Standardization of methods for pathogenicity of chilli fruit rot caused by *Colletotrichum capsici* (Syd.) Butler and Bisby

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ABSTRACT

Chilli is the foremost important spice crop of India. It is affected by many diseases, among which chilli fruit rot is a major fungal disease that causes severe yield loss. A rowing survey was conducted in different places of Tamil Nadu. The overall disease severity ranges from 19 per cent to 3.6 per cent. The highest fruit rot disease incidence was recorded in Kovilpatti and the least was recorded in Sathanoor. The pathogenicity of these isolates through different inoculation methods on unfascinated semi ripe chilli fruit variety K1 revealed that *C. capsici* CcI_{17} was most virulent with an average lesion size of 16.5 mm and a least lesion size observed in CcI_3 was 4.9 mm. Among the twenty isolates, the isolate CcI_{17} was significantly the most virulent one, which recorded the highest fruit rot intensity (65.8 %) and leaf infection (59.7 %) followed by CcI_{19} which was the least virulent. In the present study, an exhaustive survey was conducted in Tamil Nadu during 2019 to know the incidence and severity of the fruit rot disease in a large scale basis.

Keywords: *Colletotrichum capsici*, pathogenicity, virulence, chilli.

MS History: 03.09.2019 (Received) - 06.09.2019 (Revised) - 4.12.2019 (Accepted).

Citation: Charumathi, M. and Suthin Raj, T. 2019. Standardization of methods for pathogenicity of chilli fruit rot caused by *Colletotrichum capsici* (Syd.) Butler and Bisby. *Journal of Biopesticides,* **12**(2): 261-267.

INTRODUCTION

Chilli is one of the important confectionery crops known for its attractive natural colour throughout the world, which originated from South Central America. It was introduced in India by the Portuguese in Goa in the middle of 17th century (Indira *et al.*, 2007). India is its largest producer with 36% share in the global production (Sahitya *et al.*, 2014). It contains an alkaloid capsaicin which has a strong spicy pungent character with anti-bacterial, anti-carcinogenic, analgesic and anti-diabetic properties. It also reduces the LDL cholesterol levels in obese individuals. Fresh chilli peppers, red and green are rich sources of vitamin-C,vitamin A and flavonoids like ß-carotene, α-carotene, lutein, zea-xanthin, cryptoxanthin, B-complex (niacin, pyridoxine (vitamin B6), riboflavin and thiamin (vitamin B1, potassium, manganese, iron and magnesium (Parey *et al,*. 2013).

Chilli is prone to a number of fungal (Walker, 1952), bacterial and viral diseases which significantly affect its production and quality. However, huge losses to the crop are incurred mostly by fungal diseases. Of these, dieback and fruit rot have assumed the status of major disease in some important chilli growing countries. Anthracnose causes extensive pre and post-harvest damage to chilli fruits causing lesions. Even small anthracnose lesions on chilli fruits reduced its marketable value (Ahila Devi and Prakasam, 2016). *Colletotrichium capcisi* is a powerful adhesive to the plant surface and remains latent until such physiological changes occur in the fruit and cause economic loss to the farmers due to low fruit quality many post-harvest diseases of fruit exhibit the phenomenon of quiescence in which symptoms do not develop until the fruit ripens. Chilli anthracnose usually develops under high humid conditions when rain occurs after the fruits have started to ripen with reported losses of up to 84% (Goswami *et al.*, 2013). Typical fruit symptoms are circular or

angular sunken lesions, with concentric rings of acervuli that are often wet and produce pink to orange conidial masses. Under severe disease pressure, lesions may coalesce. Conidial masses may also occur scatteredly or in concentric rings on the lesions (Than *et al*. 2008). On the leaves, small-circular spots appear. Severely infected leaves fall off leading to defoliation. The infection of growing tips leads necrosis of branches which progresses backward on the diseased branches (Dieback Stage).The die back may kill the whole plant (Kumar M and Bhaskaran 2007). Economic losses caused by the disease are mainly attributed to lower quality and marketability. In this context, the present study was carried out to evaluate the virulence and pathogenicity of various isolates of *C. capsici.*

