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EFEECTS OF CINNAMON, CLOVE AND NUTMEG LEAF EXTRACTS ON THE CONTROL OF ANTHRACNOSE DISEASE IN CILLI.

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ABSTRACT

An experiment was conducted to identify the fungal pathogens associated with anthracnose disease of chilli in Sri Lanka and efficacy of leaf extracts and chemical control methods in controlling the disease. Most commonly isolated anthracnose fungi were *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *C. Capsici* (Syd.) Butler & Bisb. Leaf extracts of cinnamon (*Cinnamomum verum* J. Presl), clove (*Syzygium aromaticum* (L.) Merr. & Perry), nutmeg (*Myristica fragrans* Houtt.), betel (*Piper betle* L.) and tippili (*Piper longum* L.) were evaluated. Studies were conducted under both *in vitro* and field conditions and the experimental designs employed were completely randomized design for *in vitro* experiments and randomized complete block design for the field experiment. Cinnamon and clove leaf extracts showed the highest reduction in growth of anthracnose fungi whereas nutmeg and betel showed moderate effects. Tippili stimulated the growth of *C. gloeosporioides* but did not show an effect on *C. capsici*. Cinnamon and clove leaf extracts showed comparative efficacy to chlorothalonil 75% WP and carbendazim 500g/l SC in controlling anthracnose disease *in vitro* conditions. The field trials also revealed that the anthracnose can be controlled by using clove and cinnamon leaf extracts. Thus, leaf extracts of cinnamon and clove can be used to control anthracnose disease of chilli.

KEYWORDS: Anthracnose disease, *Capsicum*, Fungicides, Leaf extracts.

INTRODUCTION

Anthracnose is one of the fungal diseases which affect chilli production in Sri Lanka. The disease mainly affects the yield as well as the quality of the crop. Anthracnose mainly appeared on mature pods, however die-back of shoots, leaf spots and mature green pod damage have also been reported (Rajapakse, 1999). Apart from pre-harvest losses, pod quality deterioration of chilli due to anthracnose ranges from 20% - 50 % in Sri Lanka (Rajapakse, 1999). However, losses could be very high and varied with climatic conditions prevailed in the growing season (Kim and Park, 1998). Use of fungicides is a common practice to control anthracnose in Sri Lanka. Although fungicides provide an effective disease control, it could be harmful to consumers as they can accumulate in the harvest. In addition, spraying of chemicals can cause negative effects such as development of pathogen resistance and may be harmful on environmentally friendly organisms as well (Sivakumar *et al.*, 2001). Therefore present emphasis on

disease control methods are focused towards the development of environmentally friendly techniques. The objective of this experiment was to identify suitable plant extracts to effectively control anthracnose in chilli.

MATERIALS AND METHODS

A series of experiments consists of both in-vitro and field were conducted to determine the efficacy of plant extracts on control of Anthracnose. Causal agent of the disease were isolated, identified and cultured for the subsequent use in both in-vitro and field experiments. Completely randomized design was used in in-vitro experiments and randomized complete design was a used for the field experiment.

Isolation and pathogenicity of causal organisms

Chilli pods having symptoms of anthracnose were obtained from approximately 90 diseased plants grown mainly in the Wet zone districts and a few samples from the other districts. The affected areas of those pods were surface sterilized with 70 % ethanol. Five, 2 mm diameter tissues from each pod were aseptically removed from the advancing edge of the rotten lesion, and placed on potato dextrose agar (PDA, Oxoid). Cultures were incubated at 26 °C ± 2 °C for 1-7 days. When fungal growth from the tissue was visible, the fungi were sub cultured on PDA and re–incubated to obtained pure cultures for identification purposes. Identification of pathogens was carried out according to the descriptions of CAB International Mycological Institute, U.K. (Sutton *et al.*, 1970 Sutton, 1992).

