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Cross infectivity of *Colletotrichum* spp. on tropical fruit crops and *Ageratum* spp. (weed host) in Southern Karnataka and Andhra Pradesh

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ABSTRACT: Species of *Colletotrichum* are associated with many host plants of diverse ecosystems including tropical, sub- tropical and temperature climatic conditions. Isolates of *Colletotrichum* were obtained from orchards of cultivated tropical fruit crops in Andhra Pradesh and Southern parts of Karnataka, India.Cross infectivity studies proved the wide host range of different isolates in seven fruit crops and one weed host (*Ageratum* spp.) tested. Most of the isolates displayed high virulence on seven alternative fruit crops and *Ageratum* spp. also. IIHR_COL_C6 isolated from mango recorded high virulence on guava fruits than other isolates. IIHR_COL_C8 displayed unique nature with cross infection *i.e.,* except on the original host isolated from, it showed least infection on all other alternative hosts tested showing its host specificity. Isolates IIHR_COL_C2, IIHR_COL_C3, IIHR_COL_C6, IIHR_COL_C7 and IIHR_COL_C9 recorded high virulence on their original hosts of isolation *viz*., banana, custard apple, mango, papaya and pomegranate respectively indicating the adaptation to main host and less preference of alternative hosts. A weed host *Ageratum* spp.was tested for its susceptibility to *Colletotrichum* isolates and found to be susceptible to all nine *Colletotrichum* isolates in the artificial inoculation. Most virulent isolates were IIHR_COL_C7 and IIHR_COL_C1 isolated from papaya and banana (4.76 cm and 4.68 cm), indicating the ability of *Colletotrichum* spp. in colonizing weeds. Isolates of *Colletotrichum* spp. from tropical fruit crops in this study can cause disease and might survive in off season alternatively on other fruit crops and also on weeds.

Keywords: *Ageratum*, *Colletotrichum*, cross infectivity, fruits, weed

INTRODUCTION

Colletotrichum species cause anthracnose disease in many crops including tropical fruit crops.They have world-wide distribution reported in all climatic conditions. *Colletotrichum* spp. were nominated as the eighth most important plant pathogen in the world on account of its scientific and economic importance (Dean *et al.*, 2012). This disease is considered as the most important fungal disease of *Annonaceous* species due to the losses ranging up to 70-100% during the periods of extended rainfall at the time of flowering, production of fruits (Junqueira and Junqueira, 2014; Kamei *et al.*, 2014).

A single species is potent to infect many host plants, similarly multiple species are potent to infect a single host plant genus (Fuentes-Aragon *et al.*, 2020). Cross infection studies unravel the *Colletotrichum* spp. life cycle like alternative hosts and survival of pathogen required for epidemic development with different species. The implication of identification and their potential to cross infect other fruit crops has direct impact on control measures as demonstrated by the host specificity and aggressiveness by *C. kahawae* to infect even green berries of coffee (Batista *et al.*, 2017) and differential sensitivity of *C. gloeosporioides* and *C. acutatum* to benomyl (Freeman *et al.*, 2000). *C. gloeosporioides* species complex was reported in association with 283 plant species of 212 genera in which the majority share of eudicots (80.6%) (Talhinhas and Baroncelli, 2021). Cross infection potential of isolates of *Colletotrichum* isolated from different tropical fruit crops on other hosts have been reported (Alahakoon *et al.*, 1994; Sanders and Korsten, 2003; Freeman *et al.*, 2000, 2001; Lakshmi *et al.*, 2011; Rampersad, 2011; Cruz *et al.*, 2020, Wu *et al.*, 2020).

In the present study we report the cross-infection potential of *Colletotrichum* isolates isolated from banana, custard apple, grapes, guava, mango, papaya, pomegranate on other fruit crops other than the host from which they had been isolated. Besides, the ability to infect a common weed host Ageratum that helps them to survive in the tropical fruit crop ecosystem also is also reported.

MATERIALS AND METHODS

Collection of anthracnose samples and isolation of *Colletotrichum* species

Anthracnose samples from the infected fruits, leaves, shoots from different fruit crops were collected systematically based on the visible anthracnose symptoms from Andhra Pradesh and Southern Karnataka by personal visit. The samples were collected in plastic bags and brought to ICAR-IIHR, Bengaluru for isolation of *Colletotrichum* spp. and washed thoroughly with tap water and later rinsed with distilled water and dried on the blotting paper folds. All the specimens were subjected to tissue isolation.

