	64.44 (53.39)	73.33 (58.90)	35.55 (32.29)
3.	Propineb	18.89 (25.76)	32.22 (34.58)	41.66 (40.20)	55.18 (47.97)	36.99 (37.13)
4.	Zineb	17.04 (24.38)	54.44 (47.54)	60.00 (50.76)	73.70 (59.14)	51.29 (45.46)
5.	Chlorothalonil	60.00 (50.76)	72.40 (58.31)	76.67 (61.11)	81.48 (64.51)	72.63 (58.67)
6.	Captan	44.44 (41.80)	77.78 (61.87)	86.11 (68.12)	96.30 (78.90)	76.15 (62.67)
Mean		23.64 (37.42)	40.40 (55.37)	55.74 (71.81)	69.75 (86.95)	47.38 (62.89)
		Fungicides(F)		**Concentration(C)**		**Interaction(F×C)**
SEm ±		0.25		0.20		0.50
CD @ 1%		0.71		0.58		1.43

*Figures in parenthesis are arc sine transformed values.



T1. Mancozeb; T2. Copper oxy chloride; T3. Propineb; T4. Zineb; T5. Chlorothalonil; T6. Captan

Fig. 2. *In vitro* evaluation of non-systemic fungicides against *Cercospora punicae*.

C. In vitro evaluation of combi products against Cercospora punicae

Among combi-product fungicides, Carbendazim 12% + Mancozeb 63% WP was found the most effective and statistically superior over among all the tested fungi which inhibited mycelial growth of 99.16% which is followed by Tricyclazole 45%WG + Hexaconazole 10% (70.00%), Hexaconazole 5%WP + Captan 70% (48.05%) which is on par with Tricyclazole 18% + Mancozeb 62% WP (47.96%) and Metalaxyl + Mancozeb (38.88%) and Cymoxanil 22.1% + Famoxadone 16.6% SC (36.48%), whereas least inhibition of mycelial growth was recorded in Iprovalicarb 5.5% + Propineb 61.25% (24.07%) (Table 3 and Fig. 3).

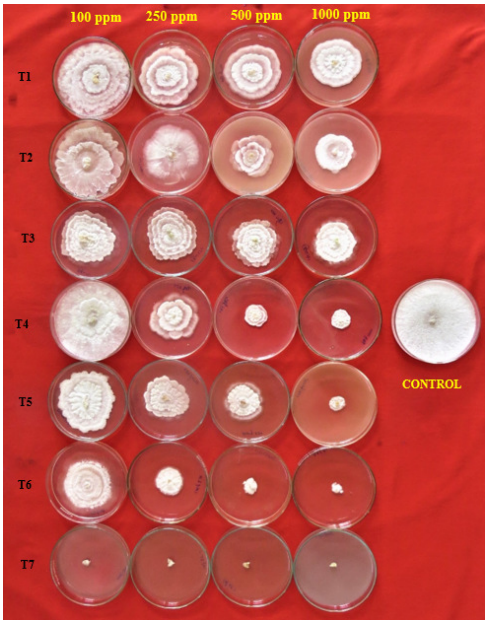
The result was compared with.

Salam *et al.* (2022) evaluated of fungicides against *Cercospora capsiciin vitro* condition. Observed that 100% inhibition of mycelial growth was found in four fungicides *viz*, Carbendazim 0.1% conc., tebuconazole at 0.2% conc., (Carbendazim 12% WP + Mancozeb 63% WP) at 0.2% conc. and mancozeb at 0.1% conc. & the least inhibition was observed in Captan at 0.2% conc. (inhibit 85.22%).