Isolated fungal strains were used to re-inoculate healthy capsicum pods, Conidia were scraped from seven day old cultures of each fungal pathogen grown on PDA and suspended in sterile distilled water. Hyphal fragments were removed by filtration through glass wool and drops of 0.02 ml conidial suspension (10^5 spores/ml) were placed on three inoculation sites of mature healthy pods. The inoculated pods were kept on wet tissues in plastic trays and covered with transparent polythene to maintain the humid conditions. Polythene covers were removed after 3 days of incubation and the pods were maintained under normal laboratory conditions at 26 °C ± 2 °C until symptoms developed.

Different plant extracts on fungal isolates

Effect of leaf extracts of cinnamon (*Cinnamomum verum* J. Presl), clove (*Syzygium aromaticum* (L.) Merr. & Perry), nutmeg (*Myristica fragrans* Houtt.), betel

(*Piper betle* L.) and tippili (*Piper longum* L.) on the growth of anthracnose isolates were tested *in vitro* with five replicates. Plant extracts were obtained by macerating 250 g fresh leaves in 1,000 ml distilled water in an electric blender at 21,000 rpm for two minutes. Thirty nine grams of PDA was added to one liter of filtered extracts and the amended medium was autoclaved at 121 °C and 1.09 kg/cm^2 pressure for 15 minutes. Agar pieces obtained from cultures of fungal isolates (0.5 cm diameter) were grown on PDA and transferred plant extract amended medium in petri-dishes and the plates were incubated at 25 °C. The test fungi were also grown under similar conditions without any amendments as a control treatment. Colony diameters were measured at 2, 4, 6 and 8 days.

Plants extracts vs. fungicides on fungal isolates: *in vitro* experiment

Two recommended fungicides, Chlorothalonil 75 % WP (contact fungicide) and Carbendazim 500 g/l SC (systemic fungicide) compared with three leaf extract of cinnamon, nutmeg and clove on the growth of fungal isolates identified under in-vitro condition. Treatments were replicated five times. The fungicide concentrations used were 1 ml/l for Carbendazim 500 g/l SC and 2 ml/l for Chlorothalonil. Plant extracts were prepared as described earlier. Thirty nine grams of PDA was added to one litre of each fungicide solutions or plant extracts and the amended medium was autoclaved at 121 °C and 1.09 kg/cm^2 pressure for 15 minutes. Fungal cultures obtained were transferred to the fungicidal amended medium in petri-dishes and incubated at 25 °C. The test fungi were also cultured without fungicides or plant extracts under similar conditions. Colony diameter was measured at 2, 4, 6 and 8 days after culturing. The percentage reductions in growth of fungi due to the addition of different plant extracts or fungicides were calculated according to the following formula:

$$\text{Reduction in growth (\%)} = 100 \times \frac{\text{diam C - diam F}}{\text{diam C}}$$

where,

diam C=The mean diameter of the growth zone of the control plate.

diam F=The mean diameter of the growth zone of the sample plate.

Efficacy of plant extracts vs fungicides on anthracnose in the field experiment

The efficacy of three plant leaf extracts and selected fungicides were evaluated against anthracnose disease of chilli in the research field of Fruit Crops Research and Development Centre, Horana. Treatments used were control (sterilized distilled water), leaf extracts of cinnamon, nutmeg and clove at 250 g/l, 2 ml/l Chlorothalonil 75 % WP

and 1 ml/l Carbendazim 500 g/l SC. Each fungicides and plant extracts were sprayed three times at 14 days interval starting from the stage of pod formation. Treatments were arranged in a randomized block design with three replications. Twenty chilli plants were included in one treatment plot. Separate experiments were conducted for varieties of MI 2 and Veraniya. Since the natural inoculum of anthracnose fungi was very low in the dry period, pods were inoculated artificially. Inoculation was performed using conidial suspensions of 10^5 spores/ml at three times in seven days interval and one inoculation site was made on each pod. Inoculated pods were incubated as described above. The ratio of total inoculated chilli pods to anthracnose diseased pod was calculated. ANOVA procedure was adopted for data analysis using SAS. Least significant difference (LSD) test was used to compare differences between treatments.

