Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(4): 642-645
© 2020 JEZS
Received: 07-05-2020
Accepted: 09-06-2020

Mahesh M
Assistant Professor, Plant Pathology, College of Sericulture, Chintamani, UAS, Bengaluru, Karnataka, India

Venkataravana P
Dean (Sericulture) and Professor, Genetics and Plant Breeding, College of Sericulture, Chintamani, UAS, Bengaluru, Karnataka, India

Narasa Reddy G
Assistant Professor, Agricultural Entomology, College of Sericulture, Chintamani, UAS, Bengaluru, Karnataka, India

Devaraja
Assistant Professor, Plant Pathology, College of Sericulture, Chintamani, UAS, Bengaluru, Karnataka, India

Ramakrishna Naika
Professor, Sericulture, College of Sericulture, Chintamani, UAS, Bengaluru, Karnataka, India

Priyadarshini SK
Assistant Professor, Genetics and Plant Breeding, College of Sericulture, Chintamani, UAS, Bengaluru, Karnataka, India

Corresponding Author:
Mahesh M
Assistant Professor, Plant Pathology, College of Sericulture, Chintamani, UAS, Bengaluru, Karnataka, India

In vitro evaluation of different fungicides against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate

Mahesh M, Venkataravana P, Narasa Reddy G, Devaraja, Ramakrishna Naika and Priyadarshini SK

Abstract
Pomegranate is extensively cultivated around the Mediterranean and other parts of world including India. It is regarded as the "Fruit of Paradise". The most popular varieties in India are Ganesh, Mridula, Arakta, Bhagwa (Kesar). Successful cultivation of pomegranate in recent years has met with different problems such as pests and diseases. Among the various fungal diseases, anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. is one of the most serious disease of pomegranate, remaining latent in early stages of fruit development and reducing fruit quality to a greater extent. Propagules of pathogen cause lesions and decay of the fruit. Keeping in view the significance of the problem, research was conducted to screen the different chemicals under *in vitro* for the management of the disease. Among different systemic fungicides evaluated, Difenoconazole 25 EC has completely inhibited (100%) mycelial growth of the pathogen at all the three different concentrations tested. Among contact fungicides, Captan 50 WP was found effective in inhibition of mycelial growth to an extent of 89.26 (70.93) per cent followed by Chlorothalonil with mean mycelial inhibition of 86.17 (68.23) per cent. Similarly under combi fungicides evaluated, Sectin (Fenamidone 10 + mancozeb 50 WP) and SAAF were found effective in inhibiting mean mycelial growth of 91.23 (73.14) and 88.40 (73.63) per cent, respectively.

Keywords: Anthracnose, *Colletotrichum gloeosporioides*, pomegranate and fungicide

Introduction
Pomegranate (*Punica granatum* L.) is an important fruit crop popularly known as "Fruit of Paradise". It is originated in Iran and extensive Pomegranate farming in done in the Mediterranean countries like Spain, Morocco, Egypt, Iran, Afghanistan, and Baluchistan. It is cultivated to some extent in Myanmar, China, USA and India. It is regarded as a "vital cash crop", commercially grown to a limited extent in selected locations of many states of India *viz.*, Maharashtra, Karnataka, Gujarat, Andhra Pradesh, Madhya Pradesh, Tamil Nadu and Rajasthan. India ranks first in pomegranate cultivation in the world. Maharashtra is leading with an area of 90,000ha and annual production of 9.45 lakh MT and productivity of 10.5 Mt/ha. Maharashtra state accounts for 78 per cent of the total area in India and 84 per cent of the total production in the country (Amar Sawant, 2019) [1]. Constant increase in area, production and productivity of Pomegranate has been observed in India since last 7 years. Record pomegranate area of 2.62 lakh ha and production of 30.36 lakh MT have been projected as third estimate for 2018-19 on Ministry of Agriculture and Farmers Welfare, Govt of India website, though it was a drought year. A pomegranate export of 67.89 thousand MT (Value Rs 68.85 x108) is also a record figure till date showing 43.41 per cent increase over previous year (Anonymous, 2019) [2].

