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Efficacy of some plant extracts on the mycelial growth of *Colletotrichum gloeosporioides*

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Abstract

The anti fungal effects of some plants extracts namely tobacco leaf, keora seed, keora, mahogoni, gaint indian milky weed, garlic and ginger at different concentrations (30%, 40%, 50%, 60% and 70%) on the growth and development of *C. gloeosporioides*, causal agent of anthracnose of mango were evaluated. Radial growth of *C. gloeosporioides* was recorded. The growth inhibition increase with the increase of concentration of all the plant extracts. Highest mycelial growth inhibition (74.35%) was observed in case of garlic extracts at 70% concentration. Garlic extract at 50% and 60% concentration were also effective than other treatments.

Keywords: *C. gloeosporioides*, Plant extracts, Inhibition

Introduction

Mango, a highly valued fruit crop in Bangladesh, is also called the king of fruit belongs to the family Anacardiaceae. In Bangladesh in terms of total area and production of fruit crops, mango ranks first in area and third in production. It occupies 50990 hectares of land and total production is 242605 tons per annum with an average yield of 4.75 tons per hectare (BBS, 2005). But the yield is very low compared to that of India, Pakistan and many other mango producing countries in the world (Hossain and Ahmed, 1994). There are many constraints that are responsible for the low yield of mango in Bangladesh. Diseases are one of them. Among all of the diseases of mango, anthracnose is the most common disease which is caused by *C. gloeosporioides* (Nelson, 2008).

Colletotrichum gloeosporioides are one of the most important pathogens affecting the flowers and fruits of mango trees causing anthracnose worldwide. In areas where rain is prevalent during flowering and fruit set, anthracnose can cause destruction of the inflorescences and infection and drop of young fruits where this can obviously lead to serious losses, reaching up to 35% of the harvested fruits (Martinez *et al.*, 2009). Excessive use of benomyl, thiophanate- methyl and thiobendazole as pre- and post-harvest sprays has led to a reduction in effectiveness in certain areas where pathogen resistance to fungicides has been reported (Spalding, 1982). Indiscriminate use of the chemicals is not only hazardous to people but also disrupt the natural ecological balance by killing the beneficial soil microbes (Ansari, 1995). So, alternatives have to be developed to control anthracnose in order to guarantee safe food production as well as reduce environmental pollution. The integration of a number of practices aiming to reduce or eliminate negative side effects caused by chemicals used for controlling major mango diseases is the most realistic option for solving the problem (Chowdury and Rahim, 2009). Research work in relation to anthracnose disease management of mango is yet to develop effective alternative/options. Hence, this study was carried out with the aim of providing broader options by evaluating the antifungal activity of botanical extracts from selected plants against *C. gloeosporioides*.

Materials and Methods

The experiment was conducted in the Plant Protection Laboratory of Agrotechnology Discipline, Khulna University, in the year of 2008-2009 to evaluate the effect of different botanical extracts on the growth of (*C. gloeosporioides*) causal agent of anthracnose of mango.

Plant extracts used

Clove of garlic, rhizome of ginger, seed of mahogani, leaf of giant indian milky weed, pulp of keora fruit, keora seed in water, tobacco leaf in water were mixed (Table 1) at different concentrations (0%, 30%, 40%, 50%, 60% and 70% respectively) as botanical extracts. In this experiment the crude extracts of the above indigenous plants were mixed with Potato Dextrose Agar (PDA) medium at different concentrations and the fungal mycelia were inoculated to grow. Data on the radial growth were recorded.

Table 1. The parts of the plants used in the experiment

Name of plants	Scientific name	Family	Plant parts used
Mahogani	<i>Swietenia macrophylla</i>	Meliaceae	Seeds
Ginger	<i>Zingibeyr officinale</i>	Zingiberaceae	Rhizomes
Tobacco leaf	<i>Nicotiana tabacum</i>	Solanaceae	Leaves
Giant indian milky weed	<i>Calotropis gigantea</i>	Asclepiadaceae	Leaves
Garlic	<i>Allium sativum</i>	Liliaceae	Cloves
Keora	<i>Sonneratia apetala</i>	Sonneratiaceae	Pulp of fruits and seeds

Collection of the sample

Diseased mango fruit (*Mangifera indica*) showing typical anthracnose symptoms of *Colletotrichum* infection was collected from market near the Khulna University campus. The fungus *Colletotrichum gloeosporioides* was isolated from the diseased mango fruit.

