Pathogenicity analysis of *Colletotrichum capsici* isolates from major Chilli (*Capsicum annuum*) growing areas of Tamil Nadu, India

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Abstract : The present objective of this study was to assess the diversity in isolates, identification and virulence of *Colletotrichum capsici* from major chilli growing areas in Tamil Nadu, India using the pathogenicity tests under pot culture conditions. Among the major chilli growing areas of Tamil Nadu, the maximum per cent disease index was recorded in Kovilpatti followed by Sattur and Sankarankovil. Whereas, the minimum disease incidence was recorded in Villupuram. Also, the nine isolates of *C. capsici* collected from various parts of Tamil Nadu exhibited a great variability with respect to mycelial growth, colony colour, colony pattern and Acervuli production. Among the nine isolates, the isolate Cc_3 collected from Kovilpatti, Tamil Nadu was significantly the most virulent one which recorded the highest fruit rot intensity and leaves infection.

Index Terms **- Disease index ,** ***Colletotrichum capsici,*** **Anthracnose, Pathogenicity tests**

I. INTRODUCTION

Chili (*Capsicum annum*.L) despite their fiery "hotness" is one of very popular spices known for medicinal and health benefiting properties. The chili plant is native to Central American region where it was used as the chief spice ingredient in Mexican cuisine for centuries. The major chilli growing countries are India, China, Korea, Nigeria, and Mexico. India ranks second among world chilli exporters and in India the state level analysis revealed Andhra Pradesh as the largest producer followed by Maharashtra and Karnataka. Chilli crop is affected by several fungal, bacterial and viral diseases, of which chilli anthracnose causes considerable damage, inflicting severe quantitative and qualitative losses (Anand *et al*., 2009; Anand *et al*., 2010). The estimated loss due to this disease ranged from 8 to 60 percent in different parts of India (Pandey and Pandey, 2003). The disease is caused by fungus *Colletotrichum capsici* Bisby and Butler that infect both unripe and ripe chilli fruits (Krairuan *et al*., 2008). *C.capsici* can survive in and on seed as acervuli and microsclerotia (Montri *et al*., 2009). *C. capsici* infection will be higher in the mature stage of chilli plant than in the early stage of plant (Krairuan *et al*., 2008). The disease is both seed borne and air borne and affects seed germination and vigour to a greater extent (Saxena *et al.,* 2016). The occurrence of different virulent strains of *C. capsici* has been well documented (Sharma *et al.,* 2005). Analysis of genetic diversity is one step towards understanding the pathogen population (Madhavan *et al.,* 2010). The objective of this study was to assess the diversity in isolates and identification of *C. capsici* from major chilli growing areas in Tamil Nadu, India using the pathogenicity tests under pot culture conditions.

II. MATERIALS AND METHODS

Survey for occurrence of fruits rots causes by *Colletotrichum capsici*

A field survey was conducted to assess the extent of fruit rot occurrence of chilly in major chilly growing areas of Tamil nadu state during 2015. The places *viz*., Ariyalur, Dharmapuri, Kovilpatti, Perambalore, Sankarankovil, Sattur, Sivapuri, Vallampadugai, and Vilupuram where chilli is traditionally grown are selected for assessing the prevalence of fruit rot disease caused by *C. capsici*. During survey plants affected due to fruit rot disease was found and also the total number of plants observed were counted and recorded. The percent disease incidence was worked out as per phytopathometry (Mayee and Datar, 1986). Also the infected plants showing the typical symptoms of leaf spot and fruit rot due to infection with *C. capsici* were collected for isolation of the pathogen.

Establishment of the isolates of *C. capsici*

The diseased chilli fruits showing the typical symptoms of fruit rot disease were collected fresh from nine conventional chilli growing areas of Tamil Nadu. The pathogens isolated from each of these localities formed one isolate of *C. capsici*. The pathogens were isolated on potato dextrose agar (PDA) medium from the diseased specimen showing the typical symptoms. The infected portion of the fruit was cut into small bits, surface sterilized in 0.1 per cent mercuric chloride solution for 30 sec., washed in repeated changes of sterile distilled water and plated onto PDA medium in sterilized Petri dishes. The plates were incubated at room temperature (28 ± 2ºC) for five days and were observed the fungal growth. The fungus was purified by single spore isolation technique (Rangaswamy, 1958) and identification of the isolate was confirmed by comparing with the culture obtained from ITCC, IARI, New Delhi and the purified isolates were maintained on PDA slants for further studies.

