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Variation in fungi toxicant sensitivity of *Colletotrichum gloeosporioides* Penz. isolates infecting major fruit crops in Khandesh region of Maharashtra

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Abstract
Twelve *Colletotrichum gloeosporioides* isolates from four fruit hosts grown in different district in Khandesh region of Maharashtra (India) expressed significant variation in their sensitivity to fungicides. All isolates expressed highly sensitive reaction to azoxystrobin. Azoxystrobin (0.04%), thiophanate methyl (0.1%) and carbendazim 12 % + mancozeb 63 % WP (0.15%) fungicides were highly sensitive against all of the isolates followed by carbendazim (0.1%) and mancozeb (0.25%) was sensitive while, propineb (0.2%) was highly resistant against all isolates. Azoxystrobin fungicide inhibit growth of all isolates was 92.19%, thiophanate methyl fungicide inhibit growth 91.01% and carbendazim + mancozeb fungicide inhibit growth was 89.64 % of all isolates. In general, *C. gloeosporioides* isolates from pomegranate, sweet orange, banana and guava are tending towards propineb non sensitive it inhibit growth was 45.05%.

Keywords: *Colletotrichum gloeosporioides*, azoxystrobin, carbendazim, mancozeb, propineb

Introduction
Colletotrichum is one of the most important genera of plant pathogenic fungi worldwide especially, in tropical and subtropical region. It causes anthrac-nose, die back, whither tip, shot hole, leaf blight and post-harvest rots in many economically important crops. Tropical and sub-tropical fruit crops are the predominant hosts and are regularly damaged by the pathogen in one or the other stage of crop development. As these crops are perennial in nature, there is regular and constant availability of the host for development of the pathogen and its survival. In India the area under fruit crops is 64.8 lakh ha by the end of 2017-18. Maharashtra is the leading producer of horticultural fruit crop as compared to other state. In Maharashtra area under fruit crops is 7.63 lakh ha and production 10378.43 MT in 2017-18. There was increase in the disease severity of anthracnose of different fruit crops in the form of localized epidemics in various parts of Maharashtra (India). Among the fungal pathogens anthracnose or wither tip or twig blight is caused by *Colletotrichum gloeosporioides* which causes 30-40% fruit losses. The anthracnose affected fruits are not fit for consumption (Singh, 2000) [10]. *C. gloeosporioides* is a ubiquitous pathogen causing substantial yield losses due to fruit decay and damage to vegetative parts in a variety of plant species (Freeman and Shabi, 1996) [5]. Anthracnose is the most prevalent disease that contributes significantly to pre and post-harvest losses in cashew, pomegranate, guava, citrus and papaya. Fungicides form an integral part of disease management under intensive cultivation of fruit crops. Survey revealed that carbendazim (50 WP) is the most widely adopted fungicide for control of diseases caused by *C. gloeosporioides* in Maharashtra. Azoxystrobin, thiophanate methyl although is not yet commercially available for general use in India, it has emerged as a promising alternative against *C. gloeosporioides* worldwide. It was therefore, felt necessary to evaluate the variation in the sensitivity level of twelve *C. gloeosporioides* isolates to six fungicides namely: azoxystrobin, carbendazim, mancozeb, propineb, thiophanate methyl and carbendazim + mancozeb (SAAF) combination fungicides.

Material and Method
Isolates of *C. gloeosporioides* were obtained from different fruit hosts namely: pomegranate (*Punica granatum*), sweet orange (*Citrus sinensis*), banana (*Musa* spp.)

and guava (*Psidium guajava*) cultivated in three district of Khandesh region of Maharashtra (India). These isolates were accessed with Cg as a prefix which is the abbreviation of the scientific name of the *C. gloeosporioides* pathogen followed by the fruit name initial letter, district name initial letter and isolate number i.e. CgPD 1 (*Colletotrichum gloeosporioides* Pomegranate Dhule). The pathogenicity of all these isolates was confirmed in the laboratory on the same host on the respective plant part from where it was isolated. The pure colony obtained from mono conidial culture of each isolate was used for further study.

