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Development of variety screening method for anthracnose disease of chilli (*Capsicum annum* L.) under field conditions

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ABSTRACT

Anthracnose disease significantly affect the yield and fruit quality of chilli (*Capsicum annum* L.). None of the chilli varieties was resistant to anthracnose but there was no reliable method to identify resistant varieties. Therefore, experiments were conducted to develop a suitable variety screening method to identify anthracnose resistant varieties of chilli under field conditions. The pathogen found in the North-Central region of Sri Lanka was *Colletotrichum capsici*. Conidia of *Colletotrichum capsici* germinate and differentiate into appressoria on fruit surfaces at all maturity stages, but the highest rates occurred on surfaces of red ripe fruits. Anthracnose lesions initiate after fruits had ripened. These findings showed that chilli fruits at red ripe stage are more susceptible to *Colletotrichum capsici* than green fruits. Suitable method of variety screening against anthracnose is spraying of spore suspensions of *Colletotrichum capsici* (10^5 conidia ml^{-1} water) at red ripe stage of fruits followed by spraying water from next day for a one week period at a frequency of two times per day. Percentage incidence of anthracnose affected fruits could be used to identify resistant varieties.

Key words: Appressoria, *Colletotrichum capsici*, Conidia, *Capsicum annum*

INTRODUCTION

Anthracnose disease is one of the major constraints to profitable cultivation of chilli (*Capsicum annum* L.) during North-East monsoon season. Recently, serious incidences of anthracnose of chilli were observed in many parts of Sri Lanka (Anon 1993,1994). Losses occurred in the field, during transit, and storage. In addition to direct losses, the disease impairs quality so that even saleable fruit commands a lower price than would be obtained in the absence of the disease. It has been reported that apart from pre-harvest losses, fruit quality deterioration of chilli due to anthracnose range from 21 - 47% in Sri Lanka (Anon 1993). Yield losses of 15% have been recorded in Korea (Kim & Park 1988) and about 50% in Malaysia (Sariah 1994). However, estimates of losses are very high and varied with climatic conditions of the growing season (Kim & Park 1988).

Anthracnose of chilli, in common with similar disease of other crops, is caused by species of the genus *Colletotrichum* (Agrios 1997). Characteristically, it is predominantly associated with lesions on mature fruits, but also causes dieback of stems and branches in chilli (Abeygunawardhana 1969). Several species of *Colletotrichum* have been implicated in the anthracnose disease of chilli. Five species, *Colletotrichum capsici* (Sydow) Butl. & Bisby, *Colletotrichum gloeosporioides* (Pent.) Penz.& Sacc., *Colletotrichum acutatum* Simmonds, *Colletotrichum coccodes* (Wallr.) Hughes, *Colletotrichum graminicola* (Ces.) Wils., have been reported to cause anthracnose of pepper from various parts of the world (Hadden & Black 1988). *C. capsici* has been identified as the major pathogen in anthracnose of chilli in some countries (Sariah 1994). *C. gloeosporioides* was also frequently isolated. *C. acutatum* and *C. coccodes* were isolated but at very low frequency, from chilli plants in Malaysia (Sariah 1994). Two species of *Colletotrichum* i.e. *C. capsici* and *C. gloeosporioides* have been identified as causal agents of anthracnose disease of chilli in Sri Lanka (Rajapakse 1998).

Models for predicting of disease progress based on weather analysis in relation to the likelihood of disease development have been constructed for

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Abbreviations: PDA-Potato Dextrose Agar, SDW-Sterile Distilled water

many diseases. Association of high relative humidity or rainfall frequency and high temperature with anthracnose epidemics on chilli plants has been recognized, but relative humidity was found to be the most important climatic parameter related to anthracnose development of chilli (Kim & Park 1988). Inocula for epidemics can come from various sources such as residues of previous crops or soil and surrounding alternate hosts. Therefore, the degree of anthracnose incidence could vary according to seasonal condition.

To date, research on anthracnose disease in Sri Lanka was mainly directed towards the development of chemical control methods and also to identify resistant varieties. As a result, several control measures have been developed and recommended to farmers. These measures included the use of systemic and contact fungicides as seed dressings and foliar sprays. However, they have not provided a satisfactory level of control, especially in wet conditions (Anon 1993). Most economical way to minimize crop losses due to disease is cultivation of resistant varieties. However, there is no reliable method to identify anthracnose resistant varieties of chilli. At present, resistance of chilli variety against anthracnose pathogen is measured under natural infection or artificial spraying of inoculum suspension onto the fruits without considering mode of anthracnose development on fruit surfaces. Thus, none of the cultivars in current use is resistant (Anon 1994). Therefore, development of an effective variety screening method is an important requirement to minimize crop losses by identifying anthracnose resistant chilli varieties.