Isolation and identification of the pathogens at genus level

Colletotrichum spp. isolations were made from the leaves, blossom or fruit samples of banana (*Musa* spp.), custard apple (*Annona squamosa* L.), grapes (*Vitis vinifera* L.), guava (*Psidium guajava* L.), mango (*Mangifera indica* L.), papaya (*Carica papaya* L.), pomegranate (*Punica granatum* L.) and showing typical symptoms of anthracnose. Isolations were made as per the standard procedures on potato dextrose agar plates. Pure cultures were maintained on PDA. To obtain the pure colonies, isolates were sub-cultured onto fresh PDA plates *i.e.,* discs of 5 mm diameter from growing tips or periphery of the colony were transferred to fresh PDA plates and incubated at 25 ± 1°C with photoperiod of 24 h for seven days.

The pathogenicity of the purified isolates was confirmed by proving the Koch's postulates. After the development of characteristic anthracnose symptoms on host parts, re-isolation was made from the infected portion of the host part and cultured on PDA. Isolates that were fulfilled the pathogenicity test were used for further studies. The identity of the pathogen was tentatively confirmed based on the morphological and cultural characteristics as described by Sanders and Korsten (2003), Damm *et al.* (2012) and Weir *et al.* (2012).

Cross inoculation on alternative fruit crops and weed host

Cross inoculation study was undertaken in order to determine the host range of the isolates within the host genera studied. For all isolates, cross infectivity was carried out as same as pathogenicity tests. Healthy susceptible, freshly harvested, untreated, mature but un-ripened fruits, leaves or flowers were washed under running tap water for 1 min followed by surface sterilization with 70% ethanol for 3 min, 1% sodium hypochlorite solution for 3 min and then rinsing three times in sterile distilled water and air dried on sterile tissue paper. The surface sterilized leaves, flowers and fruits were placed in a plastic box that contained moist blotting paper to maintain 95% relative humidity. They were inoculated by making wound method with pin-pricking on the leaves, flowers or fruits with a sterile needle and then placing mycelial disc of 5 mm diameter onto the wound and incubated at room temperature for seven days as described by Mo *et al.* (2018). Fruits of banana, papaya and guava, flowers of pomegranate and leaves of custard apple, mango and grapes were used for the inoculation. They were observed for symptom development up to 10-13 days. Isolates were considered pathogenic if lesion size expanded beyond initial wound site. Virulence was assessed by measuring lesion length at 7-11 days post inoculation (DPI). Significant differences in virulence caused were assessed by an analysis of variance, and when significant treatment effects were found, means were compared in CRD. The host range studies were carried out with a weed host *Ageratum* spp. also which was highly prevalent in the fruit orchards ecosystem at Hessaraghatta, Bengaluru.

RESULTS AND DISCUSSION

All the nine representative isolates of *Colletotrichum* spp. in this study were tested for their host range on seven fruit crops *viz*., banana, custard apple, grapes, guava, mango, papaya, pomegranate and one weed host *Ageratum* species. They produced typical anthracnose symptoms upon artificial inoculation (Fig. 1) within 7-11 days, though there was variation in virulence or aggressiveness in producing the symptoms. Symptoms like brown to black necrosis of host tissue due to infection from artificially inoculated *Colletotrichum* isolates were measured seven days post inoculation (DPI) on fruits of banana, guava and flowers of pomegranate, eight DPI on papaya fruits, nine DPI on mango leaves and *Ageratum* spp., ten DPI on grapes leaves, eleven DPI on custard apple leaves. The mean lesion diameters recorded with nine isolates of *Colletotrichum* spp. on seven fruit crops and one weed host *Ageratum* spp. is presented in Table 1. No infection was recorded in control fruits, flowers or leaves at the site of pin pricking. The response of different hosts to the nine isolates tested is described below.