The crop is prone to many fungal, bacterial, viral and nematode diseases. Among them, anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* (Penz.) is one of the most destructive diseases which contribute for low productivity and large revenue losses. The symptoms on leaves observed as pinhead size of black to brown water soaked spots with circular margin. In advanced stage, these spots enlarged, coalesced and resulted in bigger patches. In severe cases the leaves dried up and dropped down. Brown spherical depressed spots occurred in scattered form on the pericarp of fruits. In advanced stage, these spots coalesced to form necrotic patches over the surface of the fruit (Jayalakshmi, 2013) [4]. In the absence of proper management options and control measures, there is danger that the fruit

growers may shift to some other crop. Considering the economic importance of the fruit crop as well as disease, present investigation was undertaken for *in vitro* evaluation of systemic, contact and combi- fungicides against *C. gloeosporioides* at department of Plant Pathology, College of Sericulture, Chintamani during 2020-21.

Material and Methods

Isolation of the pathogen from anthracnose infected fruit sample: Anthracnose infected fruit samples were collected from Moodachintalahalli village of Chintamani Taluk, Karnataka state and were brought immediately to the Department of Plant Pathology, College of Sericulture, and Chintamani (Plate-1). The pathogen *Colletotrichum gloeosporioides* (Penz.) was isolated by following standard tissue isolation technique as suggested by Jayalakshmi *et al.* (2015) [5]. The infected tissues along with healthy portions were cut into small bits and were surface sterilized with 0.1 per cent sodium hypochlorite solution for 30 seconds followed by dipping in ethyl alcohol and washed three times in sterile distilled water and transferred to potato dextrose agar (PDA). The plates were incubated at room temperature (28 ± 1^0C) and observed periodically for fungal growth. The colonies which developed from the tissue bits were transferred to PDA slants, subcultured and used for further studies.

Plate 1: Fruit rot of Pomegranate infected by *Colletotrichum gloeosporioides*

In vitro* evaluation of systemic, contact and combi fungicides against *Colletotrichum gloeosporioides: The bio-efficacy of five each of contact (Copper oxy chloride, Captan, Mancozeb, Chlorothalonil and Zineb), systemic (Azoxystrobin, Carbendazim, Hexaconazole, Thiophanate methyl and Difenconazole) and combi fungicides (SAAF, Matco, Sectin, Melody Duo and Curzate) were evaluated under *in vitro* conditions against *Colletotrichum gloeosporioides* for inhibition of radial growth on the PDA using poisoned food technique (Sharvelle, 1961) [6]. The systemic fungicides were evaluated at 500 ppm, 1000ppm and 1500 ppm concentration, whereas contact and combi fungicides were tried at 1000ppm, 2000ppm and 3000ppm concentrations. Similar type of evaluation method was carried out by Jagtap *et al.* (2015) [3].

The requisite quantities of fungicides were incorporated aseptically to PDA medium cooled to 45°C, so as to, give the required concentrations. Twenty milli litre of the poisoned medium was poured into sterilised Petri dishes. The plates were then inoculated by cutting half cm of seven days old mycelial discs of *Colletotrichum gloeosporioides* with a sterile Cork borer and incubated at 28 ± 1^0C. Three replications were maintained for each treatment. The fungus growth on the PDA without any fungicide was served as control. The radial growth (mm) of the colony was recorded when maximum growth (7 days) in control plates was noticed. The per cent inhibition of the mycelial growth of the fungus was determined by using the Vincent's formula (Vincent, 1947) [7]. The percent values were converted into angular transformations, the data were analysed statistically.

$$I = \frac{(C-T)}{C} X\ 100$$

Where,
I = Per cent inhibition;
C = Radial growth in control;
T = Radial growth in treatment (fungicide)

Results and Discussion

Isolation of the pathogen from Anthracnose infected plant sample: The collected diseased sample of Pomegranate fruit was observed under compound microscope to know the presence of pathogen in the infected plant part. After confirming, the presences of pathogen under microscope, diseased plant samples showing the typical anthracnose symptoms were subjected to isolation. *C. gloeosporioides* was isolated on PDA medium by tissue isolation method and it produced whitish ash coloured cottony fluppy mycelial growth (Plate-2A). The pathogen produce sickle shaped conidia with oil globules (Plate-2B). The isolated fungus was further purified by single spore isolation method and the purified culture was maintained on PDA slants for further studies. Similar type of isolation method was carried out by Jayalakshmi *et al*. (2013) [4] for Pomegranate anthracnose with the use of infected fruits along with some healthy portions.

