Annals of Sri Lanka Department of Agriculture. 2007.9:119-126.

CHEMICAL FACTORS STIMULATORY TO ANTHRACNOSE DEVELOPMENT ON BRINJAL (*Solanum melongena* L.) FRUITS

R.G.A.S. RAJAPAKSE, H.V. KALUBOWILA, K.G. KANDANAMULLA and S.M.I.S.K. SAKALASOORIYA

Horticultural Crops Research and Development Institute, Gannoruwa, Peradeniya

ABSTRACT

Anthracnose disease is one of the major problems in brinjal (*Solanum melongena* L.) cultivation. Identification of resistant varieties is easy, economical and a reliable method to control the disease. Studies were undertaken to identify the causal agent of anthracnose and to study the relationship between varietal resistance/ susceptibility and influence of conidia differentiation by fruit exudates of different brinjal varieties. The causal agent of anthracnose was identified as *Colletotrichum gloeosporioides*. Stimulatory compounds which are responsible for conidia differentiation into appressoria were found to be present in fruit exudates of all tested varieties of brinjal. Percentage of conidia germination and appressoria formation in fruit exudates of varieties showed significant correlation with size of the anthracnose lesions developed on fruits. Highest stimulation of conidia differentiation of pathogen was observed in fruit exudates of the variety Padagoda which is highly susceptible to the disease. Chemical factors found in exudates obtained from fruit surface were separated into ether and water soluble fractions. Chemical factors in the water-soluble fraction enhanced conidia differentiation more than ether soluble fraction. Results revealed that the stimulatory effect of exudates on conidia differentiation could be used to compare susceptibility/resistance to anthracnose of brinjal germplasm as an *in vitro* test.

KEYWORDS: Anthracnose, Appressoria, Brinjal, *Colletotrichum*.

INTRODUCTION

Anthracnose disease of brinjal infects about 10-40% of fruit yield, causing small brown lesions on the fruit surface and deteriorating the appearance and fruit quality. The causal agent of anthracnose disease is mainly *Colletotrichum* species (Abeygunawardhana, 1969). The pathogen attacks flowers and fruits and causes stem die back under moist weather conditions. Conidia bearing bodies *i.e.* acervuli are produced as small grey to brown depressions on mature fruits. Development of fungal pathogens on the surface of the host is influenced by many factors. Of these, the role played by chemicals, normally exuded or leached by the host *i.e.* fruit peel exudates has been studied by many scientists (Adikaram *et al.*, 1983; Swinburne, 1976). Such substances can affect three distinct phases in infection process namely spore germination, germ-tube growth and the formation of infection structures, the appressoria.

Several species of the genus *Colletotrichum*, produce appressoria in response to specific chemical signals. Appressorium formation was found to be influenced by chemical signals from the plant, such as anthranilic acid in *C. musae* (Harper *et al.*, 1980), and cuticular compounds in *C. gloeosporioides* (Podila *et al.*, 1993). Kolattukudy *et al.* (1995) showed that hydroxy fatty acid

monomers in avocado wax were involved in the induction of the cutinase gene in conidia of *C. gloeosporioides* on fruit surface. Rajapakse (1999) showed that conidia germination and appressorial formation of *Colletotrichum capsici* were higher on fruit exudates of chilli varieties which are susceptible to anthracnose than in resistant varieties. Therefore, the present study was carried out to detect the relationship between varietal resistance/susceptibility and influence of conidia differentiation by exudates of fruits of different brinjal cultivars.

MATERIALS AND METHODS

Pathogen isolation and identification

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

Collection of exudates from brinjal fruits

Mature fruits of the brinjal varieties Padagoda, 558, 8890 x SM164, Anjalee and Amanda were collected from field grown healthy plants. Fruits were carefully detached from plants, washed in sterile distilled water (SDW) and wiped with cotton wool soaked in 90% ethanol to reduce microbes on pod surface. Five SDW drops of 10µl volume were separately placed using a micropipette on surface of each fruit of all tested varieties and left for 16 hr under moist chamber. Total of 6 fruits of 6 plants were used for collecting exudates from each variety. To avoid microbial activity, exudates were stored at -20°C until later analysis.