Response of different host plants to *Colletotrichum* isolates from other hosts

Banana: Most severe infection on banana was recorded with IIHR_COL_C2 isolated from same host *i.e*., banana with a mean lesion diameter of 3.14 cm followed by infection with IIHR_COL_C1 and IIHR_COL_C6 isolated from banana and mango without significant difference between them (2.37 cm and 2.37 cm respectively); IIHR_COL_C4 and IIHR_COL_C7 isolated from grapes and papaya without significant difference between them (2.06 cm and 2.06 cm respectively). IIHR_COL_C3 and IIHR_COL_C5 isolated from custard apple and guava were on par in

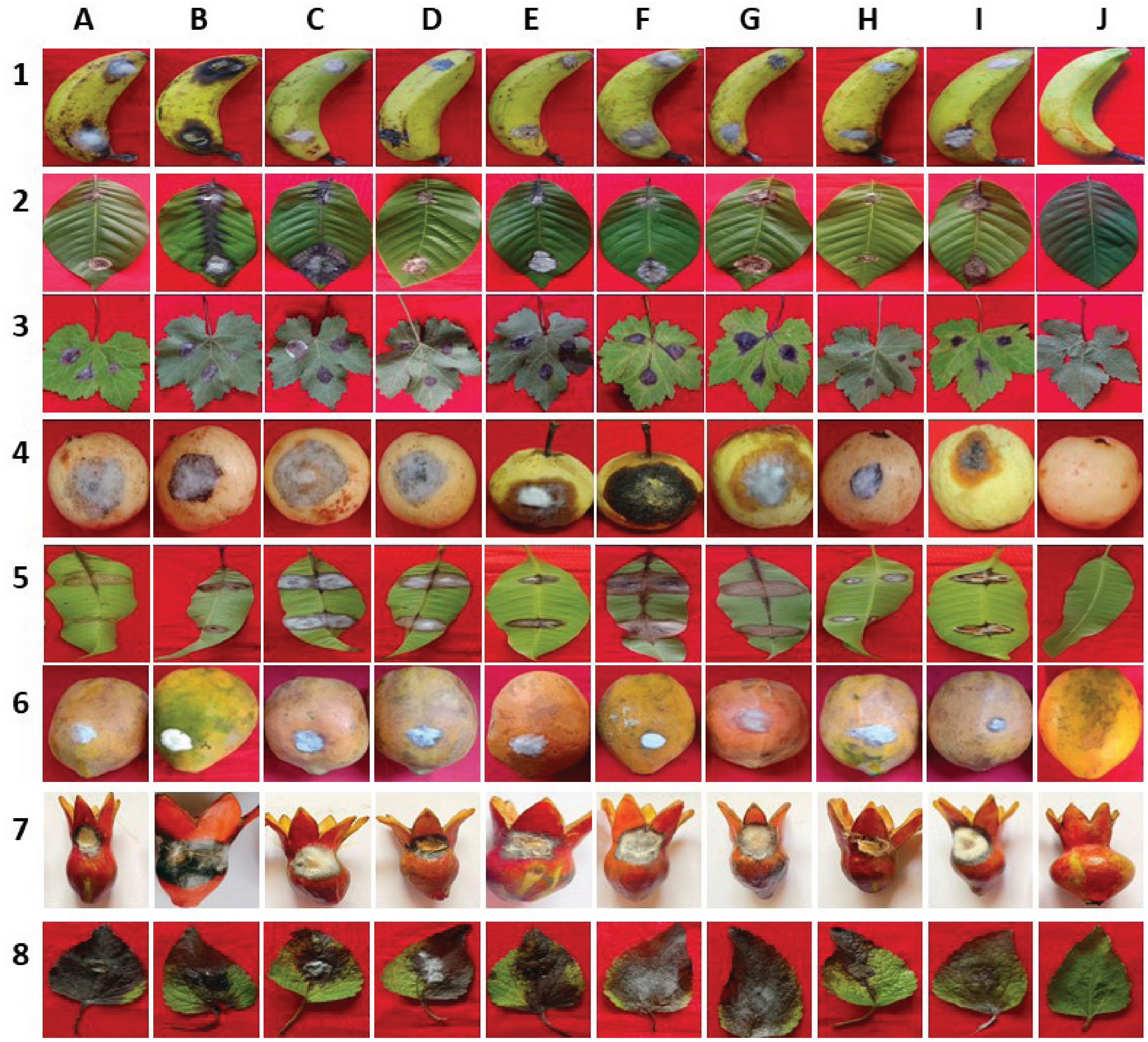


Fig. 1.Cross infection by isolates of *Colletotrichum* spp. (A to I, J-control) from different tropical fruit crops and one weed host *Ageratum*