Table 3: Evaluation of combi product fungicides against *Cercospora punicae.*

Sr. No.	Combi products	Percent inhibition over control				
		Concentration				
		100ppm	250ppm	500ppm	1000ppm	Mean
1.	Iprovalicarb 5.5% + Propineb 61.25%	3.70 (11.09)	23.33 (28.88)	28.88 (32.51)	40.37 (39.44)	24.07 (27.98)
2.	Metalaxyl 8%WP + Mancozeb 64%	10.00 (18.43)	32.22 (34.58)	50.37 (45.21)	62.96 (52.51)	38.88 (37.68)
3.	Cymoxanil 22.1% + Famoxadone 16.6% SC	17.40 (24.65)	36.66 (37.26)	39.63 (39.01)	52.22 (46.27)	36.48 (36.80)
4.	Hexaconazole 5% WP + Captan 70%	2.59 (9.26)	37.03 (37.48)	73.70 (59.14)	78.88 (62.64)	48.05 (42.13)
5.	Tricyclazole 18% + Mancozeb 62% WP	19.63 (26.29)	40.74 (39.66)	48.88 (44.36)	82.59 (65.34)	47.96 (43.91)
6.	Tricyclazole 45% WG + Hexaconazole 10%	38.14 (38.14)	66.29 (54.51)	84.81 (67.06)	90.74 (72.28)	70.00 (58.00)
7.	Mancozeb 63%WP + Carbendazim 12%	97.77 (81.42)	98.88 (83.94)	100.00 (90.00)	100.00 (90.00)	99.16 (86.34)
Mean		27.03 (29.90)	47.88 (45.19)	60.89 (53.90)	72.54 (61.21)	52.09 (47.55)
		Fungicides(F)	**Concentration(C)**	**Interaction (F×C)**		
SEm ±		0.30	0.30	0.61		
CD @ 1%		0.87	0.65	1.74		

*Figures in parenthesis are arc sine transformed values.



T1. Iprovalicar b5.5% + Propineb 61.25%WP; T2. Metalaxyl 8%WP + Mancozeb 64%
T3. Cymoxanil 22.1% +Famoxadone 16.6% SCT; 4. Hexaconazole 5% WP + Captan 70%
T5. Tricyclazole 18% + Mancozeb 62%WP; T6. Tricyclazole 45% WG+Hexaconazole10%
T7. Carbendazim 12% + Mancozeb 63% WP

Fig. 3. *In vitro* evaluation of combi-product fungicides against *C. punicae.*

D. In vitro evaluation of botanicals against Cercospora punicae

The study was carried out to know the antifungal activity nature of different plant extracts against *C. punicae.* The effectiveness of different plant extracts in reducing the mycelial growth of *C. punicae* is varied greatly. The results thus obtained are presented here under (Table 4 and Fig. 4).

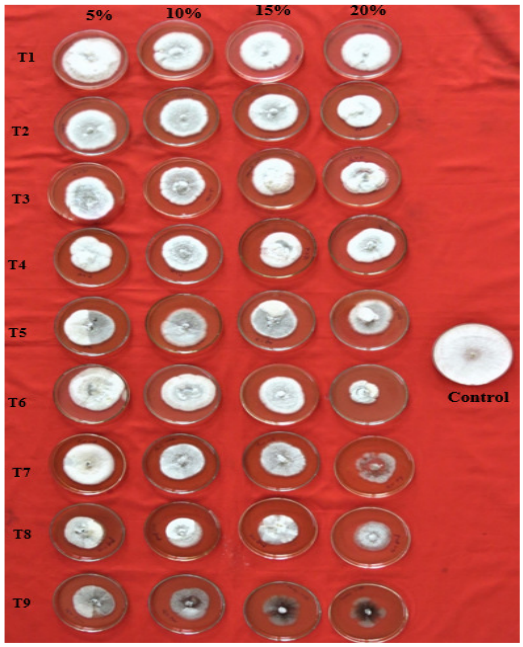
The results presented in Table 7 revealed that, statistical difference between plant extracts when per cent inhibition of four different concentrations at three replication concerned. Simarouba (52.54%) was found to be most effective and statistically on par with pongamia (51.71%), followed by subabul (44.16%), tulsi (41.89%) neem (38.84 %), garlic (36.29%), lemon grass (36.29%) and ginger (28.88%). There was least inhibition of mycelial growth in onion (26.25%). The results have been compared with reference by Kumar *et al.* (2022) tested eight botanicals at three concentrations (5, 10 and 15%) under *in vitro* condition against *C. canescens*, neem seed kernel extract was found most effective and inhibiting mycelial growth (58.00%) at 15% concentration followed by tulsi leaf extract (52.96%) and neem leaf extract (44.68%) also found effective.