MATERIALS AND METHODS

Disease survey

Rowing survey was conducted to obtain chilli fruit rot incidence in various chilli growing areas of Tamil Nadu. In each field, five plots each with 5×5m area were selected. Among them, one plot was fixed at the centre of the field and the remaining four plots were fixed at random in different places and kept away from border rows. Affected chilli plants were collected in polybags along with soil samples and labeled properly. They were brought to the laboratory and stored in a refrigerated condition for future studies. The fruit rot incidence was assessed by counting the number of infected plants out of the total number of plants in each plot ($25m^2$). In each area, three fields were assessed and the mean disease incidence was calculated. The per cent disease incidence was calculated using the following formula (Karthik Pandi et al. 2018)

$$\text{Percent disease incidence} = \frac{\text{Number of plants affected} \times 100}{\text{Total number of plants observed}}$$

Isolation of the pathogen

A small portion of the infected tissue (2-3 mm size) was cut at the juncture of the diseased and healthy portion with the help of a disinfected blade after surface sterilizing the sample with alcohol. These bits were surface sterilized in 1% per cent sodium hypochlorite solution for about 20 seconds followed by three times washing with sterilized distilled water in petriplates, under aseptic conditions using a laminar air flow chamber to remove the traces of sodium hypochlorite. After blot drying with sterilized filter paper, these bits were transferred to potato dextrose agar (PDA) medium in sterilized petriplates. Three such bits were placed in each petriplates and incubated in a BOD incubator for three days at 28±2°C. The culture thus obtained was subjected to purification. The pure culture thus obtained was maintained on PDA slants (Choi *et al.,* 1999). They were then incubated at 28±2°C in a Biological Oxygen Demand (BOD) incubator. The cultures were revived after every month and maintained throughout the study period on PDA in sealed culture tubes at 5°C in a refrigerator. The above procedure was adopted with respect to all the twenty isolates collected from different parts of Tamil Nadu.

Method of inoculation

The pathogenicity of each isolate was tested by four different inoculation methods under laboratory condition on unfastened non-fruits (turning red) of chilli (Suthin Raj, 2011). The methods of pathogenicity test were:Pin prick (PP) method – A sterilized needle was used and two pricks were given on the fruit, prior to inoculation through spraying of the spore suspension. Spore suspension spray (SSS) – The spore suspension was applied with the help of an atomizer and the entire surface of fruit was covered with the spray Spore suspension injection (SSI) – Spore suspension was injected into the fruit with the help of a sterile syringe under aseptic conditions. Spore suspension dipping (SSD) – Dipping the fruit in spore suspension. The spore suspension of different isolates was derived by adding 10 ml of sterilized distilled water to fifteen- days- old

culture grown on PDA in 30 ml test tubes. The spore concentration was determined using a haemocytometer and adjusted to 10^6 conidia / ml with sterile water. The chilli fruit collected from various places were surface sterilized with a surfactant, 1 % sodium hypochlorite solution for 30 seconds and washed with distilled water thrice to remove any trace of sodium hypochlorite. These chillies were air dried by placing sterilized blotter paper and the spore suspension was inoculated using different methods already mentioned earlier. The inoculated fruits were placed in the moist chambers maintained in glass jars under wet cotton swabs and incubated at 25± 2°C. The disease development was recorded by measuring lesion length of the diseased portion after 8 days of inoculation.

Pathogenicity and virulence of the pathogen.

In this study, 20 isolates were checked for their virulence ability by pathogenicity test. Five kilograms of top soil collected from chilli growing fields was steam pasteurized and filled in 30 cm diameter earthern pots. One - month-old seedlings of var. K2 were transplanted into pots and three replications were maintained. The isolates were cultured on PDA at 27°C under aseptic condition. Prior- to-inoculation, chilli fruits were pin pricked gently with a sterilized needle. Conidia from fifteen-day-old cultures were harvested by adding 5-10mL of sterilized distilled water to the surface of the cultures, brushing with a soft bristle brush and filtering through a double layer of cheesecloth. The spore concentration was determined using a haemocytometer and was adjusted to 10^6 conidia / ml with sterile water. Fruits used as control were inoculated with 20ul of sterile distilled water and was used as uninoculated controls. The symptoms were evaluated 7-15 days after inoculation (DAI). The number of fruits infected and the intensity of fruit rot were also calculated. The intensity of fruit rot was calculated as Per cent Disease Index (PDI) as per the grade chart using the formula given below (Karthik Pandi *et al.,* 2018)

Score description		
Grade	**Per cent fruit infection**	**Disease reaction**
0	0%	Immune
1	1-10%	Resistant
3	11-15%	Moderately resistant
5	26-50%	Moderately resistant
7	51-75%	Moderately susceptible
9	>75%	Highly susceptible

$$PDI = \frac{\text{Sum of numerical values}}{\text{Number of plants observed} \times \text{maximum disease rating}} \times 100$$

Experimental design and data analysis

The experiments were conducted following the Completely Randomized Design (CRD) with three replications. The significant difference, if any, among the means was compared by Duncan's Multiple Range Test (DMRT). Whenever necessary, the data were transformed before statistical analysis following appropriate methods.