MATERIALS AND METHODS

Isolation of pathogen

Anthracnose affected chilli fruits were collected from farmers' fields in different locations in the North-Central region. Pathogen was isolated from anthracnose lesions of disease-affected fruits cultured on Potato Dextrose Agar (PDA). Isolates of pathogen were collected from single conidia. Pathogen was identified on the basis of size and morphology of sprouting acervuli, conidia, setae and morphology on culture medium and microscopic observations. Isolates of pathogen were stored in PDA slants for further studies. Pathogenicity of all isolates was tested by wound inoculation with conidia suspension (pin prick method) and subsequent anthracnose development on fruit surfaces.

Pathogen behaviour on fruit surface

Conidia germination, appressoria formation and anthracnose development of pathogen on the surface of fruits at various stages of maturity were determined. Sets of chilli fruits belonging to light green immature (2-3 cm long fruits), dark green mature (fully grown fruits before red colour initiation) and red ripe fruits were obtained from healthy chilli plants of variety MI-2. Fruits were carefully detached from plants and washed with sterile distilled water (SDW) and then wiped with cotton wool soaked in ethanol to reduce microbes on the surface. It has been reported that suitable concentration of conidia in inoculum drop is $5x10^5$ conidia per ml of water (Rajapakse 1998). Therefore, suspension of conidia ($5x10^5$ conidia ml^{-1} water) of pathogen isolate was prepared and drops (10µ) of conidia suspension were placed on two sites of fruit surfaces. Similarly, another set of fruits was wound-inoculated with conidia suspension to observed anthracnose lesion development on fruit surfaces. Then fruits were transferred into humid plastic boxes lined with moisture paper pre-soaked in SDW. The experiment involved a completely randomised design with three replicates.

For counting of germination of conidia on the fruit surface, very thin slices were removed from the peel at the inoculated sites and these were fixed in 0.3% trypan blue lactophenol solution on glass slides. Counts for conidia germination and appressoria formation were made in two microscopic fields within the inoculated sites of each fruit at 16 hours after incubation. Wound inoculated fruits were incubated for 10 days to observe lesion development on the surface. The experiments were repeated twice and the results presented represent the bulked data analysed by ANOVA. Mean separation were done by Duncan's multiple range test using a statistical programme MSTATC, developed by Michigan State University, U.S.A.

Field studies

The experiment was conducted at the FCRDI, Maha Illuppallama during North-east monsoon season in 1998 and South-west monsoon season in 1999. One month old seedlings of chilli variety MI-2 were planted in the field at 60 cm x 45 cm spacing. Plot size was 3 m x 3 m. All cultural practices were done according to the recommendation of Department of Agriculture (Anon 1990). Weeding was done manually. Insecticide (Imidacloprid 200g/l SC) was applied for the control of leaf curl complex of chilli. Artificial inoculation was practiced on chilli plants

which survived the fruit bearing stage. Before artificial inoculation three stages of fruits belonging to light green immature, dark green mature and red ripe were kept in the plants for respective treatments and others were picked off from plants. Experiment was arranged in a Randomised Complete Block design with four replicates.

Treatments were-

T1) Plant bearing red fruits only + inoculation with conidia
T2) plants bearing dark green mature fruits only + inoculation with conidia
T3) plants bearing light green immature fruits only + inoculation with conidia
T4) Plants bearing fruits belonging to all maturity stages + inoculation with conidia
T5) Plant bearing red fruits only without inoculation
T6) plants bearing dark green mature fruits only without inoculation
T7) plants bearing light green immature fruits only without inoculation
T8) Plants bearing fruits belonging to all maturity stages without inoculation

Artificial inoculation was done by spraying suspension of conidia (5x 10^5 conidia ml^{-1} water) evenly onto the chilli plants in respective treatments using hand sprayer. Un-inoculated plots served as controls. Then, canopy of all plants was watered from next day morning up to one week period by spraying water, two times per day at morning and evening using Knapsack sprayer to stimulate conidial differentiation on fruit surfaces. Finally fruits were picked at fully red ripe stage and the number of anthracnose affected fruits were counted. The percentage incidences of anthracnose in each plot was then calculated. Data were analysed by ANOVA and mean separation were done by Duncan's multiple range test using a statistical programme MSTATC.

RESULTS AND DISCUSSION

Characters of the fungal isolates

Isolates were identified by comparison of their colony characteristics and morphology on PDA with published data. According to the colony morphology on PDA medium and the shape of conidia, setae and acervuli, all isolates were identified as *C. capsici*. Isolates produced a large number of black acervuli on culture media at early stages (3 days) which later became light brown or

Table 1. Characters of fungal isolates collected from anthracnose affected chilli fruits.