Total nine plant extracts tested in that the highest mean inhibition was found in simarouba (52.54%) and lowest mean inhibition was onion (26.25%).

Table 4: Evaluation of botanicals against *Cercospora punicae.*

Sr. No.	Botanicals	Percent inhibition over control				
		Concentration (%)				
		5 %	10%	15%	20%	Mean
1.	Onion	8.89 (17.34)	23.88 (29.25)	28.15 (32.04)	44.07 (41.59)	26.25 (30.06)
2.	Ginger	21.11 (27.35)	27.41 (31.57)	27.77 (31.80)	39.25 (38.79)	28.88 (32.38)
3.	Lemongrass	22.96 (28.63)	30.92 (33.78)	42.40 (40.63)	48.89 (44.36)	36.29 (36.85)
4.	Tulsi	37.22 (37.59)	40.18 (39.33)	44.81 (42.02)	45.37 (42.34)	41.89 (40.32)
5.	Garlic	29.81 (33.09)	32.22 (34.58)	36.11 (36.93)	47.04 (43.30)	36.29 (36.98)
6.	Neem	20.37 (26.82)	36.11 (36.93)	39.26 (38.79)	59.630 (50.55)	38.84 (38.27)
7.	Subabul	34.07 (35.71)	41.66 (40.20)	46.30 (42.87)	54.63 (47.65)	44.16 (41.61)
8.	Pongamia	46.29 (42.87)	50.18 (45.10)	52.59 (46.48)	57.78 (49.47)	51.71 (45.98)
9.	Simarouba	39.44 (38.90)	49.26 (44.57)	55.18 (47.97)	66.30 (54.51)	52.54 (46.49)
mean		28.90 (32.03)	36.87 (37.26)	41.39 (39.95)	51.44 (45.84)	39.65 (38.77)
		Botanicals(B)	**Concentration(C)**		**Interaction(B×C)**	
SEm ±		0.26	0.17		0.52	
CD @ 1%		0.74	0.49		1.48	

*Figures in parenthesis are arc sine transformed values.



T1. Onion ; T2. Garlic ; T3. Lemongrass; T4. Tulsi; T5. Ginger; T6. Neem; T7. Subabul; T8. Pongamia; T9. Simaroba

Fig. 4. *In vitro* evaluation of botanicals against *C. punicae.*

E. In vitro evaluation of bio-agents against Cercospora punicae

(a) *In vitro* evaluation of fungal bio-agents against *C. punicae.* The fungal antagonistic microorganisms were evaluated against *C. punicae* by dual culture technique to know their antagonistic effect.

The per cent inhibition of mycelial growth of fungus was calculated and results are noted in Table 5 and Fig. 5a.

Table 5: *In vitro* evaluation of fungal bio-agents against *Cercospora punicae.*

Sr. No.	Fungal bio-agents	Per cent mycelial inhibition over control*
1.	*Trichoderma viride*-3(Tv-3)	71.11 (57.49)
2.	*T. harzianum*-56 (Th-56)	67.04 (54.96)
3.	*T. harzianum*-41 (Th-41)	65.93 (54.29)
4.	*T. viride*- 1 (Tv-1)	65.19 (53.84)
5.	*T. harzianum*-14 (Th-14)	61.48 (51.64)
6.	*T. harzianum*-55 (Th-55)	59.26 (50.33)
7.	*T. viride*-2(Tv-2)	54.44 (47.55)
SEm±		**0.45**
CD @ 1%		**1.38**

*Figures in parenthesis are arc sine transformed values.

Fig. 5a. *In vitro* evaluation of fungal bio-agents against *C. punicae.*

Statistically difference among the bio-agents (Tv-1, Tv-2, Tv-3, Th-56, Th-14, Th-55, Th-41) evaluated with respect to per cent inhibition of mycelia of *C. punicae* the highest 71.11 per cent inhibition of mycelial growth was observed in *T. viride*-3 (Tv-3) found to be statistically superior when compared to other treatments which was followed by Th-56 (67.04%), Th-41 (65.93%) which was on par with the Tv-1 (65.19%), followed by Th-14 (61.48%), Th-55 (59.26%), and Tv-2 (54.44%).