Isolation of the fungus

The fungus was isolated from the infected mango fruit following standard procedures (Dasgupta, 1981; Agostini and Timmer, 1992). The infected diseased samples along with healthy tissues were cut into small pieces and were surface sterilized by dipping in 0.1% sodium hypochloride (NaOCl) solution for two minutes. The treated plant tissues were washed three times with sterilized distilled water. Excess water was decanted by soaking with sterilized blotting paper. The cut pieces were then placed onto sterilized potato dextrose agar (PDA) in glass Petridish (20ml/petridish) and incubated at 28±1°C for three days for mycelium formation in petridish.

Purification and preservation

To obtain pure culture of *C. gloeosporioides* hyphal tip was transferred aseptically onto PDA plate by using the flame sterilized tip of an inoculation needle. The plate was incubated at room temperature for three days. Mature hyphae were collected and transferred into the test-tube slants containing PDA and incubated at room temperature for seven days. After incubation, the slants were carefully checked for contamination and then preserved at 4 °C in a refrigerator for further use.

Identification of fungus isolates upto species

The fungus was identification on the basis of morphological characteristics suggested by Ellis (2009) and Agron (2009).

Treatments of the experiment

The experiment was conducted in two factor completely randomized design (CRD) where factor A = plant extracts of tobacco leaves, giant indian milky weed leaves, cloves of garlic, rhizome of ginger, and mahogani seed, keora seed, keora fruit and factor B = different level of concentrations i.e. 0%, 30%, 40%, 50%, 60% and 70%.

Preparation of botanical extracts

Botanical extracts were prepared by using a newly adapted method of standard procedure (Koul *et al.*, 1999)

Preparation of different media using botanical extracts

Fresh mahogani fruit was collected directly from the mahogani tree and fruit were washed with tap water and seeds were separated from hard seed coat and taken into mortar and pestle to collect pure extract. For preparing the stock solution of mahogani seed extract, at first 150 ml pure extracts were mixed with 150 ml distilled water and then 30, 40, 50, 60 and 70 ml extracts from stock solution were mixed with 70, 60, 50, 40 and 30 ml PDA respectively in separate 250 ml conical flask to prepare 30, 40, 50, 60 and 70% concentrations of extract. Same procedure was followed for preparing garlic, ginger and giant Indian milky weed extracts.

For preparing the stock solution of tobacco leaves 250 ml of distilled water was taken into a 1000 ml beaker by using measuring cylinder and 250 ml of tobacco crude leaves were soaked into it for 24 hours. After 24 hours, the tobacco leaves within the beaker were pressed by hand. Then the crude extract solution was filtered and collected for use. After that 30, 40, 50, 60 and 70 ml extracts from stock solution were mixed with 70, 60, 50, 40 and 30 ml PDA respectively in separate 250 ml conical flask to prepare 30, 40, 50, 60 and 70% concentrations of extract. Same procedure was followed for preparing keora seed extract.

150gm fresh fruits of keora collected from the market were washed with running tap water and taken into a pot and boiled for 15 minutes. Then the crude extract was filtered and collected for use. For preparing the stock solution of keora fruit extract, at first 150ml pure extract was mixed with 150 ml distilled water Then 30, 40, 50, 60 and 70 ml extracts from stock solution were mixed with 70, 60, 50, 40 and 30 ml PDA respectively in separate 250 ml conical flask to prepare 30, 40, 50, 60 and 70% concentrations of extract.