RESULTS

A field survey was conducted during 2018-2019 to assess the intensity of fruit rot incidence in various places of Tamil Nadu. In all the places, incidence of fruit rot was recorded during different stages of crop growth (Table 1). The disease incidence ranged from 3.6 to 19 per cent. The highest incidence was recorded at Kovilpatti (19%) while it was the least in Sathanoor (3.6 per cent). The pathogenicity test manifests the method of inoculation among the four methods like pin pricking, spore suspension assay, spore suspension injection and spore suspension dip. Among them, pin pricking method showed the highest length of disease lesion which varied from 4.9 mm to 16.5 mm while CcI_{17} showed the highest per cent of disease incidence (Table 2).

Table 1. Survey of disease incidence of chilli *C. capsici* in different localities of Tamil Nadu

Locality	Crop stage	Variety	Disease Incidence (%)
Manamadurai	Vegetative	MDU-1	10.1 [f]
Sivagangai	Fruiting	MDU-1	8.9 [h]
Sathanoor	Fruiting	CO-1	3.6 [n]
Krishnagiri	Fruiting	CO-1	7.2 [i]
Dharmapuri	Vegetative	CO-1	7.1 [i]
Naiyandipuram	Fruiting	MDU-1	9.2 [gh]
Sivapuri	Fruiting	CO-1	13.6 [d]
Balethottam	Vegetative	K-1	9.4 [g]
Namakkal	Fruiting	C0-1	12.9 [e]
Paramakudi	Fruiting	K-2	14.1 [c]
Puthumottur	Fruiting	CO-1	7.0 [i]
Kalorpathi	Fruiting	Palur	9.0 [gh]
Karadiyur	Vegetative	CO-1	13.1 [e]
Kannadhahalli	Fruiting	K-2	5.2 [kl]
Santhur	Fruiting	K-2	6.1 [j]
Theni	Fruiting	CO-1	5.4 [k]
Kovilpatti	Vegetative	K-2	19 [a]
Sattur	Fruiting	MDU-1	4.7 [m]
Rajapalayam	Fruiting	MDU-1	17 [b]
Pollachi	Fruiting	K-1	4.9 [lm]

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

The isolate CcI_{17} was significantly the most virulent one which recorded a highest fruit rot intensity and leaves infection. This was followed by CcI_{19} (63.4 PDI of fruit rot and 57.3 PDI of leaf infection) and CcI_{10} (60.2 PDI of fruit rot and 50.1 PDI of leaf infection) while CcI_3 was the least virulent one, which recorded 9.2 PDI of fruit rot and 4.2 PDI of leaf infection respectively (Table 3).

DISCUSSION

Voorrips (2004) investigated chilli fruit rot incidence in important chilli growing states of India like Andhra Pradesh (49%), Karnataka (15%) Maharasthra (6%) and Tamil Nadu (3%) which constitutes nearly 75 per cent of the total area under chilli. Karthik Pandi *et al.* (2018) observed fruit rot of chilli caused by *Colletotrichum* during his survey and recorded a maximum incidence of fruit rot (60.10%) in Perumalpatty in the Theni district and a lower incidence at Vathrappu in Virudhunagar district, Shilpa *et al.* (2017) also revealed fruit rot disease incidence in four different districts like Virudhunagar, Kovilpatti, Tirunelveli and Thootukudi in kharif 2013. The highest disease incidence was noticed in Belagari district (59.14 per cent) and the least disease incidence at Gadog district (19.21 per cent). Gupta *et al.* (2017) reported lesions produced by piercing sterilized toothpick tips infested with *Colletotrichum capsici* into chilli fruits followed by incubation of chilli fruits in a moist chamber. Bhale *et al.* (1999) reported that pin-pricking method was the best method of inoculation of *Colletotrichum dematium* on chilli fruits when compared with other methods like spore suspension spray, spore suspension dip, tooth pick prick, carborandum rub and the injection method. In fruits and leaves, *C.capsici* was inoculated [pinpricking + spore suspension spray] which recorded a

Table 2. Reaction of pathogen isolates on unfasinated semi-ripe fruits by using different inoculation technique