Fractionation of exudates

Compounds in exudates were separated into ether soluble and insoluble fractions. Exudates (5ml) were shaken with 15ml diethylether in a separating funnel and the ether soluble fraction separated from the water fraction. Process was repeated five times and the ether washings were bulked and dried over anhydrous Na_2SO_4. The ether and water-soluble fractions were evaporated to dryness using a vacuum evaporator in a water bath at 28°C and finally the solutions of ether soluble and insoluble compounds were obtained by adding 5ml of SDW.

Effect of ether soluble and water soluble fractions of exudates on conidia germination and appressoria formation

Bioassay

Conidia germination, appressoria formation and anthracnose development in fruit exudates of five brinjal varieties were determined. *Colletotrichum* isolate IS10 isolated from brinjal fruits were used for all inoculation and bioassay experiments. Conidia for all experiments were obtained from cultures on Potato Dextrose Agar (PDA) incubated for 14 days at room temperature (28°–30°C). Conidia were harvested by adding 10 ml SDW to the culture dishes, which were then gently shaken. The suspension was transferred into pre-sterilized centrifuge tubes, which were then spun at 5000 rpm for 4 min. The supernatant was discarded and the conidia containing pellets were resuspended in fresh SDW. This process was repeated four times. Density of conidia in the final suspension was measured using a haemocytometer and adjusted to the $5x10^5$ conidia/ml with SDW (Rajapakse, 1998). 10µl drops of conidia suspension were wound inoculated on three sites of fruit surfaces to observe anthracnose lesion development. 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.

To determine the effect of pod exudates, its ether soluble and water soluble fractions of fruit exudates on germination and appressoria formation by conidia: bioassays were conducted on glass slides. Suspension of conidia in SDW was mixed with equal volumes of the exudates or ether soluble or water soluble solutions and 10µl drops were placed on clean glass slides which were then placed in sealed chambers, lined with moist filter paper to prevent evaporation, and incubated for 6 and 22 hours at 28°C. At each time of inspection, slides were removed briefly dried by placing in oven at 50°C and then a drop of lactophenol containing trypan blue (0.03%) was added. Percent germination and percent germinated conidia with appressoria were assessed by microscopic observations. The experiments were repeated twice with three replicates on each occasion. Values were based on count of 200 conidia in all replicates. The data were transformed to arc sine and analysed by ANOVA using a statistical programme (MSTATC).

RESULTS AND DISCUSSION

Isolates of anthracnose pathogen of brinjal were identified by comparison of their colony morphology on PDA with published data. According to the colony morphology on PDA medium and the shape and size of conidia, all isolates were identified as *C. gloeosporioides*. Isolates produced a grey coloured mycelium on culture media (PDA) at early stages (3 days) and later became light brown or brown colour after 14 days. Acervuli of isolates on anthracnose lesions

on fruits were round and consisted of conidia (Table 1). Conidia size varied within isolates and among isolates but generally ranged from 12-18 µm in length and 2-4µm in width. Conidia from all the isolates showed a similar shape. Pring *et al.* (1993) and Sutton (1992) showed that variation between isolates is typical for many species of the genus *Colletotrichum*. However, characteristics of all tested isolates were within the published range of *C. gloeosporioides* (Sutton, 1992). Isolates tested for their ability to induce lesions in mature fruits using the pin prick method indicated that tested isolate of *C. gloeosporioides* had the ability to develop anthracnose lesions on brinjal fruits. The disease was identified by large 2-4cm diameter, brown, circular depressions and the fungus appeared as brown acervuli on the surface of inoculated fruits.

Table 1. Characters of fungal isolates collected from anthracnose affected brinjal pods.

