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Short Communication

Variation in fungi toxicant sensitivity of *Colletotrichum gloeosporioides* isolates infecting fruit crops

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Thirty *Colletotrichum gloeosporioides* isolates from nine fruit hosts grown in different agro ecological zones of Maharashtra (India) expressed significant variation in their sensitivity to fungicides. All isolates expressed sensitive to highly sensitive reaction to prochloraz. Benomyl tolerance was also not detected except the isolate *C. gloeosporioides* (Cg) 42 from mandarin orange which was found to be insensitive to Benomyl. Cg 82 from pomegranate exhibited insensitive reaction to carbendazim with highest mean IC_{50} value of 330.15 µg/ml. This was followed by Cg 21 (243.77 µg/ml) from cashew nut, Cg 62 (218.7 µg/ml) from mango and Cg 83 (256.62 µg/ml), Cg 84 (261.22µg/ml) and Cg 86 (246.97µg/ml) isolated from pomegranate. Cg 82, Cg 84 and Cg 86 recorded sporulation up to 500 µg/ml level of carbendazim. In general, *C. gloeosporioides* isolates from pomegranate are tending towards carbendazim tolerance. One isolate Cg 43 was moderately insensitive to tricyclazole with mean IC_{50} value of 222.75 µg/ml and all remaining isolates recorded sensitive to highly sensitive reaction with tricyclazole.

Key words: *Colletotrichum gloeosporioides*, carbendazim, benomyl, prochloraz, sensitivity.

INTRODUCTION

Colletotrichum gloeosporioides is one of the most important plant pathogens worldwide. It causes anthracnose, 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. With increase in area under fruit crops from 2.42 lakh ha during 1990 - 1991 to 13.66 lakh ha by the end of 2000 (Anonymous, 2006), 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). 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. Further, benomyl sensitivity is an important criterion to study the species and sub-specific grouping in *Colletotrichum* (Freeman et al., 1998). Prochloraz 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 thirty *C. gloeosporioides* isolates to four fungicides namely: carbendazim, benomyl, tricyclazole and prochloraz.

These five isolates were rated as moderately insensitive.

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MATERIALS AND METHODS

Isolates of *C. gloeosporioides* were obtained from different fruit hosts namely: mango (*Mangifera indica)*, pomegranate (*Punica granatum)*, sweet orange (*Citrus sinensis)*, mandarin (*Citrus reticulata)*, custard apple (*Annona squamosa)*, guava (*Psidium guajava)*, cashew nut *(Anacardium occidental)*, areca nut *(Areca catachue)* and jamun *(Sizigium cumini)* cultivated in different agro ecological zones 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 two digits. The first digit indicates the name of the host and second digit indicates the isolate number from that particular host. 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.

The fungi toxicant sensitivity was studied by following the method of Swart (1999) and Liyanage et al. (1992) with slight modification. Four fungicides namely: carbendazim (50 WP), benomyl (50 WP), prochloraz (45 EC) and tricyclazole (75WP) were separately amended to PDA to get fungicide concentrations of 0, 50, 100, 150, 200, 250, 500 and 1000 µg/ml with two replications against the isolates under study. The quantity required for respective concentration of each fungicide was calculated on active ingredient basis and added to the pre autoclaved medium when its temperature was about 40 to 45°C and plates were poured. Inoculations were made with 5 mm mycelial bits (five day old) from test isolates and such plates were incubated at 28°C and 90 ± 2 per cent RH for seven days. The colony diameter was measured in two directions at right angle and the growth inhibition was recorded. Mean values of radial growth inhibition due to fungicide effect with various concentrations were subjected to probit analysis (Finny et al., 1981) to calculate IC_{50} (50% inhibitory concentration) values of respective fungicide for each isolate. The isolates were categorized on the basis of IC_{50} values (Kumar et al., 2002; Gutierrez and Gutierrez, 2003).

IC_{50} up to 100µg/ml Highly sensitive
IC_{50} 101 to 200µg/ml Sensitive
IC_{50} 201 to 300µg/ml Moderately insensitive
IC_{50} 301 to 400µg/ml Insensitive
IC_{50} above 400µg/ml Highly insensitive

Further, mean IC_{50} values of each isolate for each fungicide were subjected to statistical analysis by following completely randomized design by following SAS software for individual fungicide.

