Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7586

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **7 (3):** 571-576 (2019)

Research Article

Cross Inoculation Studies of *Colletotrichum capsici* Isolates between Betelvine, Turmeric and Chilli

K. Venkata Ramesh[1*], R. Sarada Jayalakshmi Devi[1], K. Gopal[2], B.K.M. Lakshmi[3], T. Murali Krishna[4] and B. Ravindra Reddy[5]

[1]Department of Plant Pathology, S.V. Agricultural College, Tirupati - 517 502

[2]Controller of Examinations, Administrative Office, Dr.YSR Horticultural, University, Venkataramannagdem

[3]Department of Plant Pathology, Mango Research Station, Dr.YSRHU, Nuzvid - 521 201

[4]Programme co-ordinator, KVK, ANGRAU, Kalyandurgam - 515 761

[5]Department of Statistics & Computer Applications, S.V. Agricultural College, Tirupati - 517 502

*Corresponding Author E-mail: kvrameshagri@gmail.com

Received: 10.05.2019 | Revised: 14.06.2019 | Accepted: 21.06.2019

ABSTRACT

Colletotrichum capsici causes chilli anthracnose and has a wide host range betel vine, turmeric, bitter gourd, castor, cluster bean, cotton, marigold etc. Cross inoculation studies were made on three important horticultural crops, betel vine, chilli and turmeric. In all the three hosts (chilli, turmeric and betelvine) the mean lesion size (mm^2) produced by the isolates from the original host were higher compared to isolates from non hosts. Isolates also differed in their virulence levels and C. capsici isolated from turmeric is more virulent compared to other isolates. Isolates differed in the days for chlorotic and necrotic symptoms. C. capsici isolates exhibited host preference among the three hosts. Turmeric is more preferred host for C. capsici compared to betelvine and chilli.

Key words: *Colletotrichum capsici, Cross inoculation, Betel vine, Chilli and Turmeric.*

INTRODUCTION

The genus *Colletotrichum* is one of the most important plant pathogenic fungus causing huge losses and recently voted as the eighth most important group of plant pathogenic fungi in the world based on its perceived scientific and economic importance[3]. The genus includes a great number of plant pathogens causing anthracnose diseases in a variety of woody and herbaceous plants in both the tropics and subtropics[1,2,22]. Recently, Farr *et al.*[4] reported that *Colletotrichum* causes anthracnose disease in more than 121 plant genera from 45 different plant families. Fungus-host relationships are broad, imprecise and often overlapping[5]. Under natural ecosystem conditions, *Colletotrichum* infects several closely related host species but some species are able to infect a wider taxonomic range of hosts.

Cite this article: Venkata Ramesh, K., Sarada Jayalakshmi Devi, R., Gopal, K., Lakshmi, B. K. M., Murali Krishna, T. and Ravindra Reddy, B., Cross Inoculation Studies of *Colletotrichum capsici* Isolates between Betelvine, Turmeric and Chilli., *Int. J. Pure App. Biosci.* **7(3):** 571-576 (2019). doi: http://dx.doi.org/10.18782/2320-7051.7586

It is also common to find multiple hosts infected by a single species of *Colletotrichum,* and single host infected by multiple species of the pathogen[16,18]. *Colletotrichum capsici* can infect many hosts and may adapt to new environments[16,12] leading to serious cross infection problems in plant production.

Chilli anthracnose has been shown to be caused by many species of *Colletotrichum* world wide. Some of them are *C. acutatum* (Simmonds), *C. capsici* (Syd.) Butler and Bisby, *C. gloeosporioides* (Penz) Penz and Sacc, and *C. coccodes* (Wallr) S. Hughe. Than *et al.*[20] and among them, *C. capsici* is more prevalent and damaging.

