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Study on efficacy of some botanicals against *Colletotrichum gloeosporioides* causing anthracnose disease in brinjal (*Solanum melongina* L.)

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Abstract

Anthracnose disease is one of the major problems in brinjal (*Solanum melongina* L.) cultivations and the use of eco-friendly botanicals for the control of this disease is a better alternative to synthetic fungicides. Laboratory studies were carried out to test the effect of some selected botanicals against *Colletotrichum gloeosporioides* causing anthracnose in brinjal and to select the most effective concentrations. Water extracts of Garlic (*Allium sativum*) bulbs, Tumeric rhizomes (*Curcuma domestica*), Heen Aratta rhizomes (*Alpenia galanga*), Guava leaves (*Psidium guajava*) and Wathupalu leaves (*Michania spp.*), were prepared as 0.05%, 0.1%, 0.5%, 1.0% and 1.5% dilutions while sterilized distilled water was used as the control. Separate experiments for each botanical was arranged in a Completely Randomized Design (CRD) with the above five treatments with three replicates. Solutions from each concentration were incorporated to Potato Dextrose Agar (PDA) medium by the pour plate method. Those plates were centrally inoculated with 1 cm diameter mycelial discs of *C. gloeosporioides*, sealed and incubated at room temperature (28 ^{0}c ± 1 ^{0}c). Colony diameters of the growing fungus were measured every other day (starting five days after inoculation). Significantly lower ($P \leq 0.05$) colony diameter was seen in 0.5% concentration of garlic compared to other concentrations. The inhibition of fungal mycelium was highest in turmeric at 0.5%, and Heen Aratta both at 0.5% and 1.0% concentrations. All the concentrations tested with Wathupalu and Guava leaf extracts showed significantly lower mycelial growth than the control. When all those best concentrates were tested together, 0.1%, 0.5% and 1.5% concentrations of Guava leaf extract and 1.5% concentration of Wathupalu leaf extract showed significantly lower colony diameters than the other effective concentrations of Garlic bulbs, Tumeric rhizomes and Heen Aratta rhizomes.

Keywords: Anthracnose, Botanicals, Colletotrichum gloeosporioides, Solanum melongina L.

Introduction

Solanum melongina L. (Brinjal) is an important commercial vegetable crop in Sri Lanka and the disease anthracnose has become one of the major constraints for a profitable cultivation (Anonymous, 2002). Among the methods used to control this disease, systemic fungicides (e.g. Benomyl, Chlorothalonyl, *etc.*) play a major role at present (Bailey and Jeger, 1992; Chaube and Singh, 1990). However, the high cost and the associated environmental pollution cause problems in the long run with this chemical disease management (Strange, 1993). Therefore, the use of eco-friendly botanicals for the control of anthracnose is a superior alternative to the use of synthetic fungicides. Hence, laboratory studies were carried out to test the effect of crude extracts of some selected plant species against *Colletotrichum gloeosporioides* causing anthracnose disease of Brinjal and to select the most effective concentration of those botanicals.

Materials and Methods

The experiment was carried out at the Department of Agricultural Biology, Faculty of Agriculture, University of Ruhuna, during July to October, 2008.

Isolation and maintenance of Colletotrichum gloeosporioides cultures

Anthracnose affected pods were collected from different areas such as Kesbewa, Piliyandala, Mapalana and Kamburupitiya. The pieces from the advancing margins of the infected regions of pods were cut, surface sterilized with 70% Ethyl Alcohol for 1 minute, inoculated under the laminar floor cabinet on Potato

Dextrose Agar (PDA) and incubated for a one week at room temperature (28 ^{0}C ± 2 ^{0}C). *C. gloeosporioides* was identified using the colony morphology and spore characteristics. Single spore isolates of this fungus was prepared on PDA slants in test tubes as stock cultures and kept at 4 ^{0}C.

Preparation of the Plant extracts

Healthy, mature bulbs of *Allium sativum* (Garlic), rhizomes of *Curcuma domestica* (Tumeric), rhizomes of *Alpenia galanga* (Heen Aratta), leaves of *Psidium gujava* (Guava) and leaves of *Michania* spp. (Wathupalu) were selected to extract plant sap. Separate experiments were carried out with different concentrations of each of these plant species to find the most effective concentration/s.