The maximum inhibition was found in Tv-3 (71.11%) whereas minimum inhibition noticed in Tv-2 (54.44%).

Same type of work was done by Salam *et al.* (2022) used the best biocontrol agent against the *Cercospora capsica* and he was found to be *Trichoderma harzianum* with 80.66% inhibition and the least effect against the test fungus was found in *Trichoderma viride* with 75.52% inhibition.

(i) *In vitro* evaluation of bacterial bio-agents against *Cercospora punicae.* *In vitro* evaluation of bacterial bio-agents *viz., Bacillus subtilis, Pseudomonas fluorescence*, *Bacillus megatherium* isolates from different places Table 6 and Fig. 5b.

Table 6: *In vitro* evaluation of bacterial bio-agents against *Cercospora punica.*

Sr. No.	Bacterial bio-agents	Isolate	Per cent mycelial inhibition over control
1.	*Pseudomonas fluorescence*(*PfD*)	CoA, Dharwad	47.96 (43.83)
2.	*Pseudomonas fluorescence*(*PfD*)	CoS, Chintamani	38.70 (38.47)
3.	*Bacillus megatherium*(*Bm*)	GKVK, B'glore	36.38 (35.19)
4.	*Bacillus subtilis*(*Bs*)	GKVK, B'glore	33.67 (30.74)
5.	*Bacillus megatherium*(*BmD*)	CoA, Dharwad	32.27 (28.52)
6.	*Bacillus subtilis*(*BsD*)	CoA, Dharwad	22.91 (15.19)
7.	*Pseudomonas fluorescence*(*PfD*)	GKVK, B'glore	19.80 (11.48)
	SEm±	**0.43**	
	CD @ 1%	**1.32**	

*Figures in parenthesis are arc sine transformed values

Experiment was carried out by following dual plate culture method and results are presented in Table 6, Fig. 5a and b. There was statistically difference among the bacterial bio-agents evaluated with regarding to mycelial growth of *C. punicae*. Among bio-agents evaluated, *Pseudomonas fluorescence* Dharwad isolate (47.96%) was found very effective in inhibiting the mycelial growth of *C. punicae* and this was statistically superior over all other treatments.

Than which was followed by *Pseudomonas fluorescence* Chintamani isolate (38.70%), *Bacillus megatherium* Gkvk (36.38%), *Bacillus subtilis* Gkvk (33.67%), *Bacillus megatherium* Dharwad (32.27%), *Bacillus subtilis* Dharwad (22.91%) and *Pseudomonas fluorescence* GKVK (19.80%). The results were compared by

In chilli, the disease index was significantly reduced in combining the *Trichoderma harzianum* + *Pseudomonas fluorescens* treated seeds, followed by the *T. harzianum* treatment compared to the control (hydroprimed) against cercospora leaf spot of chilli. Seed Bio-priming Mediated Control of Cercospora Leaf Spot and Bacterial Wilt Disease Resistance in Chilli (*Capsicum annuum* L.) under New Alluvial Zone).

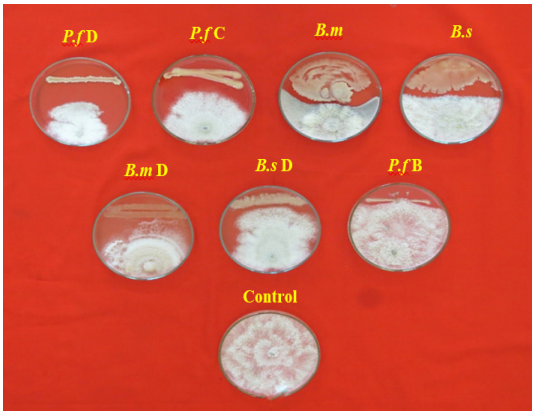


Fig. 5b. *In vitro* evaluation of bacterial bio-agents against *C. punicae.*

The maximum mycelial inhibition was recorded in *Pseudomonas fluorescence* Dharwad isolate (47.96%). The minimum inhibition was recorded in *Pseudomonas fluorescence* GKVK (19.80%).

6. *In vivo* evaluation of fungicides for the management of *Cercospora punicae*

The study was carried out in field conditions at Maralawadi and Hiriyur to know the occurrence of leaf spot and per cent infected area along with its management was done by application of seven different fungicides in 10 days' interval (Table 7a&b, Fig. 6a &b).