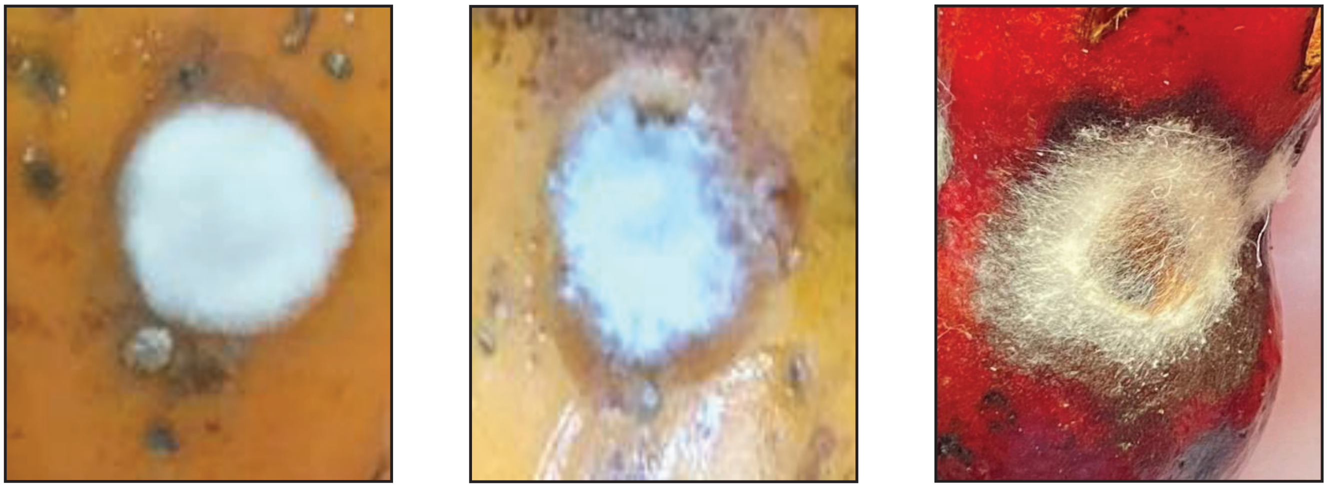
1- Banana, 2-Custard apple, 3-Grapes, 4-Guava, 5-Mango, 6-Papaya, 7-Pomegranate, 8- *Ageratum*; A. IIHR_COL_C1, B- IIHR_COL_C2, C- IIHR_COL_C3, D- IIHR_COL_C4, E- IIHR_COL_C5, F- IIHR_COL_C6, G- IIHR_COL_C7, H- IIHR_COL_C8, I- IIHR_COL_C9, J-control

lesion diameter (1.72 cm and 1.77 cm respectively). IIHR_COL_C8 isolated from papaya caused poor infection on banana (1.59 cm). Varying symptoms observed were brownish black lesion coupled with pure white mycelium growth (C1), small salmon pink acervuli on the necrotic lesions (C2), black necrotic lesion without any mycelium growth (C4) and necrotic lesion coupled with dull white mycelium growth with other isolates.

Custard apple: Most severe infection on custard apple was recorded with IIHR_COL_C3 isolated from same host *i.e.*, custard apple with a mean lesion diameter of 5.35 cm, followed by infection with IIHR_COL_C7 isolated from papaya (4.1 cm). Least cross infection on custard apple was recorded with IIHR_COL_C8 (1.8 cm) isolated from papaya. Other isolates had lesion size in between 2.29-3.6 cm. Highly virulent isolate C3 produced black necrotic, nearly spherical lesion, large in size with comparison to other isolates with small salmon pink acervuli while other isolates produced necrotic lesions without visible mycelium growth.

a. Banana with IIHR_COL_C1
b. Banana with IIHR_COL_C2
c. Custard apple with IIHR_COL_C3

d. Grapes with IIHR_COL_C7
e. Guava with IIHR_COL_C6
f. Mango with IIHR_COL_C6



g. Papaya with IIHR_COL_C7
h. Papaya with IIHR_COL_C8
i. Pomegranate with IIHR_COL_C9

Fig. 2. Close view of typical anthracnose symptoms (some with acervuli) on host tissues of fruit crops with highly virulent isolates in this study.