Characters of fungi Isolates	*Morphological and culture characters of isolates*
Colony colour on PDA	Grey initially then turned light brown or brown, colony margin wavy
Reverse colony colour on PDA	Brown or dark brown
Acervuli	Brown colour masses, conidia present inside.
Setae	Absent
Conidia	Cylindrical with round end shaped, aseptate, Fat globules present inside, 12-18µm in length and 2-4µm in width, Conidia germinate and produce appressoria at the end of germ-tube
Appressoria in fruit exudates	Oval shape, abundant and black colour

Results of germination assay (Table 2 and 3) showed there was no significant difference in conidia germination and appressoria formation of *C. gloeosporioides* in exudates of brinjal varieties for 6 hours incubation. However, number of conidia that germinated and formed appressoria in fruit exudates were significantly higher in the variety Padagoda compared to 558, 8890 x SM164, Anjalee and Amanda varieties for the 22 hour period of incubation. Conidia differentiation was very low in SDW on glass slides. 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. Rajapakse (1999) showed that *C. capsici* conidia germination and formation of appressoria varied in fruit exudates of chilli varieties. The rate of leaching of solutes from plant cells is related to the integrity of the plasma membrane (Tukey, 1970). Lesion development assessed by lesion diameter after 10 days of inoculation showed significant difference between fruits of different varieties (Table 4). Higher lesion development was observed on fruits of variety Padagoda, compared to 558, 8890 x SM164, Anjalee and Amanda. Percentage conidia germination and appressoria formation in fruit exudates had significant correlation with size of anthracnose lesion on fruits (r^2=0.94 and 0.59 respectively) (Fig. 1 and 2). Therefore, stimulatory effects of exudates of different varieties on conidia differentiation could be used to compare anthracnose susceptibility/resistance of brinjal germplasm as an *in vitro* test.

Table 2. Effect of exudates of fruits of brinjal varieties on *C. gloeosporioides* conidia germination at different incubation periods.

	*Percent conidia germination in exudates on glass slides***															
Incubation period (h)	*Padagoda*			*558*			*8890 x SM164*			*Anjalee*			*Amanda*			*SDW*
	*EX	WF	EF	EX	WF	EF	EX	WF	EF	EX	WF	EF	EX	WF	EF	
6	9.7	6 8	2.2	7.1	4.3	1.7	5.9	5.5	2.5	8.6	5.1	4.5	6.7	4.6	3.8	0
22	72.0^{a}	41.6^{c}	6.6^{g}	71.3^{a}	28.0^{d}	13.4^{fg}	62.5^{b}	25.2^{de}	11.3^{fg}	36.8^{c}	26.4^{de}	8.5^{f}	26.3^{de}	20.3^{e}	$12,6^{f}$	1.5^{g}

* EX – Fruit exudate, WF – Water fraction of fruit exudate, EF – Ether fraction of fruit exudate, SDW – Sterile distilled water

** mean of six replicates

At each incubation period: values followed by the same letter are not significantly different, by DMRT at p=0.05.

Table 3. Effect of exudates of fruits of brinjal varieties on formation of appressoria by conidia of *C. gloeosporioidesi* at different incubation periods.

	*Percent appressoria formation on glass slides***															
	Brinjal varieties															
Incubation (h)	*Padagoda*			*558*			*8890 x SM164*			*Anjalee*			*Amanda*			*SDW*
	*EX	WF	EF	EX	WF	EF	EX	WF	EF	EX	WF	EF	EX	WF	EF	
6	2.8	2.5	2.3	2.4	2.6	1.7	3.3	2.5	1.5	2.1	1.7	1.3	2.2	1.9	1.5	0
22	30.9^{a}	23.3^{b}	5.4^{ef}	14.2^{cd}	9.2^{de}	5.3^{ef}	22.4^{a}	17.2^{c}	6.1^{ef}	9.6^{d}	6.5^{ef}	4.3^{efg}	7.1^{ef}	6.1^{efg}	3.0^{fg}	1.3^{g}

* EX – Fruit exudates, WF – Water fraction of fruit exudates, EF – Ether fraction of fruit exudates, SDW – Sterile distilled water

** mean of six replicates

At each incubation period: values followed by the same letter are not significantly different, by DMRT at p=0.05.

All extracts and the original exudate significantly stimulated conidia germination relative to the water controls. Most of the chemicals responsible for conidia germination and appressorium formation were extracted by water.