Fungicide name	Concentration
Azoxystrobin 23% SC	0.04 %
Carbendazim 50% WP	0.1 %
Mancozeb 75% WP	0.25 %
Propineb 70% WP	0.2 %
Thiophanate methyl 70%WP	0.1 %
Carbendazim 12% + mancozeb 63% WP	0.15 g/lit
Untreated control	-

The experiment was conducted to study the sensitivity of 12 isolates of *C. gloeosporioides* collected from three districts of Khandesh region in Maharashtra. Sensitivity of these isolates to carbendazim and mancozeb commonly used fungicides was tested by farmers. The pathogen was grown on Potato Dextrose Agar medium prior to the setting of the experiment. The fungicide suspension was made by adding required quantity of fungicides to the melted Potato Dextrose Agar medium to obtain the desired concentration on the basis of active ingredient present in the chemical. 20 ml of poisoned medium was poured into each sterilized petriplate and suitable checks were maintained without addition of fungicides. 5 mm of ten days old fungal disc was taken from the periphery of the culture and was placed in the centre of the poisoned medium aseptically and incubated at 28^0C for seven days. *In vitro* evaluation of different chemical fungicides against different isolates of *C. gloeosporioides* was carried out by poisoned food technique. Three replications were maintained for each treatment and the diameter of the colony was measured in 2 directions and the average was recorded after incubation for seven days. Per cent inhibition of the fungus was calculated by using the formula suggested by Vincent (1947) [11].

I = C - T / T X 100

Where as,
I = Per cent inhibition
T = Growth in treatment
C = Growth in control

Based on mean radial growth, the isolates were classified as highly sensitive, sensitive, moderately resistant, resistant and highly resistant to each fungicide as given below:

Class	Per cent inhibition over control
Highly sensitive	> 90
Sensitive	> 80 – 90
Moderately resistant	> 70– 80
Less sensitive	> 50 – 70
Non sensitive	< 50

To compare different numerical observations, the data was statistically analyzed by using the appropriate statistical methods (Panse and Sukhatme, 1978).

Result and Discussion

The data on mean sensitivity in terms of inhibition values of *C. gloeosporioides* isolates to six selected fungicides is presented in Table 1. Isolates differed significantly in their sensitivity to individual fungicide. At recommended concentrations, azoxystrobin (0.04%), thiophanate methyl (0.1%) carbendazim 12 % + mancozeb 63 % WP (0.15%) (SAAF) completely inhibited the mycelial growth of the fungus which is followed by carbendazim (0.1%) and mancozeb (0.25%). While, propineb (0.2%) recorded the least inhibition of the fungus growth.

Azoxystrobin (0.04%)

It was observed that azoxystrobin 0.04% was found to be highly effective fungicide against all isolates. Out of 12 isolates, 9 isolate were highly sensitive, 3 isolate were sensitive Table 2. Maximum per cent inhibition was (96.62%) observed in *Colletotrichum gloeosporioides* Sweet orange Nandurbar (CgSN 6) which was followed by CgGD 10, CgGN 12, CgGJ 11, CgSD 4, CgSJ 5, CgPJ 2, CgPN 3 and CgPD 1, these are highly sensitive isolates while, lowest (82.81%) per cent inhibition was observed in isolate *Colletotrichum gloeosporioides* Banana Nandurbar (CgBN 9).

Carbendazim (0.1%)

Out of 12 isolates, 5 isolates were highly sensitive, 6 isolates were sensitive and 1 isolate was moderately sensitive Table 2. Maximum per cent inhibition was (94.38%) observed in *Colletotrichum gloeosporioides* Sweet orange Nandurbar (CgSN 6) which was followed by CgGN 12, CgGJ 11, CgPN 3 and CgSJ 5, while, minimum (78.12%) inhibition was observed in *Colletotrichum gloeosporioides* Banana Nandurbar (CgBN 9) which is moderately sensitive.

Mancozeb (0.25%)

It was observed that out of 12 isolates, 3 isolate were highly sensitive, 5 isolates was sensitive, 3 isolates was moderately sensitive while 1 isolate was resistant Table 2. Maximum per cent inhibition was (96.62%) observed in *Colletotrichum gloeosporioides* Sweet orange Dhule (CgSD 4) and *Colletotrichum gloeosporioides* Sweet orange Nandurbar (CgSN 6) isolates followed by CgSJ 5 while, minimum per cent (69.12%) inhibition was observed in CgGD 10 which found resistant.

Propineb (0.2%)

It was observed that out of 12 isolates 5 isolates were less sensitive and 7 isolates were non sensititve Table 2. Maximum per cent inhibition was (61.36%) observed in *Colletotrichum gloeosporioides* Sweet orange Jalgaon (CgSJ 5) followed by CgSD 4 (59.55%), CgBD 7 (57.35%), CgSN 6 (53.93%) and CgGD 10 (50.61%), while, minimum per cent (22.22%) inhibition was observed in *Colletotrichum gloeosporioides* Pomegranate Dhule (CgPD 1) which was found highly resistant.