Table 1. Host range of *Colletotrichum* isolates on fruit crops and *Ageratum* spp. on artificial inoculation

Isolate	Host	Mean lesion diameter (cm) on inoculated leaves, flowers and fruits							
		Banana (Fruit)	Custard apple (Leaves)	Grapes (Leaves)	Guava (Fruit)	Mango (Leaves)	Papaya (Fruit)	Pomegranate (Flower)	*Ageratum* spp. (Leaves)
IIHR_COL_C1	Banana	2.37	2.34	2.40	3.53	2.08	2.56	1.72	4.68
IIHR_COL_C2	Banana	3.14	2.56	1.85	2.40	2.33	3.27	1.98	3.41
IIHR_COL_C3	Custard apple	1.72	5.35	3.09	3.70	2.82	3.82	2.05	3.46
IIHR_COL_C4	Grapes	2.06	2.29	2.15	2.92	2.25	3.17	1.95	4.45
IIHR_COL_C5	Guava	1.77	3.00	2.35	3.39	1.76	3.41	2.02	4.08
IIHR_COL_C6	Mango	2.37	3.64	3.23	4.15	3.13	2.88	1.95	4.30
IIHR_COL_C7	Papaya	2.06	4.10	3.75	2.81	2.65	4.13	2.22	4.76
IIHR_COL_C8	Papaya	1.59	1.80	1.29	1.87	1.70	3.45	1.33	1.96
IIHR_COL_C9	Pomegranate	2.19	3.30	2.46	2.92	2.35	2.42	2.28	3.98
SE(m)±		0.02	0.02	0.03	0.02	0.04	0.03	0.02	0.09
C.D (0.05)		0.08	0.08	0.10	0.08	0.12	0.09	0.06	0.28
C.V %		2.35	1.59	2.43	1.55	3.20	1.61	1.79	4.25

Grapes: Maximum cross infectivity on grapes was recorded with IIHR_COL_C7 isolated from papaya with a mean lesion diameter of 3.75 cm followed with IIHR_COL_C6 (3.23 cm) isolated from mango. Least cross infection on grapes was recorded with IIHR_COL_C8 isolated from papaya (1.29 cm). Other isolates produced lesions of intermediate range. Purplish black necrotic, nearly circular lesions with sparse mycelium over the lesions with salmon pink colour sporulation (acervuli) were recorded with all the nine isolates upon artificial inoculation on grapes leaves. Shot hole like breaking of the infected lesions was observed due to drying of the necrotic region of lesions with IIHR_COL_C1.

Guava: Maximum cross infection on guava was recorded with IIHR_COL_C6 isolated from mango with a mean lesion diameter of 4.15 cm followed with IIHR_COL_C3 from custard apple (3.70 cm). Minimum cross infection on guava was documented with IIHR_COL_C8 isolated from papaya (1.87 cm). Other isolates resulted in lesions of intermittent range of 2.4 – 3.5 cm. The symptoms observed were dull or pure white mycelium growth with all isolates and acervuli with mango and guava isolates.

Mango: Maximum infection on mango was recorded with IIHR_COL_C6 (3.13 cm) isolated from same host *i.e.*, mango. It was followed with IIHR_COL_C2, IIHR_COL_C4 and IIHR_COL_C9 isolated from custard apple and papaya respectively (2.33, 2.25 and 2.35 cm) which did not differ significantly in cross infectivity on mango. Least cross infection on mango was recorded with IIHR_COL_C5 (1.76 cm) and IIHR_COL_C8 (1.70 cm) isolated from papaya and guava. The symptoms included brown colour necrotic lesions surrounded with dark chocolate brown colour margins with no visible mycelium growth over the lesions. All lesions were nearly spherical in shape. Many small salmon pink sporulation's (acervuli) were observed in the necrotic regions of lesions for all isolates except with IIHR_COL_C8.

Papaya: Maximum infection on papaya was recorded with IIHR_COL_C7 isolated from same host *i.e.*, papaya with a mean lesion diameter of 4.13 cm followed with IIHR_COL_C3 isolated from custard apple (3.82 cm). Least infectivity on papaya was recorded with IIHR_COL_C9 isolated from pomegranate (2.42 cm). The lesion diameter of other isolates ranged between 2.42 – 3.17cm. Ample dull white mycelium growth covered over the sunken brown colour lesions were recorded in all isolates while with C8 black colour sporulation was observed.