Colony colour on PDA	White initially then turned brown or light brown, colony margin smooth
Reverse colony colour on PDA	Brown or dark brown
Acervuli	Black colour masses, conidia present inside.
Setae	Black colour, straight, septate, 60-192 µm in length & 4-6 µm in width
Conidia	Sickle shaped, aseptate, vacuole present in centre, 16-32 µm in length & 2-4 µm in width. Conidia germinate and produce appressoria at the end of germ-tube on plant surfaces, but rarely in distilled water
Appressoria on pod surface	Oval shape, abundant and black colour

brown after 14 days. Acervuli of all isolates were round to elongate and consisted of conidia and setae (Table 1). Conidia size varied within isolates and between isolates but generally ranged from 16-32 µm in length, 2-4 µm in width. Setae were dark brown, septate, normally with 5 cells, rigid, sharp at the tip and swollen at the base. Setae size varied within the isolates and among the isolates, but generally ranged from 60-192 µm in length, 4-6 µm in width (Table 1). Conidia from all isolates showed a similar shape. Colonies on PDA were at first white; the older mycelium rapidly became brown. Variation between isolates is typical for many species in the genus *Colletotrichum* (Pring *et al.*, 1995; Sutton 1992). However, all isolates studied possessed two key features in cultures, which Sutton (1992) described as typical for *C. capsici*. These were characteristic sickle shaped conidia and the presence of prominent setae. The sizes of both these structures varied between isolates, but all were within the published range (Pring *et al.* 1995; Sutton 1992).

Isolates tested for their ability to induce lesions in ripe fruits using the pin prick method indicated that all isolates of *C. capsici* have the ability to develop anthracnose lesions on red ripe chilli fruits. The disease was identified by small, brown, circular depressions and the fungus appeared as minute black acervuli on the surface of inoculated chilli fruits.

Artificial inoculation of detached fruits

Results of germination assay (Table 2) showed that considerable numbers of conidia germinated and formed appressoria on fruit surfaces but very low in SDW on glass slides. Maximum conidia

Table 2. Conidia germination, appressoria formation by germinated conidia and lesion development of anthracnose on chilli fruit surfaces, after inoculation of detached fruits with *C. capsici*

Maturity stage of fruits	Conidia germination (%)	Appressoria formation (%)	Mean Diameter of anthracnose lesion on fruits (mm)
Immature fruits	18.5[b]	23.5[b]	0.0
Mature Fruits	41.4[c]	53.8[c]	0.48
Red ripe fruits	69.2[d]	77.3[d]	1.22
Glass slides (Control)	2.6[a]	0.0[a]	-

In each column, values followed by same letters are not significantly different according to Duncan's multiple range test at p=0.05

differentiation into appressoria was observed on surface of red ripe fruits compared to fruits belonging to the other maturity stages and they were significantly different at P=0.05. Similar observations were made by Swinburne (1976) with *Colletotrichum musae* on banana, which was ascribed to the presence of solutes leaching into the inoculum drop from the host cells. The rate of leaching of solutes from plant cells is related to the integrity of the plasma membrane, which breaks down with fruit ripening (Tukey 1970). The lesion development was assessed as diameter of the lesion after 10 days of inoculation. Significant difference in lesion development was found between fruits at different maturity stages (Table 2). Lesion development occurred when fruits were fully riped. Highest lesion development rate was observed on red ripe fruits. Anthracnose lesions were initiated on green fruits when it became red ripe stage. No lesion development was observed on inoculated sites of light green immature fruits during incubation period. But conidial differentiation occurred. This quiescence of anthracnose pathogen on immature fruits can occur due to a number of pre-formed antifungal compounds detected in unripe fruits, which declined during ripening (Rajapakse 1998).

Artificial inoculation under field condition

Variation of anthracnose development on chilli fruits at various maturity stages when artificial inoculation was practiced under field conditions is given in Table 3. Significantly higher percentages of anthracnose lesions were observed in inoculated plots over uninoculated plots. Among inoculated plots, highest incidences of anthracnose lesion were observed on chilli fruits when inoculation was practiced at red ripe stage (Table 3). Generally, under dry conditions with low relative humidity when chilli is cultivated with irrigation, occurrence of anthracnose frequency

Table 03. Anthracnose incidences of chilli fruits under field conditions, during North-easy monsoon in 1998 and South-west monsoon in 1999 in Sri Lanka.

Treatments	North-east monsoon (1998)	South-west monsoon (1999)
T1 -Red fruits + inoculation	48.7[a]	52.5[a]
T2 -Dark green mature fruits + inoculation	34.0[b]	36.3[b]
T3 -Light green immature fruits + inoculation	20.5[c]	11.5[d]
T4 -Fruits of all maturity stages + inoculation	35.0[b]	21.3[c]
T5 -Red fruits without inoculation	16.7[c]	6.3[d]
T6 -Dark green mature fruits without inoculation	19.3[c]	6.8[d]
T7 -Light green immature fruits without inoculation	20.5[c]	5.3[d]
T8 -Fruits of all maturity stages without inoculation	31.5[b]	7.3[d]
CV %	13.7	17.4

In each column, values followed by same letters are not significantly different according to Duncan's multiple range test at p=0.05

is low. Therefore, water spraying, from next day after artificial inoculation is an important requirement for conidial differentiation into infective structure on fruit surface.

CONCLUSION

Artificial inoculation of anthracnose by spraying conidial suspention of *C. capsici* onto the canopy of chilli plants with fruits at red ripe stage, followed by spraying water using knapsack sprayer from next day morning for one week period at two times per day seems to be a good method for variety screening of chilli against the disease.

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