In India, anthracnose or ripe fruit rot caused by *Colletotrichum capsici* (Syd.) Butler and Bisby has been reported to be the most important pathogen causing anthracnose of chilli[17,10,6]. The fungus *C. capsici* has a wide host range and was reported to infect chilli, bell pepper, aristolochia, bengalgram, brinjal, cotton, jute, tomato, turmeric and many other plants from a wide range of families. Infection of *C. capsici* on various hosts has been reported by various workers *viz.,* on betel vine by Gupta and Sen[7] on bitter gourd by Samita and Dubey[15]; on castor by Sinha and Singh; on *Cicer arietinum, Lupines angustifolius, Phaseolus vulgaris, Pisum sativum, Vigna radiata, Vigna unguiculata* by Pring *et al.*[13]; on cluster bean by Kothari and Bhatnagar$_8$; on cotton by Chopra *et al.*; on marigold by Sherf and Mac Nab. In Andhra Pradesh and Telangana states *Colletotrichum capsici* is known to infect many other commercially grown crops including betel vine, chilli and turmeric and causes huge losses in different districts in which these crops are often grown in contiguous areas. It is necessary to know whether, the *C. capsici* occuring on these crops is same or different from each other. Also, these isolates have the same infection potential on their original hosts and on other crops or not. Information on this will help in better understanding of the pathogen and the epidemics occurring on these crops, and to plan for better and effective management strategies. Thus the present investigation was carried out to study the cross infection potential between the *C. capsici* isolates of betelvine, chilli and turmeric.

MATERIAL AND METHODS

Isolation and proving the pathogenicity:

Pathogen isolates were isolated from the diseased parts showing typical symptoms of anthracnose on betelvine, chilli and turmeric by tissue segmentation method[14] on PDA medium. Small bits measuring about three mm size were cut from the leaves showing lesions in such a way that it contains both infected and healthy portions. Such bits were surface sterilized in 1 per cent sodium hypochlorite (NaOCl) solution for one minute followed by three washings in sterile distilled water for 30 seconds each. These bits were further transferred to sterile discs of blotting paper for removing excess water. The bits were subsequently transferred to sterile PDA medium in petri plates under aseptic conditions. The petri plates were incubated at 27 ± 2°C and observed periodically for growth of the fungus. Isolates are further purified by single spore isolation. The pathogenicity of the respective isolates was proved by using detatched leaf assay method as described below. The isolates were designated as *C. capsici* isolates of betel vine (CCB), chilli (CCC) and turmeric (CCT).

Cross inoculation studies:

Inoculum preparation:

The pure cultures obtained by single spore isolation were used for pathogenicity test under glasshouse conditions. Conidial suspension was prepared from sporulated culture. About 5 ml of sterile distilled water was added to each plate and the conidia were loosened by gentle scraping with the help of a camel hair brush. Washing was repeated to get maximum spores into the suspension. The spore suspension was filtered through three layers of cheese cloth. Tween 20 (Polyoxyethylene sorbitol monoleate), a

surfactant was added @ 0.1 per cent to the suspension that enable uniform spread of inoculum on the plants. Concentration of spores was adjusted to 10^6 conidia ml^{-1} established by haemocytometer was utilized for pathogenicity studies.

Detatched leaf assay method:

The experiments was conducted in Completely Randomized Design with seven replications. The studies were done by using detached leaf assay method by following the method described by Tu[21] by using the leaves of betel vine (cv. Chennur), chilli (cv. Sindhur), turmeric (cv. Duggirala). The leaves were placed in a plastic tray lined with three layers of blotting paper and covered with a polythene sheet to avoid evaporation losses and to serve as incubation chamber. Conidial suspensions of the respective *C. capsici* isolates from betelvine, chilli and turmeric were prepared using 10 day old cultures and the numbers of conidia were adjusted to 10^6 conidia ml^{-1} using haemocytometer. Apparently healthy and disease free leaves of betelvine, turmeric and chilli were thoroughly washed under running water, swabbed with 70% (v/v) ethanol and air-dried in the laboratory. Inoculum droplets (10 µl) containing 10^6 conidia ml^{-1} were separately placed onto the carefully marked areas in the center of leaves the of each crop. Each of the three isolates from respective crops were placed onto a separate leaf with seven replications. Leaves receiving only sterile distilled water droplet serves as control. Inoculated leaves will be placed in moist chamber at room temperature. The experiments were arranged using a Completely Randomized Design with seven replications. Data was collected regularly, on lesion size (mm^2), days to chlorotic lesions and necrotic lesions on each leaf tested and analyzed statistically.