Pomegranate: Maximum infection on pomegranate with a mean lesion diameter of 2.28 cm was caused with IIHR_COL_C9 isolated from same host i.e., pomegranate followed with IIHR_COL_C7 (2.22 cm) isolated from papaya. Least infection of 1.33 cm on pomegranate was recorded with IIHR_COL_C8 isolated from papaya. Other isolates resulted in lesion size of 1.33 – 2.05cm. Black colour necrotic sunken lesions, with sparse mycelium growth over the lesions were recorded. Many small salmon pink sporulation's (acervuli) were observed in the necrotic regions of lesions for all isolates except with IIHR_COL_C8 with sparse mycelium growth with little infection.

Ageratum species- The weed host

A weed host *Ageratum* spp. observed to be widely prevalent in the ecosystem of the fruit orchards in the areas of collection was tested for its susceptibility to all nine isolates of *Colletotrichum* spp. and was found susceptible to all nine *Colletotrichum* isolates in the artificial inoculation. Among the isolates tested, most virulent were IIHR_COL_C7 and IIHR_COL_C1 isolated from papaya and banana with mean lesion diameters of 4.76 cm and 4.68 cm respectively. Least infection on *Ageratum* spp.was recorded with IIHR_COL_C8 that was isolated from papaya (1.96 cm). Other isolates resulted in lesion size of intermediate range. Lesions were irregular shape. They were with brown or black necrotic in nature. to nearly spherical lesion shape in cross infectivity studies on fruit crops. No visible sporulation was observed within the necrotic lesions with all isolates on *Ageratum* spp. suggesting their endophytic life style (Freeman *et al.*, 2000).

As a whole, most of the isolates displayed high variation in aggression when inoculated on alternative fruit crops and one weed host tested. A close view of typical anthracnose symptoms with acervuli on host tissues of fruit crops with maximum infecting isolates in this study are presented in Fig. 2. In banana, custard apple, mango and pomegranate, the isolates from original hosts caused maximum lesion size. In case of grapes, isolate C7 from papaya caused severe infection with purplish-black lesion with presence of numerous small salmon pink acervuli formed in concentric rings. Similarly isolate from mango caused severe infection on guava. This clearly indicates that the pathogen can survive on other hosts and can move to other hosts depending on the availability of the host during the crop season. The results suggest that these isolates have good adaptation or specificity to certain hosts and less preference of other alternative hosts.

Another significant observation was that IIHR_COL_C8 isolated from papaya had falcate shaped conidia and greyish- black mycelium. Apart from the distinctiveness of morphological and cultural characters with other isolates, it also showed unique nature in cross infection. Except on the original host isolated from, it showed least infection on all other alternative hosts tested. IIHR_COL_C8 isolate's poor infection on alternative hosts also gives implications of host- specificity, possibility of pathogen elicitors ability to receipt only certain hosts before infection. The isolate from papaya resembled *C. truncatum* while all others resembled C. *gloeosporioides* complex that was confirmed with molecular data (Teja, unpublished data).

Cross infection between two or three hosts viz., mango and custard apple (Lakshmi *et al.*, 2011), mango and sweet pepper reported the cross infection between anthracnose pathogens of mango, papaya and custard apple (Rampersad, 2011); and avocado, durian and mango (Alahakoon *et al.*, 1994). Host range studies reported earlier also suggest the wide host range of *Colletotrichum* especially in fruit crops viz. avocados, mango, strawberry, guava, citrus and papaya (Sanders and Korsten, 2003; De Souza *et al.*, 2013, Cruz *et al.*, 2020, Wu *et al.*, 2020).

High variation in virulence observed in the present study is indicating the isolate variations from tropical fruit crops. Therefore, the isolates of *Colletotrichum* spp. from tropical fruit crops in this study can cause disease and might survive alternatively on other fruit crops and also on weeds. It has implications for researchers to identify the interaction responsible for poor cross infectivity, host specific nature and impart resistance to the cultivars.

REFERENCES

Alahakoon, P. W., Brown, A. E. and Prasad, S.S. 1994. Cross-infection potential of genetic groups of *Colletotrichum gloeosporioides* on tropical fruits. *Physiological and Molecular Plant Pathology*, **44** (2): 93-103.