Plate 2A: Culture on potato dextrose agar, B. Spores of *Colletotrichum*

In vitro* evaluation of systemic, contact and combi fungicides against *Colletotrichum gloeosporioides

Evaluation of fungicides *in vitro* is a handy tool to screen large number of fungicides at different concentrations. In the present study, the laboratory evaluation of fungicides by poison food technique revealed significant results for various fungicides evaluated with different concentrations. Among systemic fungicides evaluated at 500 ppm, 1000 ppm and 1500 ppm, complete inhibition (100%) of mycelial growth was observed in Difenconozole 25 EC with all concentrations followed by Hexaconazole 5 SC with mean mycelial growth inhibition of 95.74 per cent. These two fungicides were also exhibited significant difference over other chemicals screened. The next best chemical found was Carbendazim with mean per cent inhibition of 83.70 per cent. Thiophanate methyl 70 WP and Azoxystrobin 23 SC recorded least mean per cent inhibition of 71.11 and 69.63 per cent respectively (Table 1 and Plate 3). Similar results were obtained by Jayalakshmi *et al*., (2013) [4] and Jagtap *et al*,. (2015) [3] for Pomegranate anthracnose with the use of infected fruits.

Table 1: *In vitro* evaluation of systemic fungicides against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate.

Sl. No.	Fungicideds	Concentration / Per cent Inhibition			Mean
		500 ppm	1000 ppm	1500 ppm	
1	Azoxystrobin 23 SC	69.63 (56.67)	78.15 (62.14)	83.70 (66.22)	69.63 (56.67)
2	Carbendazim 50 WP	83.70 (66.39)	86.30 (68.28)	89.26 (70.87)	83.70 (66.39)
3	Hexaconazole 5 SC	95.74 (78.16)	96.67 (79.48)	100.00 (90.00)	95.74 (78.16)
4	Thiophanate methyl 70 WP	71.11 (57.52)	75.19 (60.12)	87.78 (69.62)	71.11 (57.52)
5	Difenconazole 25 EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
6	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean		84.04 (69.75)	87.26 (72.00)	92.15 (77.34)	87.81 (73.03)
				S. Em±	C.D @1%
Fungicide (A)				0.61	2.35
Concentration (B)				0.43	1.66
Fungicide x Concentration (A x B)				1.06	4.06

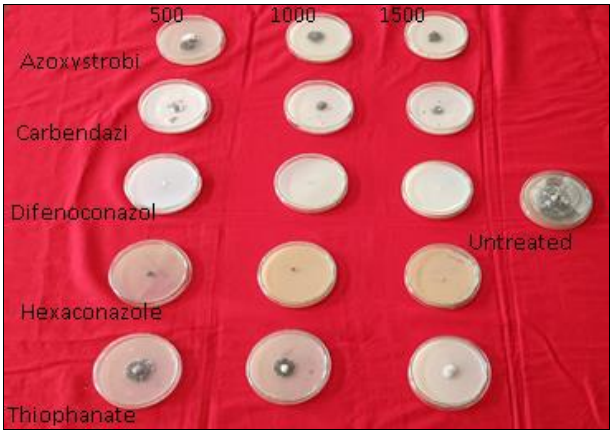

Plate 3: *In vitro* evaluation of systemic fungicides against fruit rots of Pomegranate caused by *Colletotrichum gloeosporioides*

Out of three different concentrations tested, 1500 ppm showed maximum mean per cent inhibition of mycelial growth of 92.15 (77.34) per cent and was found significantly superior over other two concentrations *viz*., 1000 ppm and 500 ppm which recorded mean per cent mycelial inhibition of 87.26 (72.00) and 84.04 (69.75) per cent respectively.

In the interactions of both fungicides and concentrations, Difenconazole 25 EC at all the three concentrations completely inhibited mycelial growth of the pathogen (100%) followed by Hexaconazole 5 SC which inhibited 100 per cent in 1500 ppm which is on par with 1000 ppm and 500 ppm which recorded mycelial inhibition of 96.67 (79.48) per cent and 95.74 (78.16) per cent, respectively. Least per cent inhibition was observed in Azoxystrobin 23 SC at 500 ppm (69.63%). Similar results were obtained by Jayalakshmi *et al*. (2013) [4] who stated that Propiconazole, Iprobenfos and Difenconazole were the most effective fungicides followed by Hexaconazole. However, Azoxystrobin and Carbendazim were the least effective at all concentrations to control Pomegranate fruit rot disease. Contradictorily, Jagtap *et al.* (2015) [3] observed that the least mycelial growth inhibition was observed in Hexaconazole (32.62%), nevertheless Propiconazole recorded maximum mean mycelial growth inhibition of 74.86 per cent.