RESULTS AND DISCUSSION

Isolation and Identification of causal organisms

The fungi isolated from diseased pods of chili obtained from different districts were *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.and *C. Capsici* (Syd.) Butler & Bisby Pathogenicity of *C. gloeosporioides* and *C. capsici* was confirmed and the symptoms of anthracnose lesions reproduced. Pathogen identification was performed according to the CMI descriptions (Sutton, 1970 and Sutton, 1992).

Effect of plant extracts on the growth of fungal inoculants *in vitro*.

The growth of anthracnose fungi *C. gloeosporioides* and *C.capsici* on different plant extracts amended medium during the incubation period is shown in Figure 1 and 2. Figures 1 and 2 show the effect of different plant extracts on *C. gloeosporioides* and *C. capsici* Growth of *C. gloeosporioides was* stimulated by tippili extract whereas nutmeg and betel leaf extracts reduced the fungal growth. Cinnamon and clove leaf extracts totally retarded the fungal growth. However, tippili did not promote the growth of *C. capsici.*

In vitro effect of plant extracts vs fungicides

Effect of plant extracts and the two fungicides (Chlorothalonil and Carbendazim) on the growth of *C. gloeosporioides* and *C. capsici* are shown in Table 3 and 4. The percentage reduction in growth of the pathogens due to the effect of plant leaf extracts and fungicides are shown in Table 5. *In vitro* studies of *C. gloeosporioides and C. capsici*

effectively reduced the fungal growth similar to chlorothalonil or carbendazim. However, nutmegs leaf extract was not effective in controlling the growth of both fungi. These results were similar to the observations made in experiment II by the leaf extracts. The percentage reduction in growth of the pathogens is shown in Table 5. It shows that clove or cinnamon leaf extracts could effectively reduce the fungal growth similar to that of the fungicides used whereas the nutmeg leaf extract showed only a moderate effect.

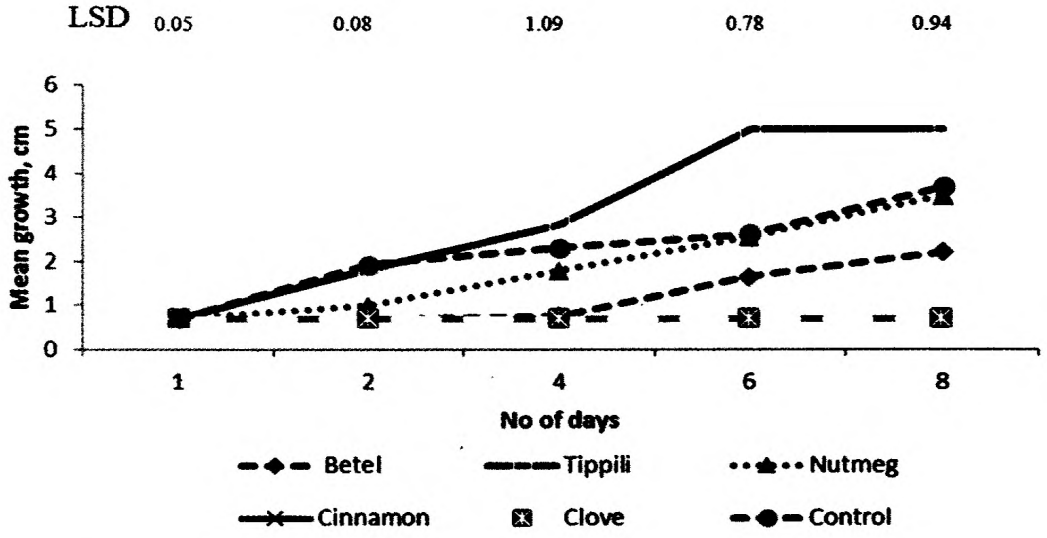

Figure 1. Effect of plant extracts on the growth of Colletotrichum glocosporioides.