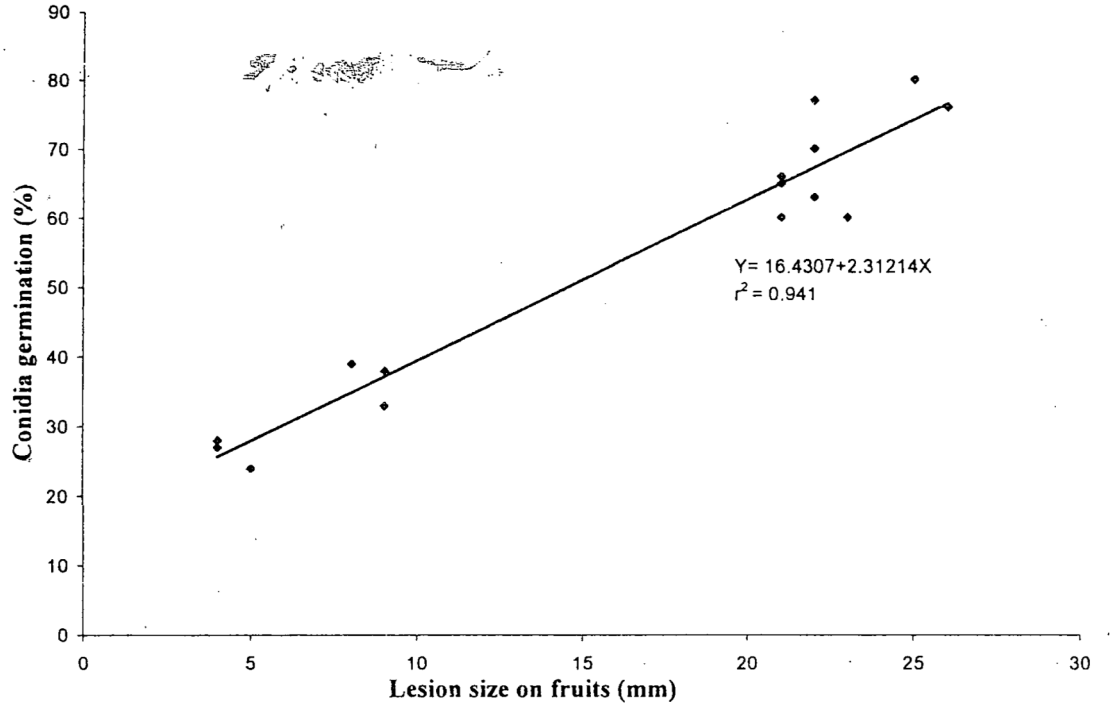

Figure 1. Relationship between conidia germination on fruit exudates and anthracnose lesion size in fruits of brinjal varieties.