Response of the identified *C. gloeosporioides* to seven different plant extracts

Different concentrated media in conical flasks were sterilized in an autoclave at temperature of 121 °C for 20 minutes. After autoclaving about 20 ml of the medium was poured in each 9cm sterilized petridishes. Mycelial discs were prepared using a cork borer (5mm diameter) from the tip of 5 days old cultures of the isolates. One disc of the isolate was placed at the center of a petridish after solidification of the PDA. Each treatment was replicated three times. The medium without plant extract served as control.

Collection and analysis of data

After three days of incubation, radial growth (mm) of *C. gloeosporioides* in petridishes was recorded. The radial growth (mm) of mycelium of each plate was measured by taking average of the two diameters taken right angles for each colony.

Percentage inhibition of growth was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{x}{x - y} \times 100$$

Where,

- x = Average growth (cm) of *C. gloeosporioides* in control petridish
- y = Average growth (cm) of *C. gloeosporioides* in each plant extract treated petridish

Data were analyzed by using MSTAT-C program. The significant difference, if any, among the means were compared by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Main effect of different plant extracts on growth inhibition of *C. gloeosporioides*

The mycelial growth of was inhibited significantly (Table 2). The highest percentage inhibition (60.46%) was observed in garlic extracts which was statistically different from all other treatments. The second highest inhibition (40.40%) was observed in case of keora extract which was also statistically different from the other treatments. The lowest percentage inhibition (18.54%) was observed in case of tobacco leaf extract which was statistically similar to mahogani extract (26.25%) and giant indian milky weed (24.38%) (Table 2).

Table 2. Effect of different plant extracts on growth inhibition of *C. gloeosporioides*

Plant extract (Treatment)	Percent inhibition
Tobacco	18.540e
Keora seed	29.644cd
Keora	40.395b
Mahogani	26.248e
Giant Inadian Milky Weed	24.380e
Garlic	60.455a
Ginger	34.878bc

Means followed by a different letter are significantly different at 1% level

Main effect of different concentrations on growth inhibition of *C. gloeosporioides*

The mycelial growth was inhibited significantly over control at 1% level of significance (Table 3). The highest percentage inhibition (41.04%) was observed at 70% concentration and that was statistically different from the other treatments at 1% level of significance. The second highest percentage inhibition (37.99%) was observed at 60% concentration which was statistically similar with 50% concentration (37.62%).

The lowest percentage inhibition (21.51%) was observed at 30% concentration which was statistically different from the other treatments. The mycelial growth inhibitions in different concentrations significantly increased with the increase of concentrations (Table 3).

Table 3. Effect of different concentration on growth inhibition of *C. gloeosporioides*

Plant extract (Dose)	Percent inhibition
30%	21.505d
40%	29.368c
50%	37.623b
60%	37.996ab
70%	41.036a

Means followed by a different letter are significantly different at 1% level

Interaction effect of seven plant extracts at the different concentrations on growth inhibition of *C. gloeosporioides*

The mycelial growth inhibition in different concentrations was significantly different with the increase of concentration for all plant extracts at 1% level of significance. The highest percentage inhibition (74.35%) was observed in garlic extract at 70% concentration which was statistically similar with to 60% (72.61%) and 50% (68.77%) concentrations of garlic respectively (Plate 6). The second highest percentage

inhibition (47.11%) was found at 40% concentration of garlic extract which was statistically similar with 70% (46.67%) concentration of ginger extract. The lowest percentage inhibition (6.32%) was observed at 30% concentration in tobacco leaf extract which was statistically different from the other treatments combinations. In general, the percentage inhibition increased with increasing of the concentrations for all the plant extracts.

The results obtained from the *in vitro* study showed that garlic extract could be used effectively against *C. gloeosporioides*. The results support the observation of other scientists. Garlic is effective to control fungal growth at 20% concentration in case of *Colletotrichum dematium* (Bhuiyan *et al*, 2008). Tobacco was found more effective to control *Colletotrichum destructivum* (Akinbode and Ikotun, 2008). But in this experiment, the performance of tobacco is inferior to the other treatments.

Conclusion

In conclusion, botanical extracts that exhibited good potential as fungicides of *C. gloeosporioides* should be evaluated further in in-depth to study the phytoextracts for their potentiality under in vivo condition.

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