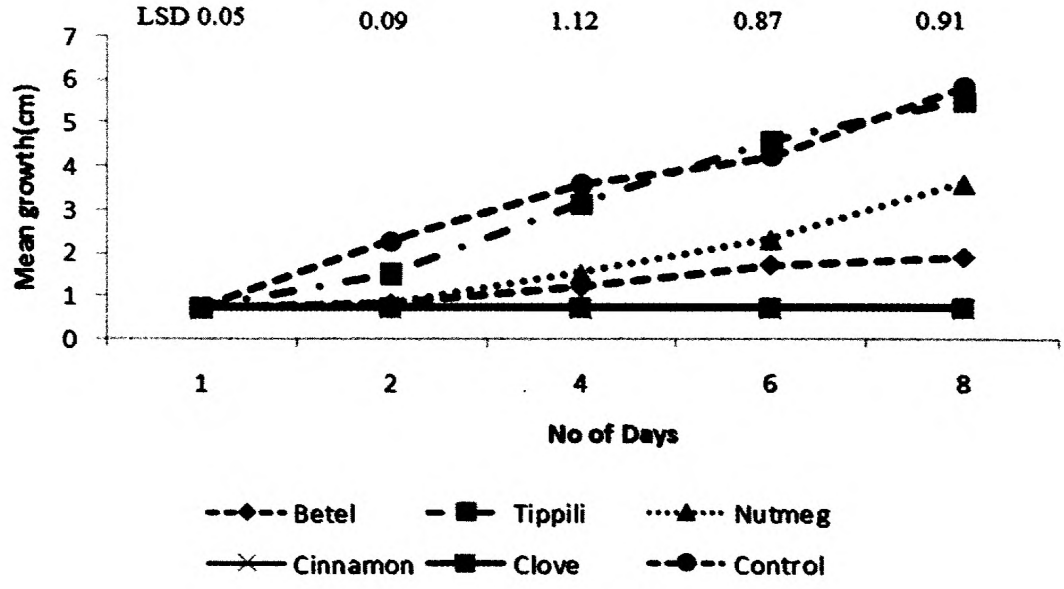

Figure 2. Effect of plant leaf extracts on the growth of *Colletotrichum capsici*.

Efficacy of leaf extracts and the two recommended fungicides in controlling anthracnose (Table 6). Both cinnamon leaf extract and the clove leaf extract showed similar efficacy in reducing the fungal growth in MI-2 and Varaniya varieties. It is interesting to note that the efficacy of the above two leaf extract were comparable to the synthetic fungicides Chlorothalonil and Carbendazim. Nutmeg leaf extract however showed only moderate effect. Cinnamon has been also found to be effective on the growth of fruit rot pathogenic fungi of Guava (Alahakoon *et al.*, 2008).

Table 3. **The growth of anthracnose fungus, *Colletotrichum gloeosporioides* on PDA containing leaf extracts or fungicides *in-vitro*.**

Treatment	*Mean colony diameter (cm) during inoculation period, days*				
	1	*2*	*4*	*6*	*8*
Nutmeg	0.70	0.99	1.78	2.55	3.48
Cinnamon	0.70	0.70	0.70	0.70	0.70
Clove	0.70	0.70	0.70	0.70	0.70
Chlorothalonil	0.70	0.70	0.70	0.70	0.70
Carbendazim	0.70	0.70	0.70	0.70	0.70
Control	0.70	1.91	2.30	2.62	3.68
LSD		1.08	0.09	0.97	1.09
CV%		7.186	5.146	5.171	3.531

Table 4. **The growth of anthracnose fungus, *Colletotrichum capsici* on PDA containing leaf extracts orfungicides *in vitro*.**