Table 7a: Occurrence of leaf spot of *Cercospora punicae* in field condition at Maralawadi during 2020-21.

			Number of leaf spots/ plant				
Sr. No.	**Fungicides**	**Concentrations (%)**	**Before spray**	**'10' days****	**'20' days****	**'30' days****	**Mean**
1.	Mancozeb	0.2	42.33 (40.57)	38.33 (38.23)	30.33 (33.40)	25.33 (30.26)	34.08
2.	Captan	0.2	33.00 (35.04)	31.00 (33.81)	25.33 (30.19)	20.67 (27.02)	27.50
3.	Carbendazim	0.1	27.66 (31.72)	24.67 (29.76)	18.33 (25.34)	14.33 (22.23)	21.24
4.	Azoxystrobin	0.1	24.66 (29.76)	21.67 (27.72)	13.00 (21.09)	9.00 (17.43)	17.08
5.	Difenoconazole	0.1	28.00 (31.93)	25.00 (29.98)	22.00 (27.95)	16.33 (23.81)	22.83
6.	Propineb	0.3	33.00 (35.04)	30.00 (33.19)	22.67 (28.41)	18.67 (25.58)	26.08
7.	control		47.66 (43.64)	50.00 (44.98)	52.00 (46.12)	51.33 (45.74)	50.24
Mean			33.76	31.52	26.24	22.24	28.44
SEm			1.08				
CD @ 5%			3.24				
CV			6.81				

*Figures in parenthesis are arc sine transformed values; ** After the 1st spray

Table 7b: Severity of cercospora leaf spot under field condition at Maralawadi during 2020-21.

			Per cent area affected				
Sr. No.	**fungicides**	**Concentrations(%)**	**Before spraying**	**'10' days****	**'20' days****	**'30' days****	**Mean**
1.	Mancozeb	0.2	34.84 (36.15)	22.29 (28.16)	12.00 (20.26)	6.00 (14.17)	18.78
2.	Captan	0.2	33.87 (35.57)	15.44 (23.12)	10.50 (18.89)	9.65 (18.09)	17.36
3.	Carbendazim	0.1	32.50 (34.74)	21.65 (27.72)	10.25 (18.66)	8.33 (16.77)	18.18
4.	Azoxystrobin	0.1	32.44 (34.70)	14.14 (22.07)	8.54 (16.98)	8.33 (16.77)	15.86
5.	Difenoconazole	0.1	42.51 (40.67)	23.72 (29.13)	16.20 (23.72)	16.00 (23.57)	24.60
6.	Propineb	0.3	31.56 (34.16)	24.45 (29.62)	21.29 (27.46)	18.14 (25.19)	23.86
7.	control		29.26 (32.73)	39.08 (38.67)	48.03 (43.85)	48.83 (44.19)	41.25
Mean			33.85	22.97	18.11	16.46	22.84
SEm			2.69				
CD @ 5%			8.07				
CV			19.45				

*Figures in parenthesis are arc sine transformed values; ** After the 1st spray

Table 8a: Occurrence of leaf spot of *Cercospora punicae* in field condition at Hiriyur during 2020-21.

Sr. No.	fungicides	Concentrations (%)	No. of leaf spot/ leaf/plant				
			Before spraying	'10' days**	'20' days**	'30' days**	Mean
1.	Mancozeb	0.2	25.33 (30.20)	22.00 (27.95)	21.00 (27.26)	16.67 (24.08)	21.25
2.	Captan	0.2	16.67 (24.07)	16.00 (23.56)	14.33 (22.23)	12.33 (20.54)	14.83
3.	Carbendazim	0.1	17.67 (24.84)	16.33 (23.81)	15.67 (23.30)	11.33 (19.66)	15.25
4.	Azoxystrobin	0.1	19.00 (25.82)	15.33 (23.04)	14.00 (21.95)	3.33 (10.49)	12.91
5.	Difenoconazole	0.1	26.00 (30.64)	25.00 (29.98)	24.00 (29.31)	19.00 (25.82)	23.50
6.	Propineb	0.3	24.00 (29.31)	19.33 (26.07)	17.33 (24.58)	14.67 (22.50)	18.83
7.	Control		28.30 (32.14)	33.33 (35.25)	34.67 (36.05)	38.00 (38.04)	33.57
mean			22.42	21.04	20.14	16.47	20.01
SEm			1.35				
CD @ 5%			4.05				
CV			10.35				

*Figures in parenthesis are arc sine transformed values; ** After the 1st spray

Table 8b: Severity of cercospora leaf spot under field condition at Hiriyur 2020-21.