Thiophanate methyl (0.1%)

Out of 12 isolates, 7 isolates were highly sensitive and 5 isolates were sensitive Table 2. Maximum per cent inhibition was (97.53%) observed in *Colletotrichum gloeosporioides* Guava Dhule (CgGD 10) which was found at par with CgGN 12 and followed by CgBJ 8, CgPD 1, CgSD 4, CgSN 6 and CgGJ 11, while, minimum per cent (82.95%) inhibition was observed in *Colletotrichum gloeosporioides* Sweet orange Dhule (CgSD 4) which was found sensitive.

Carbendazim 12 % + mancozeb 63 % WP (0.15%)
Out of 12 isolates, 6 isolates were highly sensitive and 6 isolates were sensitive Table 2. Maximum per cent inhibition was (96.87%) observed in *Colletotrichum gloeosporioides* Banana Nandurbar (CgBN 9) followed by CgSD 4, CgSJ 5, CgBJ 8, CgSN 6 and CgGJ 11, while, minimum per cent (80.89%) inhibition was observed in *Colletotrichum gloeosporioides* Pomegranate Nandurbar (CgPN 3) which was sensitive. Saju *et al.* (2012) reported the *in vitro* tests showed that, the pathogen was highly sensitive combined formulation of carbendazim + mancozeb (12 + 63) WP (0.3 %).
Azoxystrobin (0.04%), thiophanate methyl (0.1%) and carbendazim 12 % + mancozeb 63 % WP (0.15%) fungicides were highly sensitive against all of the isolates followed by carbendazim (0.1%) and mancozeb (0.25%) was sensitive while, propineb (0.2%) was highly resistant against all isolates.
These results are confirmation with Ashoka (2005) [2], Devamma *et al*. (2012) [3], Filoda (2008) [4], Joshi *et al.* (2013) [7] and Gaikawad (2000).

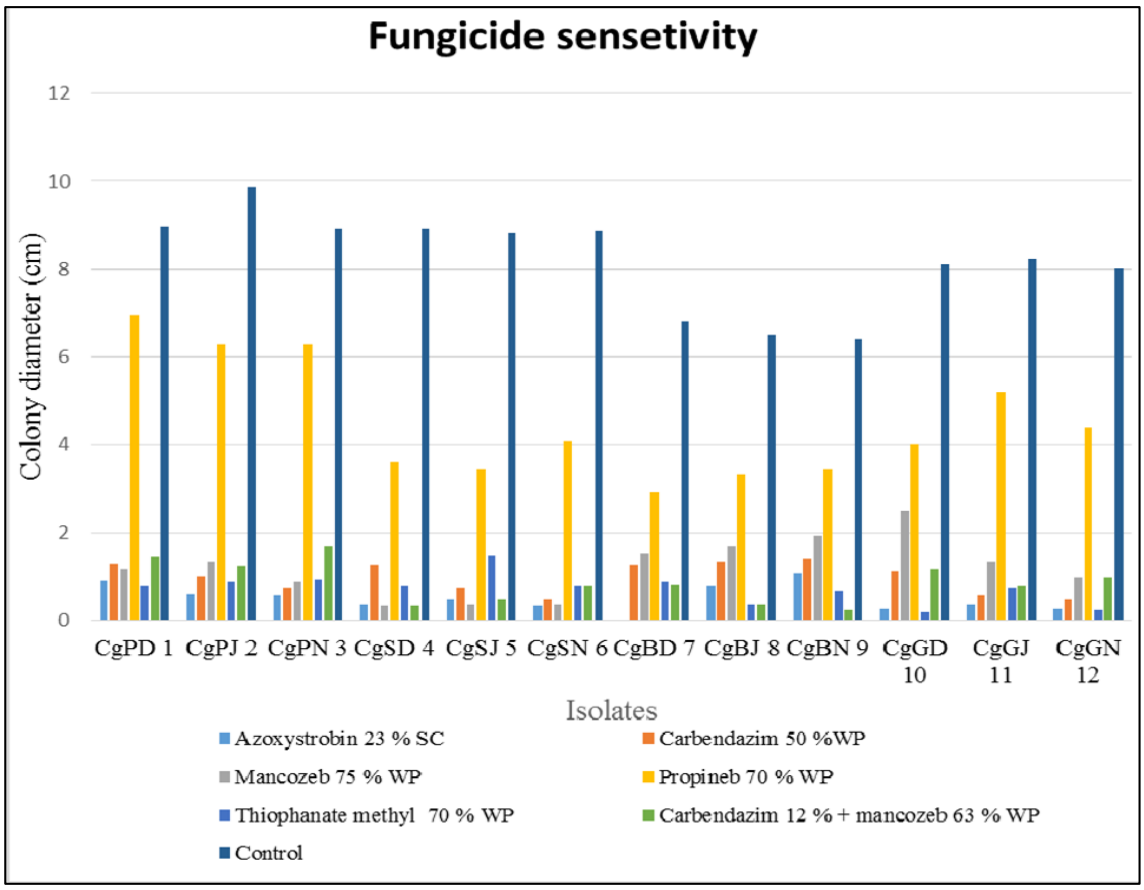

Fig 1: *In vitro* evaluation of fungicides against different isolates of *C. gloeosporioides*