RESULTS AND DISCUSSION

Isolation and proving the pathogenicity:

All the three three isolates of *C. capsici* isolated from betelvine (CCB), chilli (CCC) and turmeric (CCT) were identified using morphological characters and cultural characters like size and shape of the conidia, size of the setae, number of setae per acervulus, formation of acervuli, colony colour, type of mycelial growth, colony margins, sectoring, colour of conidial mass, growth rate, mycelial dry weight, and sporulation. The pathogenicity of the isolates was proved by the production of typical symptoms on leaves when inoculated in detatched leaf assay method.

Cross inoculation studies:

Host specificity

The isolates of *C. capsici* from different hosts are not host specific which is evident from the disease reaction (Table 1) of different isolates on hosts. All the isolates infected the other hosts.

Mean lesion size:

Studies on cross infection of three isolates of *C. capsici* isolated from betelvine (CCB), chilli (CCC) and turmeric (CCT) (Table 2), revealed the pathogenic variability and host preference among the isolates from different hosts. Highest mean lesion size on a particular host (Betelvine/Chilli/Turmeric) was observed when the isolate of *C. capsici* from the same host was inoculated. Highest mean lesion sizes by the isolates from the same hosts, 121.57 mm^2 on betel vine by isolate CCB, 61.57 mm^2 on chilli by isolate (CCC), 2116.14 mm ^{2} on turmeric by isolate (CCT). Lesion size of isolates from non hosts were statistically on par on betelvine and chilli leaves where as significantly different on turmeric leaves.

Days to chlorotic and necrotic symptoms:

The average number of days for production of chlorotic symptoms on leaves by different isolates (Table 3) differed on hosts. On betel vine, time taken by the isolates of betelvine (CCB 2.29 days) and turmeric (CCT 2.71 days) was less compared to chilli isolate (3.14 days). On chilli, the isolates did not differ in number of days for production of chlorotic lesions statistically. On turmeric, isolates of turmeric (CCT 2.71 days) and chilli (CCC

3.14 days) took less time compared to betel vine isolate (CCB 3.29 days). With regard to the necrotic symptoms, on all the three crops, the isolates from the same host took less time compared to the isolates from other hosts. The isolates did not differ among themselves, regarding the average number of days for chlorotic and necrotic symptoms on all the hosts together was considered.

In all the three hosts (chilli, turmeric and betelvine) the lesions produced by the isolates from the original host were higher compared to isolates from non hosts.This indicates the specificity of the hosts and isolates. Similar such reports of more aggressiveness on original hosts were reported by other workers also. Simmonds also demonstrated that *C. gloeosporioides* isolates were more aggressive in attacking the host from which they were originally isolated. Sanders and Korsten[16] also reported that the isolates of *C. gloeosporioides* from avocado and mango produced lesions on other hosts but showed larger lesions on their original host. Lakshmi et *al*.[9] also reported that the level of host preference among *C. gloeosporioides* isolates from seven subtropical fruit crops and the susceptibility of the hosts varied significantly. *C. gloeosporioides* isolated from a fruit could cross-infect other fruits, and the fungus isolate was most aggressive when inoculated to its original host.

The isolates also differed in their virulence levels across the hosts which is evident from the mean lesion size of the isolates on different hosts. *C. capsici* isolated from turmeric is significantly more virulent compared to other isolates (mean lesion size on all three hosts together). This might be due to the intrinsic differences in the genetic make up of the isolates which result different virulence potential of the isolates. Such genetic variations in same species of a pathogen isolated from different hosts were reported earlier. Mills *et al*.[11] reported that the isolates of *C. gloeosporioides* obtained from avacado, mango and papaya did not have the same ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) restriction pattern or random amplified polymorphic DNA (RAPD) banding patterns.