Pathogen / isolate	Lesion size (mm) after 8 days of incubation with inoculation method				
	PP*	SSS*	SSI*	SSD*	Average
CcI_1	9.3	7.1	9.0	6.6	8.0^{efg}
CcI_2	8.3	5.9	8.2	5.3	6.9^{ghj}
CcI_3	4.9	1.9	4.5	3.0	3.6^{n}
CcI_4	8.1	5.0	7.9	5.0	6.5^{hij}
CcI_5	7.8	5.3	7.2	4.7	6.3^{hijk}
CcI_6	8.9	6.5	8.8	5.9	7.5^{fgh}
CcI_7	12.1	7.9	10.5	7.3	9.5^{bcd}
CcI_8	9.5	6.8	9.3	6.1	7.9^{efg}
CcI_9	10.5	7.4	9.7	6.7	8.6^{def}
CcI_{10}	13.4	8.4	10.8	7.5	10.0^{abc}
CcI_{11}	7.4	4.4	6.9	4.4	5.8^{ijkl}
CcI_{12}	8.7	6.3	8.5	5.5	7.2^{gh}
CcI_{13}	11.3	7.7	10.1	7.0	9.0^{cde}
CcI_{14}	6.2	3.6	5.8	3.9	4.9^{lmn}
CcI_{15}	7.0	4.0	6.3	4.2	5.4^{jkl}
CcI_{16}	6.5	3.9	6.0	4.0	5.1^{klm}
CcI_{17}	16.5	9.1	11.3	8.1	11.3^{a}
CcI_{18}	5.2	2.0	5.0	3.2	3.9^{mn}
CcI_{19}	15.2	8.9	11.0	7.7	10.7^{ab}
CcI_{20}	5.9	3.2	5.5	3.5	4.5^{lmn}

PP -Pin prick, SSS - Spore suspension assay, SSI - Spore suspension injection, SSD - Spore suspension dip

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

Isolation	Per cent fruits infected	PDI	Per cent leaves infected	PDI
CcI_1	$68.00(55.55)^{f}$	51.1	$61.00(51.35)^{f}$	42.7
CcI_2	$59.00(50.18)^{j}$	40.8	$49.00(44.43)^{j}$	39.9
CcI_3	$10.00(18.43)^{t}$	9.2	$6.00(14.18)^{t}$	4.2
CcI_4	$57.00(49.02)^{k}$	37.3	$47.00(43.28)^{k}$	32.1
CcI_5	$54.00(47.29)^{l}$	34.1	$45.00(42.13)^{l}$	30.5
CcI_6	$63.00(52.54)^{h}$	47.6	$55.00(47.87)^{h}$	37.4
CcI_7	$76.00(60.67)^{c}$	58.1	$69.00(56.17)^{c}$	48.3
CcI_8	$65.00(53.73)^{g}$	49.3	$57.00(49.02)^{g}$	40.3
CcI_9	$70.00(56.79)^{e}$	54.7	$63.00(52.54)^{e}$	44.2
CcI_{10}	$79.00(62.73)^{b}$	60.2	$70.00(56.79)^{c}$	50.1
CcI_{11}	$50.00(45.00)^{m}$	30.8	$40.00(39.23)^{m}$	27.4
CcI_{12}	$60.00(50.77)^{i}$	44.4	$52.00(46.14)^{i}$	35.2
CcI_{13}	$73.00(58.69)^{d}$	56.3	$66.00(54.33)^{d}$	46.7
CcI_{14}	$40.00(39.23)^{q}$	23.2	$30.00(33.21)^{q}$	18.4
CcI_{15}	$47.00(43.28)^{n}$	29.6	$37.00(37.46)^{n}$	24.7
CcI_{16}	$44.00(41.55)^{op}$	25.1	$34.00(35.67)^{op}$	20.2
CcI_{17}	$84.00(66.42)^{a}$	65.8	$76.00(60.67)^{a}$	59.7
CcI_{18}	$20.00(26.57)^{s}$	18.1	$12.00(20.27)^{s}$	9.4
CcI_{19}	$80.00(63.43)^{b}$	63.4	$74.00(59.34)^{b}$	57.3
CcI_{20}	$30.00(33.21)^{r}$	20.9	$20.00(26.57)^{r}$	11.3

mean per cent infection as 60 and 50 per cent respectively and the isolate *C.capsici* (Cc1) was significantly the most virulent one which recorded the highest fruit rot intensity (69.6 PDI) and leaf infection. The incidence of infection on chilli leaves was found to be less when compared to that of its fruits (Suthin Raj *et al*. 2013).

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