Sr. No.	Fungicides	Concentrations (%)	Per cent Affected area				
			Before spraying	'10' days**	'20' days**	'30' days**	Mean
1.	Mancozeb	0.2	27.56 (31.65)	24.58 (29.71)	21.85 (27.85)	13.99 (21.95)	21.99
2.	Captan	0.2	28.64 (32.34)	22.02 (27.97)	16.57 (24.01)	13.08 (21.19)	20.07
3.	Carbendazim	0.1	29.40 (32.82)	25.44 (30.28)	17.71 (24.87)	11.10 (19.45)	20.91
4.	Azoxystrobin	0.1	26.96 (31.26)	29.23 (32.71)	12.79 (20.94)	8.00 (16.42)	19.24
5.	Difenconazole	0.1	28.41 (32.19)	24.68 (29.77)	23.65 (29.08)	22.00 (27.96)	24.68
6.	Propineb	0.3	26.58 (31.02)	25.02 (30.00)	18.12 (25.18)	15.97 (23.54)	21.42
7.	Control		27.81 (31.81)	32.45 (34.71)	40.03 (39.23)	42.18 (40.48)	35.61
Mean			27.91	26.20	21.53	18.04	23.42
SEm			1.95				
CD @ 5%			5.85				
CV			13.67				

*Figures in parenthesis are arc sine transformed values; ** After the 1st spray

Fig 6a: General view of cercospora leaf spot infected plant before spraying at Maralawadi 2020-21.

Fig. 6b. General view of cercospora leaf spot infected plant after spraying of azoxystrobin at 0.1% at Maralawadi 2020-21.

The fungicide trail was taken in field condition at Maralawadi at recommended concentrations during sever infection of cercospora leaf spot. The fungicides were sprayed with 10 day's interval, the observation on no. of leaf spot per leaf and per cent leaf area affected was recorded. The fungicide, azoxystrobin reduced no. of leaf spot from 24.66 to 9.00 (Table 7a) with per cent leaf area coverage was reduced from 32.44 to 8.33 per cent (Table 7b). Next best fungicide was carbendazim so in this treatment no. of leaf spot reduced from 27.66 to 14.33 (Table 7a) with per cent leaf area affected was reduced to 32.50 to 8.33 percent (Table 7a). in unsprayed plant no. of leaf spots increased from 28.30 to 38.00 with per cent leaf area coverage also increased from 29.26 to 48.03 percent. Based on these results fungicide azoxystrobin is effective against cercospora leaf spot of pomegranate so this fungicide can have exploited for the management of cercospora leaf spot disease.

Results were compared with reference of El-Housni *et al.* (2022) conducted *in vivo* tests in the Gharb area to monitor the severity of *Cercospora beticola* on the host under different fungicide treatments by measuring the area under the disease progress curve. The fungicide molecules all had better efficacy in preventive treatment, with the best results for trifloxystrobin, difenoconazole, and epoxiconazole.

Abdullah *et al.* (2022) conducted experiment against sugar beet cercospora leaf spot *in vivo* result show that the treated sugar beet plants with plant extracts before inoculated then by *Cercospora beticola* give the best result for reduction the disease severity of CLS disease comparing with the control.

The fungicide trail was taken in field condition at Hiriyur at recommended concentrations during sever infection of cercospora leaf spot. The fungicides were sprayed with 10 days' interval, the observation on number of leaf spot per leaf and per cent leaf area affected was recorded.