Treatment	*Mean colony diameter (cm) during inoculation period, days*				
	1	*2*	*4*	*6*	*8*
Nutmeg	0.70	0.89	1.81	2.64	3.68
Cinnamon	0.70	0.70	0.70	0.70	0.70
Clove	0.70	0.70	0.70	0.70	0.70
Chlorothalonil	0.70	0.70	0.70	0.70	0.70
Carbendazim	0.70	0.70	0.70	0.70	0.70
Control	0.70	2.24	3.58	4.22	5.82
LSD		1.21	0.19	0.96	1.21
CV%		6.81	4.93	5.21	3.63

Similar behaviour of plant extracts on different pathogenic fungi was reported by Sivakadadchum and Dodanwela (1987) and Alahakoon *et al.*, (1990). According to their observations certain plant extracts stimulated the fungal growth while others reduced it. Similar results were recorded elsewhere; through the causal agents were different species of the genus *Colletotrichum* (Agrios, 1988). Several species of *Colletotrichum*, i.e. *C. capsici*, *C. gloeosporioides*, *C. acutatum* Simmonds, *C. cocoides* (Wall.) Hughes, *C. graminicola* (Ces.) Wils., have reported to cause the anthracnose disease in capsicum in

other parts of the world (Hadden and Black, 1988). In Sri Lanka, *C. capsici* and *C. gloeosporioides* have been identified as causal agent of anthracnose disease of capsicum (Rajapakse *et al.*, 2008).

Table 5. Effect of plant extracts and fungicides on the percentage reduction in growth of *Colletotrichum gloeosporioides* and *C. capsici* after 8 days *in vitro* experiment.

Treatment	*Mean percentage reduction in growth*	
	C. gloeosporioides	*C. capsici*
1.Cinnamon leaf extract	100 [a]	100 [a]
2.Clove leaf extract	100 [a]	100 [a]
3. Nutmeg leaf extract	21.7 [b]	36.77 [b]
4. Carbendazim 500g/l SC	100 [a]	100 [a]
5.Chlorothalonil 5%wp	100 [a]	100 [a]
6.Control	0 [c]	0 [c]
LSD	11.01	9.68
CV%	3.53	0.94

Within a column, the means followed by same letter are not significantly different at p=0.01

Table 6. Efficacy of plant extracts and fungicides against the anthracnose disease development in MI-2 and Veraniya varieties (pods were artificially inoculated).

Treatment	*Development of disease (%)*	
	MI-2	*Veraniya*
1. Chlorothalonil	0 [c]	0 [c]
2. Carbendazim	4.16 [c]	0 [c]
3.Clove leaf extract	0 [c]	0 [c]
4. Nutmeg leaf extract	63.20 [b]	56.57 [b]
5. Cinnamon leaf extract	0 [c]	0 [c]
6.Control(Distilled sterilized water)	88.60 [a]	74.88 [a]
CV%	18.36	20.06

Note: Within a column, the means followed by same letter are not significantly different at p=0.01 according in LSD

Our experiments at both *in vitro* and field conditions showed that clove and cinnamon leaf extracts could effectively control the growth of fungal mycelium and the anthracnose disease of chilli. The efficacy of both clove and cinnamon leaf extracts were comparable to the recommended fungicides, Chlorothalonil 75% WP and Carbendazim 500g/1 SC. Nutmeg leaf extract retarded the fungal growth and controlled anthracnose disease to a lesser extent compared to clove or cinnamon leaf extract. The plant extracts are cheaper and does not produce health hazards to human and animals. In addition, they are environmental friendly. Therefore, promotion of the use of plant leaf extracts such as clove or cinnamon is necessary as they prevent the environmental contamination in Sri

Lanka. However, it is necessary to implement a pilot scale testing trials to ascertain the effects further.

CONCLUSIONS

Two fungal species associated with the anthracnose disease of chilli in Sri Lanka are *Colletotrichum gloeosporiodes* and *C. capsici*. Results of the study suggest that clove or cinnamon leaf extracts effectively suppressed the growth of two fungal pathogens and their efficacy was equal to the presently recommended fungicides in Sri Lanka.

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