Table 1: *In vitro* evaluation of fungicides against different isolates of *C. gloeosporioides*

Isolates	Azoxystrobin 23 % SC		Carbendazim 50 %WP		Mancozeb 75 % WP		Propineb 70 % WP		Thiophanate methyl 70 % WP		Carbendazim 12 % + mancozeb 63 % WP	
	Colony diameter (cm)	% inhi - bition	Colony diameter (cm)	% inhib -ition	Colony diameter (cm)	% inhi - bition	Colony diameter (cm)	% inhi - bition	Colony diameter (cm)	% inhib - ition	Colony diameter (cm)	% inhibition
CgPD 1	0.90	90.00	1.30	85.55	1.17	86.66	6.97	22.22	0.77	91.18	1.43	84.44
CgPJ 2	0.60	93.25	1.00	88.76	1.33	85.39	6.30	29.21	0.87	89.88	1.23	86.51
CgPN 3	0.57	93.25	0.73	92.13	0.87	89.88	6.30	29.21	0.93	89.88	1.70	80.89
CgSD 4	0.37	95.50	1.27	85.39	0.33	96.62	3.60	59.55	0.77	91.01	0.33	96.62
CgSJ 5	0.47	94.31	0.73	92.04	0.37	96.59	3.43	61.36	1.47	82.95	0.47	94.31
CgSN 6	0.33	96.62	0.47	94.38	0.37	96.62	4.07	53.93	0.77	91.01	0.77	91.01
CgBD 7	0.9 7	85.29	1.27	80.88	1.53	77.94	2.93	57.35	0.87	86.76	0.80	88.23
CgBJ 8	0.77	87.69	1.33	80.00	1.70	73.74	3.33	49.23	0.37	93.84	0.37	93.84
CgBN 9	1.07	82.81	1.40	78.12	1.93	70.31	3.43	46.87	0.67	89.06	0.23	96.87
CgGD 10	0.27	96.29	1.10	86.41	2.50	69.13	4.03	50.61	0.20	97.53	1.17	85.18
CgGJ 11	0.37	95.12	0.57	92.68	1.33	84.14	5.20	36.58	0.73	91.46	0.77	90.24
CgGN 12	0.27	96.25	0.47	93.25	0.97	87.5	4.37	45.00	0.23	97.50	0.97	87.50
Mean	0.58	92.19	0.97	87.46	1.20	84.54	4.50	45.09	0.72	91.01	0.85	89.64
											SE ±	CD @ 5%
Fungicides											0.01	0.04
Isolates											0.004	0.01
Fungicide X Isolates											0.05	0.14

Table 2: Sensetivity group of isolates against the fungicides

	Highly sensitive	Sensitive	Moderately sensitive	Less sensitive	Non sensitive
Azoxystrobin 23 % SC	CgPD 1, CgPJ 2, CgPN 3, CgSD 4, CgSJ 5, CgSN 6, CgGD 10, CgGJ 11, CgGN 12	CgBD 7, CgBJ 8, CgBN 9			
Carbendazim 50 %WP	CgPN 3, CgSJ 5, CgSN 6, CgGJ 11, CgGN 12	CgPD 1, CgPJ 2, CgSD 4, CgBD 7, CgBJ 8, CgGD 10	CgBN 9		
Mancozeb 75 % WP	CgSD 4, CgSJ 5, CgSN 6,	CgPD 1, CgPJ 2, CgPN 3, CgGJ 11, CgGN 12	CgBD 7, CgBJ 8, CgBN 9	CgGD 10	
Propineb 70 % WP				CgSD 4, CgSJ 5, CgSN 6, CgBD 7, CgGD 10	CgPD 1, CgPJ 2, CgPN 3, CgBJ 8, CgBN 9 CgGJ 11, CgGN 12
Thiophanate methyl 70 % WP	CgPD 1, CgSD 4, CgSN 6, CgBJ 8, CgGD 10, CgGJ 11, CgGN 12	CgPJ 2, CgPN 3, CgSJ 5, CgBD 7, CgBN 9			
Carbendazim 12 % + mancozeb 63 % WP	CgSD 4, CgSJ 5, CgSN 6, CgBJ 8, CgBN 9, CgGJ 11	CgPD 1, CgPJ 2, CgPN 3, CgBD 7, CgGD 10, CgGN 12			

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