Evaluation of virulence of *C. capsici* isolates in pot culture condition

Five kilograms of top soil collected from chilli growing field was steam pasteurized and filled in 30 cm diameter earthen pots. One month old seedlings of var. K2 were transplanted in pots. The spore suspension ($5 \times 10^{-5} ml^{-1}$) of *C. capsici* was prepared from 20 days old culture grown on PDA slants using sterile distilled water (Rajapakse, 1998). Ninety days old plants were inoculated with spore suspension of *C. capsici* thoroughly over the plant canopy by pinpricking method. The inoculated plants were incubated in a growth chamber maintained at 28ºC. The intensity of fruit rot was calculated as per cent disease index (PDI) as per the grade chart proposed by Reddy (1982) using the formula proposed by Mc Kinney (1923).

Method of assessment of fruit rot incidence

Category value	Percent fruit area diseased
0	0
1	1-10
3	11-15
5	16-25
7	26-50
9	51 above

The per cent disease index (PDI) was worked out by using Mc Kinney (1923) infection index.

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Total no. of fruits observed}} \times \frac{100}{\text{Maximum disease grade in the score}}$$

III. RESULTS

Survey for the disease severity of chilli anthracnose incited by C. *capsici* (2015) in different locality of Tamil Nadu

The data presented in table 1 on the fixed plot survey conducted in Chilli growing areas of Tamil Nadu revealed the prevalence of fruit rot disease in all the villages surveyed. The maximum per cent disease index (18.42%) was recorded in Kovilpatti followed by Sattur (16.45%), Sankarankovil (14.47%), Sivapuri (13.33%), Ariyalur (11.11%), Dharmapuri (8.95 %), Perambalore (7.57%), Vallampadugai (6.41%) and Viluppuram (5.17%) in the decreasing order.

Culture variability among the isolates of *C. capsici* causing anthracnose in chilli

The results depicted in table 2 revealed that chilli fruits showing typical fruit rot and leaf spots symptoms were collected from nine conventional chilli growing areas of Tamil Nadu with a view to find out the pathogenic fungi involved in leaves and fruit rot of chilli in the different places. From the samples of fruit and leaves, the pathogen was isolated and purified. All the nine isolates of *C. capsici* exhibited a great variability with respect to mycelial growth, colony colour, colony pattern and Acervuli production. Maximum mycelial growth was recorded by Cc_3 (89.84 mm) isolate, followed by Cc_6 (86.87 mm), Cc_5 (84.92 mm), Cc_7 (79.86 mm), Cc_1 (75.16 mm), Cc_2 (69.48 mm), Cc_4 (65.86 mm), Cc_8 (63.42 mm) and Cc_9 (55.04). Colony colour and colony pattern also varied between the test isolates. Cc_3 isolates showed greyish and cottony growth, whereas Cc_6 was black and cottony growth. No of acervuli production also varied between the test isolates Cc_3 isolates showed 53.00 acervuli.

Virulence of *C. capsici* isolates

The isolate Cc_3 was significantly the most virulent one which recorded the highest fruit rot intensity (75.68 PDI) and leaves infection (65.09 PDI). This was followed by Cc_6 (71.43 PDI of fruit rot and 61.80 PDI of leaf infection), Cc_5 (67.17 PDI of fruit rot and 57.68 PDI of leaf infection) and Cc_7 (63.77 PDI of fruit rot and 53.56 PDI of leaf infection) while Cc_8 and Cc_9 were the least virulent one, which recorded 44.21, 39.96 PDI of fruit rot and 40.37, 36.25 PDI of leaf infection respectively (Table 3).

IV. DISCUSSION

Survey for the anthracnose disease severity in different localities of Tamil Nadu

The survey conducted to assess the anthracnose index of chilli in the major growing areas in Tamil Nadu revealed that the disease index range from 5.17 to 18.42 per cent showing the endemic nature of the disease (Table 1). The variation in the extent of the disease incidence might be due to the prevalence of the isolates of the pathogen differing in their virulence and the susceptibility of the host (Hossain *et al.*, 2010). Also, the results of the present study are similar to the findings of Pandey and Pandey (2003) that reported maximum disease incidence in Punjab and attributed growing of susceptible variety as the reason. In the present survey, the disease incidence was maximum in fruit ripening stage of the crop. Similar such observation was made by Sujatha bai (1992) who reported that secondary infection of chilli fruit mostly occur in matured ripening fruit.

Culture variability among the isolates of *C. capsici* causing anthracnose in chilli

In the present study, all the nine isolates of *C. capsici* exhibited a great variability in respect of colony diameter, colony colour, colony pattern and Acervuli production. These results on culture variability of *C. capsici* isolates observed in the present study are similar to the findings of several earlier workers. This variability may be due to variation in environment and biotypes, or strains of the pathogen (Cannon *et al*., 2000). Similar cultural variability among the isolates of *C. capsici* grown on PDA medium were reported earlier by several workers (Freeman *et al*., 1998; Grahovac *et al*., 2012; Sahitya *et al*., 2014).