Among the five contact fungicides evaluated, maximum mean per cent inhibition of 89.26 (70.93) per cent was observed in Captan 50 WP followed by Chlorothalonil 75 WP which inhibited mean mycelial inhibition of 86.17 (68.23) per cent (Table-2 and Plate-4). Zineb 78 WP, Mancozeb 75 WP and Copper Oxy Chloride 50 WP recorded significantly less mean mycelial growth per cent inhibition of 78.77 (62.68), 76.67 (61.34) and 76.17 (61.06) per cent respectively. Among the three concentrations evaluated, maximum mean per cent inhibition was observed in 3000 ppm which recorded 86.00 (68.20) per cent followed by 2000 ppm with mean per cent inhibition of 82.15 (65.19) per cent. Least per cent inhibition was observed in 1000 ppm concentration with mean per cent inhibition of 76.07 (61.15) per cent. Similar type of results were obtained by Jayalakshmi *et al.* (2013) [4], with maximum mycelial per cent inhibition observed in Captan with 73.88 (59.49) per cent and least per cent inhibition was recorded by Copper oxy chloride 50 WP with 1.55 (7.26) per cent only.

Table 2: *In vitro* evaluation of Contact fungicides against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate

Sl. No.	Fungicideds	Concentration / Per cent Inhibition			Mean
		1000 ppm	2000 ppm	3000 ppm	
1	Copper oxy chloride 50 WP	65.93 (54.30)	78.89 (62.65)	83.70 (66.22)	76.17 (61.06)
2	Captan 50 WP	87.78 (69.58)	88.89 (70.54)	91.11 (72.68)	89.26 (70.93)
3	Mancozeb 75 WP	67.78 (55.430	78.89 (62.65)	83.33 (65.94)	76.67 (61.34)
4	Chlorothalonil 75 WP	84.44 (66.8)	85.93 (67.98)	88.15 (69.90)	86.17 (68.23)
5	Zineb78 WP	74.44 (59.64)	78.15 (62.130	83.70 (66.26)	78.77 (62.68)
6	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean		76.07 (61.15)	82.15 (65.19)	86.00 (68.20)	81.41 (64.85)
				S. Em±	C.D @1%
Fungicide (A)				0.46	1.76
Concentration (B)				0.32	1.25
Fungicide x Concentration (A x B)				0.79	3.05

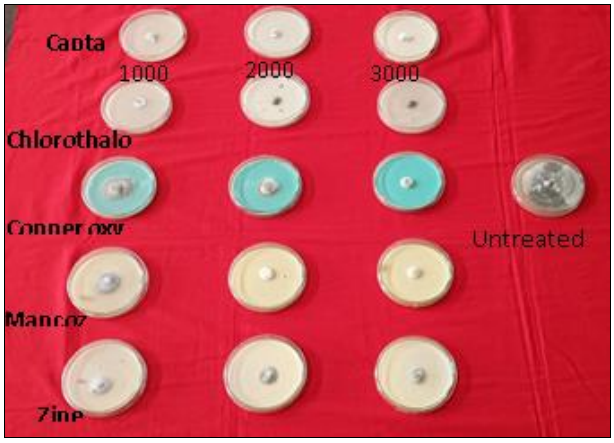

Plate 4: *In vitro* evaluation of contact fungicides against fruit rots of Pomegranate caused by *Colletotrichum gloeosporioides*

Among combi fungicides screened, SECTIN (Fenamidone 10 + Mancozeb 50 WG) and SAAF (Carbendazim 12 + Mancozeb 63 WP) inhibited maximum mean mycelial growth of 91.23 (73.14) and 88.40 (73.63) per cent respectively (Table 3 and plate 5) followed by CURZATE (Cymoxanil 8 + Mancozeb 63 WP) with 86.54% and MELODY Duo (Iprovalicarb 5.5 + Propineb 61.25 WP) with 86.17 per cent mycelial inhibition. Least per cent inhibition was observed in MATCO (Metalaxyl 8 + Mancozeb 64 WP) with 83.21 per cent. Among the three concentrations evaluated, concentration of 3000 ppm (93.41%) and 2000 ppm (86.52%) were found effective and the least inhibition of mycelial growth was observed in 1000 ppm (81.41%). Similarly, SAAF (Carbendazim + Mancozeb) recorded maximum mycelial growth inhibition of 81.88 (64.79) Jayalakshmi *et al.* (2013) [4].