The fungicide, azoxystrobin reduced no. of leaf spot from 19 to 3.33 (Table 8a, Fig. 7a) with per cent leaf area coverage was reduced from 26.96 to 8.0 per cent (Table 8b). Next best fungicide was Captan so in this treatment no. of leaf spot reduced from 16.67 to 12.33 (Table 8a) with per cent leaf area affected was reduced to 28.64 to 13.08 percent (Table 8b) in unsprayed plant no. of leaf spots increased from 28.30 to 38.00 with per cent leaf area coverage also increased from 27.81 to 42.18. based on these results fungicide azoxystrobin is effective against cercospora leaf spot of pomegranate so this fungicide can have exploited for the management of cercospora leaf spot disease.

Fig. 7a. General view of cercospora leaf spot infected plant before spraying at Hiriyur 2020-21.

Fig. 7b. General view plant after spraying azoxystrobin at 0.1% at Hiriyur 2020-21.

DISCUSSION

Pomegranate is one of the commercially growing fruit crop in India, affected by many diseases caused by various pathogens, which affects yield and quality of the fruit. The research done with reference to management of disease.

Leaf spot pathogen was managed by testing different chemicals, botanicals and bio-agents against *Cercospora punicae.*

In vitro evaluation of different systemic fungicides carbendazim showed maximum mycelial inhibition of the *C. punicae.* similar results were recorded by Ram *et al.* (2018) had used different systemic fungicides among those fungicides Roko sprayed plots showed lowest disease severity and higher yield and thousand grain weight followed by Bavistin.

In vitro evaluation of different non-systemic fungicides Captan showed maximum mycelial inhibition of the *C. punicae.* The results were compared in accordance with Sharma (2018) recorded *Cercospora punicae* growth by using different contact fungicides among those maximum mean mycelial growth inhibition (91.17 per cent) was recorded in Bordeaux mixture at all the three concentrations (250, 500 and 1000ppm) tested, followed by 86.23 per cent inhibition in Captan.

In vitro evaluation of different combi product fungicides Carbendazim + mancozeb showed maximum mycelial inhibition of the *C. punicae.* The similar types of results were recorded by Hegde *et al.* (2013) were recorded the complete inhibition of mycelial growth of the *Cercospora canescens* by using combination of fungicide *i.e.* Carbendazim + Mancozeb @ 0.025%.

In vitro evaluation of nine different botanicals or plant extract among them, Simarouba leaf extract showed maximum mycelial inhibition of the *C. punicae* Similar observation was reported by Sharma (2018), that the eight plant extracts among them, maximum mycelial inhibition of (55.92%) was observed in neem extract (25%) and the least inhibition (5.37%) was recorded in bougainvillea against *Cercospora punicae.*

In vitro evaluation of different fungal bio-agents like, *Trichoderma* isolates were tested. Among them *T. viridae-3* was found most effective, whereas in bacterial bio-agents like, *Pseudomonas fluorescence, Bacillus megatherium, Bacillus subtilis* isolates were tested among them *Pseudomonas fluorescence* Dharwad isolate showed maximum mycelial growth inhibition. Similar types were recorded by Vasava and Patel (2020) tested the seven fungal bio-agents against *C. malayensis.* They recorded out of seven antagonists, *T. viride* showed significantly maximum per cent growth inhibition (86.86 per cent) with the lowest fungal colony diameter of pathogen 10.33mm, whereas in bacterial bio-agents 36.86 and 25.00 per cent growth inhibition was recorded in *Bacillus subtilis* and *Pseudomonas fluorescence*, respectively.

The cercospora leaf spot management practices were carried out under field conditions at Maralawadi and Hiriyur by spraying of different fungicides with ten day's intervals. Among the tested fungicides azoxystrobin 23% SC was found to be the most effective in reducing the leaf spots severity in both the locations (17.08% & 12.91%) and it also reduce severity of cercospora leaf spot disease in both the locations (15.86 to 19.24%) when compared to untreated (control) plant.

SUMMARY

The research conducted on Studies on leaf spot of pomegranate caused by *Cercospora punicae* Henn. was managed under *in vitro* conditions by best chemicals like carbendazim, captan and combination of carbendazim + mancozeb whereas in bio-agents *Trichoderma viridae*-3 (fungal) and *Pseudomonas fluorescence* Dharwad isolate (bacteria) found most effective. In case of botanicals Simarouba leaf extract showed maximum per cent of mycelial inhibition over the control. *In vivo* or in field condition we found one of the best chemical for managing the disease was azoxystrobin 23% SC fungicide which was reducing no. of spot on leaves and disease severity in both locations.