Garlic and the rhizomes of Tumeric and 'Heen Aratta' were peeled off, cleaned and washed thoroughly first with soap water and then with running tap water for 20 minutes. The leaves of Guava and 'Wathupalu' were also cleaned separately in the same manner first with soap water and next with running tap water for 20 minutes.

Those specimens were then cut into small pieces, ground thoroughly using a mortar and a pestle to form a thick paste then squeezed and filtered using a cheese cloth to extract the sap. The extract of each 50 g of the botanical specimen was volumed up to 500 ml with sterilized distilled water and from that, extracts of different concentrations *i.e.* 0.05%, 0.1%, 0.5%, 1.0% and 1.5% were prepared by diluting appropriately.

Assessment of the effect of the botanicals on C. gloeosporioides

One milliliter of each concentration of plant species was incorporated into 15 ml of PDA in Petri plates by pour plate method. After solidification of the medium, mycelial discs of 1 cm diameter were cut from the edge of an actively growing colony of the fungal isolate and transferred aseptically into the centre of each plate. PDA plates incorporated with one ml of sterilized distilled water served as a control. Plates were then sealed with parafilm and incubated at room temperature for a one week.

Experiment consists of three replicates and arranged in a Completely Randomized Design (CRD). Five days after the inoculation, the diameters of the growing fungal colonies were measured every other day.

Selection of the most effective concentration/s

The results of the above experiment were used to find the most effective concentration of each plant extract. In the other experiment all those effective concentrations were tested together in the same manner as described earlier, to identify the most effective one out of them.

Data Analysis

Analysis of variance (ANOVA) was performed on the data, using the Statistical Analysis System (SAS) software package to identify differences between treatments. Duncan Multiple Range Test (DMRT) procedure for comparison of means was applied to separate means ($P<0.05$).

Results and Discussion

The effect of different concentrations of crude extracts of each selected plant species on the growth of *C. gloeosporioides* is presented in the table 1. According to the results, Garlic, 0.5% concentration had significantly lower mycelial growth compared to other treatments including control.

Among different concentrations tested, 0.5% Tumeric significantly reduced the mycelial growth of *C. gloeosporioides.* It is also observed that 0.1% and 1.5% concentrations of Turmeric reducing the mycelial growth of the fungus significantly compared with control.

Considering 'Heen Aratta', significantly ($P<0.05$) lower colony diameters were seen both in 0.5% and 1.0% concentrations. However, the rest of the concentrations also found effective in reducing the mycelial growth compared with the control.

All the concentrations of crude extracts of Guava were responsible for significantly lower colony diameters than the control. The slowest mycelial growth of *C. gloeosporioides* was observed with the concentration of 1.5% Wathupalu leaves while all the other concentrations showing significantly slower mycelial growth compared to control.

Based on the above experiments, concentrations of 0.5% Turmeric, 0.5% and 1% Heen Aratta, 0.05%, 0.1%, 0.5%, 1% and 1.5% of Guava and 1.5% Wathupalu were selected as effective concentrations inhibiting mycelial growth of *C. gloeosporioides* and used in the second experiment to identify the most effective one.

The concentrations responsible for the greatest inhibition of the fungus were 0.1%, 0.5% and 1.5% Guava leaf extract and 1.5% of the 'Wathupalu' leaf extract when the selected concentrations of plant extracts were tested together (Fig. 1). Those 4 concentrations affected the growth of the fungus similarly as there was no significant difference between them according to the statistical analysis.

The growth of *C. gloeosporioides* was least affected with 0.5% Garlic extract and the control. Although mycelial growth in 0.5% Garlic was higher than the control, there is no significant difference between these two treatments.

The antifungal activity of crude extracts such as *Eucaliptus citriodora* was confirmed against *Colletotrichum graminicola* and several other pathogens in a previous study (Schwan-Estrada *et al.*, 1998). However, relatively little information on the efficiency of plant species tested in this study against *C. gloeosporioides* is available. The antifungal activity of tested plant extracts was confirmed in the experiment although different plant extracts varied in their effectiveness in inhibiting the mycelial growth of C. *gloeosporioides.* Guawa and Wathupalu leaves found to be most effective in reducing the mycelial growth of the fungus.

Conclusion

The present results show that crude extracts of Guawa leaves and Wathupalu leaves are able to alter the myelial growth of C. *gloeosporioides* and also indicate their potential as an alternative control of that pathogen.

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