It can also be observed that within the isolates, the lesion size produced by betelvine and chilli isolates on turmeric leaves were larger than those produced on their original hosts. This indicates the host preference of *C. capsici* to turmeric compared to other hosts. This might be due to the composition of leaves, which encourage the growth of a pathogen. Suparman *et al*.[19] during their studies on cross infection of *Colletotrichum* spp. on papaya, brinjal (eggplant), and chilli observed that brinjal (eggplant) is more susceptible to anthracnose pathogen than papaya, or the anthracnose pathogens, *Colltotrichum* spp. are more virulent when inoculated to brinjal (eggplant). Also, the isolates have lowest days for chlorotic and necrotic symptoms on their original hosts except in case of chilli in which all the isolates behaved similarly. This might be due to the host pathogen specific factor involved in pathogenesis. Similar view of virulence of anthracnose pathogens affected by host conditions such as availability of nutrients and enzymes required by the pathogens, or the presence of anti fungal compounds was expressed by Lakshmi *et al*.[9]. Lakshmi *et al*.[9] reported that cross inoculation studies on *C. gloeosporioides* isolates from seven fruit crops viz., mango, acid lime, custard apple, pomegranate, papaya, cashew and guava revealed that among different fruit crops mango, cashew, pomegranate and custard apple were highly susceptible to the anthracnose disease.

The results of the present study it can be concluded that *C. capsici* are not host specific in production of disease which is evident from the production of symptoms on all hosts by all the isolates. However, with in the species host specificity and differences in virulence levels exists and also the susceptibility levels among the hosts also differ from each other.

Table 1: Cross infectivity between isolates of *C. capsici* from betel vine, chilli and turmeric

Isolate	Host		
	Betel vine	Chilli	Turmeric
Betel vine isolate (CCB)	+	+	+
Chilli isolate (CCC)	+	+	+
Turmeric isolate (CCT)	+	+	+

Table 2: Mean lesion size in cross inoculation of *C. capsici* isolates between betelvine, chilli and turmeric

Isolate	Mean lesion size mm^2			
	Betelvine	Chilli	Turmeric	Mean
Betelvine isolate (CCB)	121.57 [a]	36.71 [b]	1421.857 [b]	526.71 [b]
Chilli isolate (CCC)	56.857 [b]	61.57 [a]	1121.28 [c]	413.23 [c]
Turmeric isolate (CCT)	60.57 [b]	39.28 [b]	2116.14 [a]	738.66 [a]
CD (0.05)	11.12	7.12	211.58	70.91
CV	12.43	13.83	12.13	11.28

Table 3: Days to chlorotic and necrotic lesions in cross inoculation of *C. capsici* isolates between betelvine, chilli and turmeric

Isolates	Days to chlorotic symptoms on leaves				Days to necrotic symptoms on leaves			
	Betelvine	Chilli	Turmeric	Mean	Betelvine	Chilli	Turmeric	Mean
Betelvine Isolate (CCB)	2.29 [a]	3.14 [a]	3.29 [b]	2.9	7.71 [a]	8.43 [b]	8.86 [b]	8.33
Chilli Isolate (CCC)	3.14 [b]	2.57 [a]	3.14 [a]	2.95	8.57 [b]	7.29 [a]	8.71 [b]	8.19
Turmeric Isolate (CCT)	2.71 [a]	2.86 [a]	2.71 [a]	2.76	8.57 [b]	8.86 [b]	7.57 [a]	8.33
C.D. (0.05)	0.51	0.57	0.53	NS	0.7	0.53	0.65	NS
SE(m)	0.17	0.19	0.17		0.23	0.18	0.21	
C.V.	16.74	17.93	15.71		7.45	5.76	6.89	

REFERENCES

1. Cannon, P. F., Damm, U., Johnston, P. R. and Weir, B. S., Colletotrichum - Current status and future directions. *Studies in Mycology*. **73(1):** 181-213 (2012).
2. Damm, U., Cannon, P. F., Woudenberg, J. H. C and Crous, P. W., The *Colletotrichum acutatum* species complex. *Studies in Mycology*. **73:** 37-113 (2012a).
3. Dean, R., Van Kan, J. A. L., Pretotius, 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., The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*. **13:** 414-430 (2012).
4. Farr, D. F., Rossman, A. Y., Palm, M. E. and Mccray, E. B., Fungal databases, systematic botany and mycology laboratory, ARS, USDA. Available online at *http:llnt.ars-grin.govt/fungaldatabases* (2016).
5. Freeman, S., Shabi, E. and Katan, T., Characterization of *Colletotrichum acutatum* causing anthracnose of anemone (*Anmone coronaria* L.) *Applied and Environmental. Microbiology*. **66(12):** 5267 5272 (2000).
6. Garg, R., Loganathan, M., Saha, S. and Roy, B. K., Chilli anthracnose: A review of causal organism, resistance source and mapping of gene, In *Microbial Diversity and Biotechnology in Food Security*, India, Springer. 589-610 (2014).
7. Gupta, B. D. and Sen, C., Sensitivity of six isolates of *Colletotrichum capsici* towards fungicides. *Pestology*. **12:** 19-23 (1988).
8. Kothari, K. L. and Bhatnagar, M. K., Evaluation of fungicides against crop diseases - 1 on *Colletotrichum capsici*