Evaluation of virulence of *C. capsici* isolates in pot culture condition

Among the isolates tested, the isolate Cc_3 was significantly the most virulent one followed by Cc_6 while Cc_8 and Cc_9 were found to be the least virulent as evidenced by the degree of disease intensity on the host. The variation in virulence of *C. capsici* isolates was also established by (Denoyes *et al.*, 2003). Ali *et al*., (2002) reported that virulence of pathogen differed from locality to locality with the change of temperature, humidity and rainfall. The plant which shows disease resistant or susceptible depends on genotypes of variety. The resistance to disease should be based on knowledge of infection and pathogenicity of the fungus. All these earlier reports corroborated and lend support to the present finding.

Table 1. Survey of disease severity of chilli anthracnose in different localities of Tamil Nadu

S. No.	Locality	Variety	Crop stage	* Disease severity (%)
1.	Ariyalur	Juwala	Fruiting	11.11[e]
2.	Dharmapuri	K-1	Fruiting	8.95[f]
3.	Kovilpatti	K-2	Fruiting	18.42[a]
4.	Perambalore	Mdu-1	Vegetative	7.57[g]
5.	Sankarankovil	K-2	Fruiting	14.47[c]
6.	Sattur	K-2	Fruiting	16.45[b]
7.	Sivapuri	Co-1	Fruiting	13.33[d]
8.	Vallampadugai	K-1	Vegetative	6.41[h]
9.	Viluppuram	Co-1	Vegetative	5.17[i]

* Values in the column followed by same letters not differ significantly by MRT(P=0.05)

Table 2. Isolation and identification of various isolates of *C. capsici* causing anthracnose in chilli from different parts of Tamil Nadu

Isolates	Locality	Plant part used for isolation	*Colony diameter (mm)	Colony colour/ pigmentation	Colony pattern	Acervuli production	
						No. of setae/acervuli	No. of septa/setae
Cc_1	Ariyalur	Fruits	75.16[e]	White	Cottony growth	38.00	2-5
Cc_2	Dharmapuri	Fruits	69.48[f]	White	Cottony growth	34.00	3-5
Cc_3	Kovilpatti	Fruits	89.84[a]	Greyish	Cottony growth	53.00	2-6
Cc_4	Perambalore	Leaves	65.86[g]	Light brownish	Fluffy growth	33.00	4-6
Cc_5	Sankarankovil	Fruits	84.92[c]	Black	Fluffy growth	44.00	2-5
Cc_6	Sattur	Fruits	86.87[b]	Black	Cottony growth	49.00	4-5
Cc_7	Sivapuri	Fruits	79.86[d]	White	Cottony growth	42.00	2-6
Cc_8	Vallampadugai	Leaves	63.42[h]	White	Fluffy growth	31.00	2-4
Cc_9	Viluppuram	Leaves	55.04[i]	White	Fluffy growth	30.00	3-4

* Values in the column followed by same letters not differ significantly by DMRT (P=0.05)

Table 3. Evaluation of virulence of *C. capsici* isolates in pot culture condition

Isolates	*Percent fruits infected	Percent disease index	*Percent leaves infected	Percent disease index
Cc_1	73.34[e]	58.67	62.83[e]	50.26
Cc_2	68.03[f]	54.42	58.71[f]	46.96
Cc_3	94.60[a]	75.68	81.37[a]	65.09
Cc_4	62.71[g]	50.16	54.59[g]	43.67
Cc_5	83.97[c]	67.17	72.10[c]	57.68
Cc_6	89.29[b]	71.43	77.25[b]	61.80
Cc_7	79.72[d]	63.77	66.95[d]	53.56
Cc_8	55.27[h]	44.21	50.47[h]	40.37
Cc_9	49.96[i]	39.96	45.32[i]	36.25

***Values in the column followed by same letters not differ significantly by DMRT (P=0.05)

REFERENCES

[1] Ali, M.A., Fakir, G.A. and sarker, A.K. 2002. Research on seed borne fungal diseases of spices in Bangladesh Agricultural University. *Bangladesh J. Train. Develp.* 15: 245-250

[2] Anand, T., Chandrasekaran, A., Raguchander, T., Prakasam, V. and Samiyappan, R. 2009. Chemical and biological treatments for enhancing resistance in chilli against *Colletotrichum capsici* and *Leveillula taurica*. *Archives of Phytopathol*. and *Pl. Prot.*, 42(6): 533- 551.