Batista, D., Silva, D. N., Vieira, A., Cabral, A., Pires, A. S., Loureiro, A., Guerra-Guimaraes, L., Pereira, A.P., Azinheira, H., Talhinhas, P. and Silva, M.D.C. 2017. Legitimacy and implications of reducing *Colletotrichum kahawae* to subspecies in plant pathology. *Frontiers in Plant Science*, **7:** 2051.

Cruz, A. F., Barka, G. D. and Blum, L. E. B. 2020. Cross-pathogenicity of *Colletotrichum* isolates on tropical fruit species in Brazil. *Archives of Phytopathology and Plant Protection*.**54** (5-6): 295-307.

Damm, U., Cannon, P. F., Woudenberg, J. H. C. and Crous, P.W. 2012. The *Colletotrichum acutatum* species complex. *Studies in Mycology*, **73:** 37-113.

De Souza, A., Delphino-Carboni, R.C., Wickert, E., de Macedo Lemos, E.G. and de Goes, A. 2013. Lack of host specificity of *Colletotrichum* spp. isolates associated with anthracnose symptoms on mango in Brazil. *Plant Pathology*, **62** (5): 1038-1047.

Dean, R., Van Kan, J. A., Pretorius, Z. A., Hammond Kosack, K. E., Di Pietro, A., Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J. and Foster, G.D. 2012. The top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*, **13** (4): 414-430.

Freeman, S., Horowitz, S. and Sharon, A. 2001. Pathogenic and non-pathogenic lifestyles in *Colletotrichum* acutatum from strawberry and other plants. *Phytopathology*. **91**(10): 986-992.

Freeman, S., Shabi, E. and Katan, T. 2000. Characterization of *Colletotrichum acutatum* causing anthracnose of anemone (*Anemone coronaria* L.). *Applied and Environmental Microbiology*, **66** (12): 5267-5272.

Fuentes-Aragon, D., Guarnaccia, V., Rebollar Alviter, A., Juarez Vazquez, S.B., Aguirre Rayo, F. and Silva Rojas, H. V. 2020. Multilocus identification and thiophanate methyl sensitivity of *Colletotrichum gloeosporioides* species complex associated with fruit with symptoms and symptomless leaves of mango. *Plant Pathology*, **69** (6): 1125-1138.

Junqueira, N. T. V. and Junqueira, K. P. 2014. Main Annonaceous diseases in Brazil: description and control. *Revista Brasileira de Fruticultura,* **36**: 55-64.

Kamei, S. H, Costa, J. F. D. O, Brito Netto, M. D. S, Assuncao, I. P. and Lima, G. S. D. A. 2014. Identification and characterization of *Colletotrichum* species associated with anthracnose of annonaceae in the state of Alagoas. *Revista Brasileira de Fruticultura*, **36:** 209-216.

Lakshmi, B. K. M., Reddy, P. N. and Prasad, R. D. 2011. Cross-infection Potential of *Colletotrichum gloeosporioides* Penz. isolates causing anthracnose in sub-tropical fruit crops. *Tropical Agricultural Research*, **22** (2): 183-193.

Mo, J., Zhao, G., Li, Q., Solangi, G.S., Tang, L., Guo, T., Huang, S. and Hsiang, T. 2018. Identification and characterization of *Colletotrichum* species associated with mango anthracnose in Guangxi, China. *Plant Disease*, **102**(7):1283-1289.

Rampersad, S. N. 2011. Molecular and phenotypic characterization of *Colletotrichum* species associated with anthracnose disease of papaya in Trinidad. *Plant Disease*, **95** (10): 1244-1254.

Sanders, G. M. and Korsten, L. 2003a. Comparison of cross inoculation potential of South African avocado and mango isolates of *Colletotrichum gloeosporioides*. *Microbiological Research*, **158** (2): 143-150.

Talhinhas, P. and Baroncelli, R. 2021. *Colletotrichum* species and complexes: geographic distribution, host range and conservation status. *Fungal Diversity*, **110** (1): 109-198.

Weir, B. S., Johnston, P. R. and Damm, U. 2012. The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology*, **73**: 115-180.

Wu, C. J, Chen, H. K. and Ni, H. F. 2020. Identification and characterization of *Colletotrichum* species associated with mango anthracnose in Taiwan. *European Journal of Plant Pathology*, **157** (1): 1-15.

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