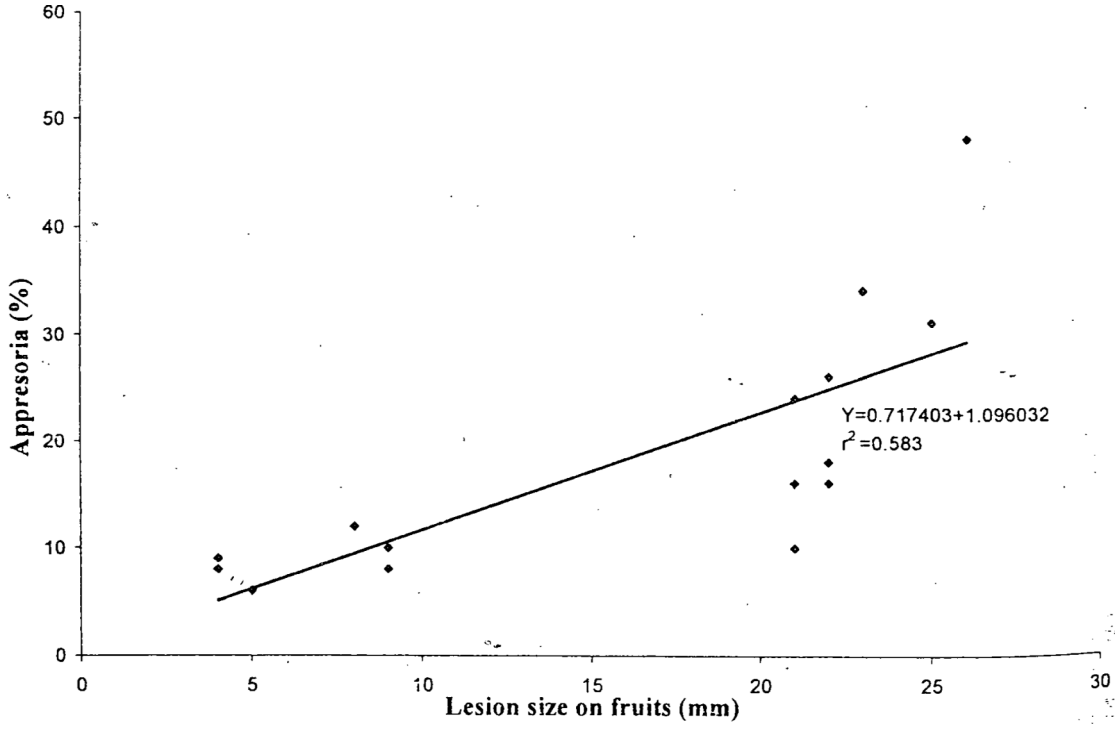

Figure 2. Relationship between appresoria (%) in fruit exudates and anthracnose lesion size on fruits of brinjal varieties.

Table 4. Lesion size of anthracnose on fruits of brinjal varieties.

Variety	*Lesion size (mm)*
Padagoda	25.4[a]
558	22.0[ab]
8890 x SM164	21.5[b]
Anjalee	9.2[c]
Amarak	4.3[d]

Values followed by the same letter are not significantly different, following DMRT at p=0.05.

CONCLUSIONS

The causal agent of brinjal anthracnose disease was identified as *Colletotrichum gloeosporioides*. Stimulatory compounds which are responsible for conidia differentiation into appressoria are present in fruit exudates of brinjal. Highest stimulation for conidia differentiation was observed in fruit exudates of brinjal variety Padagoda which is highly susceptible to anthracnose disease. Rate of conidia differentiation by exudates could be used as an *in vitro* test to compare anthracnose susceptibility/ resistance in brinjal germplasm.

REFERENCES

Abeygunawardhana, V.W. 1969. Diseases of cultivated plants. Colombo Apothecaries LTD, Colombo, 167-169.

Adikaram, N.K.B., A.E. Brown and T.R. Swinburne. 1983. Observations on infection of *Capsicum* annuum fruit by *Glomerella cingulata* and *Colletotrichum capsici*. Trans. Br. Mycol. Soc. 80(3): 395-401.

Harper, D.B., T.R. Swinburne, S.K. Moore, A.E. Brown and H. Graham. 1980. A role for iron in germination of conidia of *Colletotrichum musae*. J. of General Microbiology, 121: 169-174.

Kolattukudy, P.E., L. Daoxin, H. Cheng-Shine and M.A. Flaishman. 1995. Host signals in fungal gene expression involved in penetration into the host. Can. J. Bot. 73:1160-1168.

Podila, G.K., L.M. Roger and P.E. Kolattukudy. 1993. Chemical signals from Avocado surface wax trigger germination and appressorium formation in *Colletotrichum gloeosporioides*. Plant Physiology, 103:267-272.

Pring, R.J., C. Nash and J.A. Baily. 1993. Infection process and host range of *Colletotrichum capsici*. Physiological and Molecular Plant Pathology. 46, 132- 152.

Rajapakse, R.G.A.S. 1998. Some observations of anthracnose disease cased by *Colletotrichum* species in Sri Lanka. PhD thesis, University of London, UK.

Rajapakse, R.G.A.S. 1999. Mode of anthracnose development in chilli (*Capsicum annuum* L.) pods. Annals of Sri Lanka Department of Agriculture. 1: 247-266.

Sutton, B.C. 1992. The genus *Glomerella* and its anamorph *Colletotrichum*. *Collototrichum* biology, pathology and control. CAB international, UK, 1-26.

Swinburne, T.R. 1976. Stimulation of germination and appressoria formation by *Colletotrichum musae* (Berk. and Curt.) Arx. in banana leachete. Phytopathologische Zeitschrift, 87:74-90.

Tukey, H.B. 1970. The leaching of substances from plants. Annual. Revive of Plant Physiology.21,305-324.