RESULTS AND DISCUSSION

The data on mean sensitivity in terms of IC50 values of *C. gloeosporioides* isolates to four selected fungicides is presented in Table 1. Isolates differed significantly in their sensitivity to individual fungicide. Out of 30 isolates, Cg 82 from pomegranate exhibited insensitive reaction to carbendazim with highest mean IC50 value of 330.15 µg/ml. This was followed by Cg 21 (243.77 µg/ml), Cg 62 (218.7 µg/ml), Cg 83 (256.62 µg/ml), Cg 84 (261.22 µg/ml) and Cg 246.97µg/ml). Out of these isolates, Cg 83, Cg 84 and Cg 86 were from pomegranate while Cg 21 was isolated from cashew and Cg 62 from mango. to carbendazim. In addition to low fungistatic effect of carbendazim, isolates from pomegranate namely: Cg 82, Cg 84 and Cg 86 recorded good amount of sporulation (5 - 10 × 103 conidia ml-1) up to 500 µg/ml level of carbendazim. Isolates having the mean IC50 value in the range of 101 to 200 µg/ml include Cg 11, Cg 33, Cg 54, Cg 55, Cg 61, Cg 63, Cg 64, Cg 65, Cg 68, Cg 71, Cg 81 Cg 85 and Cg 91. These isolates were referred as sensitive while remaining isolates Cg 31, Cg 32, Cg 41, Cg 42, Cg 43, Cg 51, Cg 52, Cg 53, Cg 56, Cg 66 and Cg 67 were highly sensitive to carbendazim. This is in accordance with the findings of Mohan et al. (2005), Xu et al. (2004) and Kumar et al. (2002) who reported the variation in carbendazim sensitivity of Gloeosporium ampelophagum isolates infecting grapes from different parts of Punjab, *C. gloeosporioides* isolates infecting mango in China and *C. capsici* isolates infecting chilli in Andhra Pradesh respectively. It was observed that out of 30 isolates, Cg 42 from mandarin orange was found to be insensitive to Benomyl with mean IC50 value of 318.85 µg/ml; while Cg 41 and Cg 43 form the same host and was highly sensitive to benomyl. In addition, all six isolates from sweet orange were highly sensitive to benomyl. Peres et al. (2004) found that benomyl resistant *C. gloeosporioides* isolates remained unaffected by concentration lower than 10 µg/ml and growth was about 20 and 10% of control at 100 and 1000 µg/ml respectively. Liyanage et al. (1992) also emphasized that benomyl concentration effect was strain dependent while differentiating *C. gloeosporioides* isolates of Citrus sp.

Isolates Cg 11, Cg 21, Cg 51, Cg 54, Cg 55, Cg 68, Cg 71, and Cg 85 were sensitive to benomyl where IC50 mean ranged between 101 to 200 µg/ml. All isolates from mango and pomegranate expressed sensitive to highly sensitive reaction to benomyl. Freeman et al. (1998) differentiated *C. gloeosporioides* isolates from almond and avocado on the basis of their sensitivity to benomyl and stated that almond isolates were insensitive as compared to avocado.

Prochloraz was found to be highly effective fungicide against all isolates. Most of the isolates recorded highly sensitive reaction whereas three isolates Cg 54, Cg 61, and Cg 67 recorded sensitive reaction to prochloraz. These results agree with the findings of Kuo (2001) who studied the reaction of 545 *C. gloeosporioides* isolates infecting mango from Taiwan and concluded that no significant resistance was found in the field even with higher frequency of prochloraz application. In the present study, Cg 43 was the only isolate which was moderately insensitive to tricyclazole with mean IC50 value of 222.75 µg/ml and all remaining isolates expressed highly sensitive to sensitive reaction.

It is revealed from the present investigation that different *C. gloeosporioides* isolates possess variable sensitivity against fungi toxicants. Four out of six isolates of pomegranate are tending towards carbendazim insensitivity and all these isolates are from the major pomegranate growing area of Maharashtra (India). Carbendazim is extensively and frequently used fungicide against wide variety of crop diseases whereas use of benomyl is quite

Table 1. Variation in sensitivity of *C. gloeosporioides* isolates to selected fungicides**.**

Isolate	Host	Mean IC_{50} values (µg/ml)			
		Carbendazim	**Benomyl**	**Prochloraz**	**Tricyclazole**
Cg 11	Areca nut	142.88	136.57	79.12	102.98
Cg 21	Cashew nut	243.77	134.06	82.7	151.39
Cg 31	Custard apple	94.5	76.05	37.06	114.51
Cg 32	Custard apple	96.07	84.12	61.88	86.26
Cg 33	Custard apple	137.37	96.12	43.09	123.67
Cg 41	Mandarin	58.14.	82.29	86.31	168.87
Cg 42	Mandarin	61.71	318.85	52.7	133.04
Cg 43	Mandarin	72.93	67.25	48.21	222.75
Cg 51	Sweet orange	52.15	119.28	22.52	61.87
Cg 52	Sweet orange	55.74	89.64	75.29	114.81
Cg 53	Sweet orange	92.35	96.82	69.1	77.92
Cg 54	Sweet orange	168.13	108.82	117.78	132.06
Cg 55	Sweet orange	130.71	100.01	41.69	160.1
Cg 56	Sweet orange	79.58	72.08	52.63	110.06
Cg 61	Mango	186.91	82.74	104.54	150.13
Cg 62	Mango	218.7	72.87	22.89	191.91
Cg 63	Mango	157.27	68.61	51.56	152.01
Cg 64	Mango	125.45	88.64	90.77	122.45
Cg 65	Mango	164.85	92.46	56.86	76.02
Cg 66	Mango	78.42	67.95	78.45	81.31
Cg 67	Mango	94.01	93.22	106.77	196.42
Cg 68	Mango	124.18	123.15	78.45	80.36
Cg 71	Guava	125.18	100.83	79.93	122.39
Cg 81	Pomegranate	117.05	72.27	83.78	124.59
Cg 82	Pomegranate	330.15	62.86	52.0	124.95
Cg 83	Pomegranate	256.62	92.71	51.39	84.51
Cg 84	Pomegranate	261.22	86.31	14.51	135.69
Cg 85	Pomegranate	182.65	108.87	38.11	48.01
Cg 86	Pomegranate	246.97	97.25	47.8	136.81
Cg 91	Jamun	157.64	111.7	74.27	104.93
S.E. ±		4.12	2.26	1.42	6.18
C.D. $(p_{= 0.01})$		13.02	6.28	4.43	19.03

limited in Maharashtra. Moorman and Lease (1992) emphasized on extended use of benzimidazoles that has resulted in selection for resistant pathogen genotypes.

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