[3] Anand,T., A. Chandrasekaran, S. Kuttalum, S. Senthilraja, and R. Samiyappan. 2010. Integrated control of fruit rot and powdery mildew of chilli using the biological agent *Pseudomonas fluorescens* and a chemical fungicide. *Bio control*., 52:1-7.

[4] Cannon, P.F., Bridge , P.D. and Monte, E. 2000. Linking the past, present and future of *Colletotrichum* systamatics. In: Prusky D, Freeman, S., Dickman M.B (eds) *Colletotrichum gloesporioides* host specificity , Pathology, and pathogen intraction :APS press, Minnesota, USA, pp 1-20.

[5] Denoyes, B., Guérin, G., Délye, C., Smith, B., Minz, D., Maymon, M and Freeman, S. 2003. Genetic diversity and pathogenic variability among isolates of *Colletotrichum* species from strawberry. *Phytopathology*, 93: 219-228.

[6] Freeman, S., Katan, T. and Shabi, E. 1998. Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. *Plant Dis.* 82: 596-605.

[7] Grahovac, M., Indic, D., Vukovic, S., Hrustc, J., Gvozdenac, S., Mihajlovic, M. and Tanovic, B. 2012. Morphological and ecological features as differentiation criteria for *Colletotrichum* species. *Zemdirbyste Agric*. 99(2): 189- 196.
[8] Hossain, M.T., Hossain, M.M.S., Bakr, A.M., Matiar Rahman, M.K.A. and Uddin, N.S., 2010. Survey on major diseases of vegetable and fruit crops in chittagong region. *Bangladesh J. Agril. Res.* 35: 423-429.
[9] Krairuan, S., Sangchote, and S. Sukprakarn. 2008. Effect of capsicum on germination of *Colletotrichum capsici* conidia. *Kasetsar J. Nat. Sci*., 42: 417-422.
[10] Madhavan, S., Paranidharan, V. and Velazhahan, R. 2010. RAPD and virulence analyses of *Colletotrichum capsici* isolates from chilli (*Capsicum annuum*). *Journal of Plant Disease and Protection.* 117 (6): 253-257.
[11] Mayee, D.C. and Datar, V.V. 1986. Phytopathometry. Technical Bull- 1. Marathwada Agricultural University, Parbhani. Pp:144.
[12] Mc Kinney, H.H. 1923. A new system of growing grading plant diseases. *J. Agric. Res.* 26: 195-218.
[13] Montri, P., Taylor, P.W.J. and Mongkolporn, O. 2009. Pathotype of *Collectotrichum capsici*, the causal agent of chilli anthracnose, in Thailand. *Plant disease* 93:17-20.
[14] Pandey, K.K. and Pandey, P.K. 2003. Survey and surveillance of vegetable growing areas for prevalence of major diseases. *Vegetable sci*., 30: 128-134.
[15] Rajapakse, R.G.A.S .1998. Observation on the anththracnose of chilli pepper(*Capsicum annum* L.) caused by *Colletotrichum* species in Srilanka. Ph.D. Thesis University of of London, UK.
[16] Rangaswamy, N.S. 1958. Culture of nucellar tissue of citrus *in vitro*. *Experienta* 14:11-12.
[17] Reddy, R.M. 1982. Evaluation of fungicides against major diseases of chilli. *M.Sc. (Ag.), Thesis*, Tamil Nadu Agric. Univ., Coimbatore, India, p. 64.
[18] Sahitya, L., Sri Deepthi, R., Kasim, P., Suneetha, P. and Krishna, M.S.R. 2014. Anthracnose a Prevalent Disease in *Capsicum. Res. J. Pharm. Biol. Chem. Sci.* 5(3): 1-22.
[19] Saxena, A., Raghuwanshi, R., Gupta, V.K. and Singh, H.B. 2016. Chilli Anthracnose: The Epidemiology and Management. *Front. Microbiol. 7:1527. doi: 10.3389/fmicb.2016.01527*.
[20] Sharma, P.N., Kaur, M., Sharma, O.P., Sharma, P. and Pathania, A. 2005. Morphological, pathological and molecular variability in *Colletotrichum capsici*, the cause of fruit rot of chillies in the subtropical region of north-western India. *J Phytopathol*. 153: 232-237.
[21] Sujatha bai, E. 1992. Studies on fruit rot of chilli (*Capsicum annuum* L.) caused by *Alternaria tenuis* Nees. *M.Sc. Thesis*, Tamil Nadu Agric. Univ., Madurai, India, 173pp.