Table 3: *In vitro* evaluation of combi fungicides against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate

Sl. No.	Fungicideds	Concentration / Per cent Inhibition			Mean
		1000 ppm	2000 ppm	3000 ppm	
1	SAAF (Carbendazim 12 + Mancozeb 63 WP)	79.26 (62.91)	85.93 (67.98)	100.00 (90.00)	88.40 (73.63)
2	MATCO (Metalaxyal 8 + Mancozeb 64 WP)	79.26 (62.91)	80.37 (63.71)	90.00 (71.58)	83.21 (66.07)
3	SECTIN (Fenamidone 10 + Mancozeb 50 WG)	87.41 (69.23)	90.74 (72.29)	95.56 (77.89)	91.23 (73.14)
4	MELODY DUO (Iprovalicarb 5.5 + Propineb 61.25 WP)	78.89 (62.65)	88.15 (69.88)	91.48 (73.04)	86.17 (68.52)
5	DuPont CURZATE M8 (Cymoxanil 8 + Mancozeb 63 WP)	82.22 (65.09)	87.41 (69.23)	90.00 (71.58)	86.54 (68.63)
6	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean		81.41 (64.56)	86.52 (68.62)	93.41 (76.82)	87.11 (70.00)
				S. Em±	C.D @1%
Fungicide (A)				0.32	1.24
Concentration (B)				0.23	0.88
Fungicide x Concentration (A x B)				0.56	2.15

Plate 5: *In vitro* evaluation of Combi fungicides against fruit rots of Pomegranate4 caused by *Colletotrichum gloeosporioides*

Conclusion

Screening of different chemicals under *in vitro* for the management of fruit rot of Pomegranate concluded that, among different systemic fungicides evaluated, Difenoconazole 25 EC has completely inhibited (100%) mycelial growth of the pathogen at all the three different concentrations tested. Among contact fungicides, Captan 50 WP was found effective in inhibition of mycelial growth to an extent of 89.26 (70.93) per cent followed by Chlorothalonil with mean mycelial inhibition of 86.17 (68.23) per cent. Similarly under combi fungicides evaluated, Sectin (Fenamidone 10 + mancozeb 50 WP) and SAAF were found effective in inhibiting mean mycelial growth of 91.23 (73.14) and 88.40 (73.63) per cent, respectively.

References

1. Amar Sawant, The Ultimate Guide for Pomegranate farming. www.Agriculture guruji.com/pomegranate farming, 2019.
2. Anonymous, Present scenario on cultivation of Pomegranate, Directors desk, ICAR-National Research Center on Pomegranate, Solapur, Maharashtra, 2019.
3. Jagtap NM, Ambadkar CV, Bhalerao GA. *In vitro* evaluation of different fungicides against *Colletotrichum gloeosporioides* causing anthracnose of Pomegranate. 2015; 11(2):273-276.
4. Jayalakshmi K, Naragund VB, Raju J, Benagi VI, Effect of fungicides and plant extracts on growth of *Colletotrichum gloeosporioides* (Penz.) causing anthracnose of Pomegranate. Bioinfolet. 2013; 10(2A):502-506.
5. Jayalakshmi K, Naragund VB, Raju J, Benagi VI, Raghu S, Madhu Giri S. Pomegranate anthracnose caused by *Colletotrichum gloeosporioides:* A menace in quality fruit production. Journal of Pure and Applied Microbiology. 2015; 9(4):3093-3097.
6. Sharvelle EG. The Nature and Use of Modern Fungicides. Burgess Publishing Co., Minnesota, USA, 1961, 308.
7. Vincent J M, Distortion of fungal hyphae in the presence of certain inhibitor. Nature. 1947; 159:800.