FUTURE SCOPE

—Screening of different germplasm for the management of *C. punicae*

—Development of integrated disease management (IDM) strategies for management of *C. punicae.*

REFERENCES

Abdullah, A. A., Nabi, H. M. A. E., Arnous, M. and Bayoumi, T. (2022). The Inhibitory Effect of Haloxylon, *Cichorium* spp and *Capparis* spp on Cercospora leaf spot disease on sugar beet *in vitro* and *in vivo.*

El-Housni, Z., Ezrari, S., Tahiri, A. and Ouijja, A. (2020). Resistance of *Cercospora beticola* Sacc isolates to thiophanate methyl (benzimidazole), demethylation inhibitors and quinone outside inhibitors in Morocco. *EPPO Bulletin*, *50*(2), 350-357.

El-Housni, Z., Tahiri, A. and Ezrari, S. (2022). Occurrence of *Cercospora beticola* Sacc populations resistant to benzimidazole, demethylation-inhibiting, and quinone outside inhibitors fungicides in Morocco. *Eur J Plant Pathol.*, *165*, 73–83.

Gaikwad, A. P. (2000). Synergy between carbendazim and mancozeb in controlling leaf and fruit spots of pomegranate. *J. Maharashtra Agric. Univ., 25*(2), 165-167.

Hegde, Y. R., Math, G. and Kumar, A. G. (2013). Bioefficacy of fungicides against *Cercospora canescens* causing leaf spot of greengram. *Crop Res.*, *46*(3), 74-78.

Hennings, P., (1906). Fungi paraenses II. I. D. J. Huber collecti. *Beiblatt zur Hedwigia*, *41***,** 15-18.

Kumar, N. (2017). Leaf Spot and Dry Fruit Rot of Pomegranate: Biology, Epidemiology and Management. *Int. J. Econ. Plants., 4*(1), 31-36.

Kumar, R., Meena, A. K., Kumar, V., & Singh, J. (2022). Isolation of *Cercospora canescens* and Management of Cercospora Leaf Spot (*Cercospora canescens*) of Mothbean through Botanicals. *Journal of Experimental Agriculture International*, *44*(5), 57-63.

Poornima, Y. R., Prashanthi, S. K. and Venugopal, C. K. (2011). Antifungal effect of botanicals against *Cercospora beticola*, the incitant of leaf spot of palak. Karnataka Journal of Agricultural Science, *24*(4), 575-576.

Praveen, B., Adinarayana, M., Krishna Prasadji, J. and Jayalalitha, K. (2016). *In vitro* evaluation of fungicides on radial growth of *Cercospora canescens.*

Ram, C., Ram, L. and Kushwaha, K. P. S. (2018). Efficacy of fungicides, biocontrol agents and botanicals on severity of cercospora leaf spot of pigeon pea. *Int. J. Curr. Microbiol. Appl. Sci.*, *7*(8), 1727-1732.

Salam, R., Kripalini, N., Singh, R. I., Supriya, L. and Bumpy, K. (2022). Studies on Leaf Spot of Chilli caused by *Cercospora capsici* and its management in Manipur.

Sharma, S. K. (2018). Studies on cercospora leaf and fruit spot of pomegranate (Doctoral dissertation, UHF, NAUNI), pp.1-78.

Vasava, K. I. and Patel, P. R. (2020). *In vitro* evaluation of various antagonists against *Cercospora malayensis* causing leaf spot of okra. *J. pharmacogn. Phytochem.*, *9*(5), 2649-2651.

Vincent, J. M. (1947). Distortion of fungal hyphae in presence of certain inhibitors. *Nature*, 159-850.

How to cite this article: Shilpa, Somasekhara Y.M., Ravichandra, Mahesh M. and Shalini M. (2023). Pomegranate Cercospora Leaf Spot as an Emerging Problem, Tackle through Biological, Botanical and Fungicidal Approach, both *in-vitro* and Field Condition. *Biological Forum – An International Journal, 15*(3): 553-563.