causing blight of Gaur. *Indian Phytopathology*. **19:** 116 (1966).

9. Lakshmi, B. K. M., Reddy, P. N. and Prasad, R. D., Cross-infection potential of *Colletotrichum gloeosporioides* Penz. isolates causing anthracnose in subtropical fruit crops. *Tropical Agricultural Research.* **22:** 183-193 (2011).
10. Madhavan, S., Paranidharan, V. and Velazhahan, R., RAPD and virulence analyses of *Colletotrichum capsici* isolates from chilli (*Capsicum annuum*). *Journal of Plant Diseases and Protection.* **117(6):** 253-257 (2010).
11. Mills, P. R., Sreenivasa Prasad, S., Brown, A. E., Detection and differentiation of *Colletotrichum gloeosporioides* isolates using PCR FEMS. *Microbiology Letters.* **98:** 137-144 (1992).
12. Photita, W., Lumyong, S., Lumyong, P., McKenzie, E. H. C., Hyde, K. D., Are some endophytes of *Musa acuminata* latent pathogens? *Fungal Diversity*. **16:** 131-140 (2004).
13. Pring, R. J., Nash, C., Zakaria, M. and Bailey, J. A., Infection process and host range of *Colletotrichum capsici*. *Physiological and Molecular Plant Pathology*. **46:** 137-152 (1995).
14. Rangaswamy, G. and Mahadevan, A., Diseases of crop plants in India. *Prentice Hall of India Pvt. Ltd. New Delhi*. **4:** 607 (1999).
15. Samita, E. and Dubey, S. C., *Colletotrichum* blight – a new disease of bitter gourd. *Indian Phytopathology*. **56:** 503 (2003).
16. Sanders, G. M. and Korsten, L., A comparative morphology of South African avocado and mango isolates of *Colletotrichum gloeosporioides*. *Botany*. **81:** 877-885 (2003).
17. Sharma, P. N., Kaur, M., Sharma, O. P., Sharma, P. and Pathima, A., Morphological, pathological and molecular variability in *Colletotrichum capsici*, the cause of fruit rot of chillies in the subtropical region of north-western India. *Journal of Phytopathology*. **153:** 232 -237 (2005).
18. Shivas, R. G. and Tan, Y. P., A taxonomic reassessment of *Colletotrichum acutatum*, introducing *C. fioriniae* comb. et stat. nov. and *C. simmondsii* sp. nov. *Fungal Diversity*. **39:** 111-122 (2009).
19. Suparman, S., Rahmiyah, M., Pujiastuti, Y., Gunawan, B. and Arsi, Cross inoculation of anthracnose pathogens infecting various tropical fruits. IOP Conference Series: *Earth and Environmental Science*. 102. 012080. 10.1088/1755-1315/102/1/012080 (2018).
20. Than, P. P., Prihastuti, H., Phoulivong, S., Taylor, P. W. and Hyde, K. D., Chilli anthracnose disease caused by *Colletotrichum* species. *Journal of Zhejiang University Science B*. **9(10):** 764-788 (2008).
21. TU, J. C., A detached leaf technique fur screening beans (*Phaseolus vulgaris* L.) *in vitro* against anthracnose. *Canadian Journal of Plant Science*. **66:** 805-809 (1986).
22. Weir, B. S., Johnston, P. R. and Damm, U., The *Colletotrchum gloeosporioides* species complex. *Studies in Mycology*. **73:** 115–180 (2012).