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Research Article
Antifungal Potential of Some Plant Extracts Against *Colletotrichum gloeosporioides* Causal Organism of Papaya Anthracnose Disease

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Abstract

Background and Objective: The chemical control of papaya anthracnose used by *Colletotrichum gloeosporioides* is a crucial problem. Plant extracts are emerging as safer alternatives to conventional fungicides for the control of plant diseases. The present investigation was conducted with the objectives of evaluating six different plant extracts for their antifungal activity against *C. gloeosporioides*. **Materials and Methods:** Plant species, *Cassia fistula*, *Lantana camara*, *Moringa oleifera*, *Ocimum tenuiflorum*, *Ricinus communis* and *Solanum torvum* were screened for inhibition of mycelium growth and conidia formation. The *C. gloeosporioides* spore suspension of 1×10^5 spores mL^{-1} was sprayed evenly on treated papaya fruits with each plant extract and control fruits were sprayed with sterile distilled water. Diseases severity, soluble solids content, pH and fruit mass loss were evaluated. **Results:** Methanol extract of *L. camara* of 100 µL from the concentration of 50 mg mL^{-1} resulted in the highest inhibition of mycelia growth and conidia formation (90.71:70.85%) followed by *O. tenuiflorum* (45.71%) and *M. oleifera* (44.76%) against *C. gloeosporioides*. Out of tested extracts, *L. camara* gave the lowest disease severity index at 20% concentration and maintained optimum quality of papaya fruit during 7 days experimental period. **Conclusion:** The study revealed that *L. camara* exhibit strong antifungal activities against *C. gloeosporioides* and has potential for being formulated into botanical fungicides against anthracnose of papaya.

Key words: Antifungal, *Carica papaya*, *Colletotrichum gloeosporioides*, *Lantana camara*, plant extracts

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Papaya (*Carica papaya*) is considered one of the most important fruits among the millions of people in the tropical and subtropical area due to its taste, nutrition value and medicinal use. It provides a cheap source of vitamins and minerals in the daily diet of the people. It is an excellent source of provitamin-A, ascorbic acid and also a good source of Calcium. But, it is very susceptible to diseases caused by many micro-organisms especially fungi, because this fruit has a very thin skin and high in moisture and nutrients[1].

Anthracnose caused by *Colletotrichum gloeosporioides* is the major postharvest disease of papaya around the world. Post-harvest losses of Papaya approximately 40-100% have been generally reported in developing countries[2]. The *C. gloeosporioides* fungus can infect immature green fruits attached to the plant and remain quiescent until the fruit begins to ripen. A single isolate can cause both symptoms. The first symptoms are small well defined dried pink spots on the surface of ripening fruit. Later, these lesions grow to 5 cm diameter, become rounded, sunken (from 3-5 mm deep) and brown to black in color[3].

The control of papaya anthracnose is a crucial problem since the fungicide treatments, affect the ripening process, resistant development and increases the risk of high levels of toxic residues. The intensive and frequent applications of fungicides are often required to control fungal pathogens casing post-harvest diseases of crops. The great public awareness of environmental and health issues, as well as the development of fungicide resistance in plant pathogens against synthetic fungicide, has stimulated an increasing demand to shift away from the reliance on conventional fungicides. Plant-based botanical extracts either as pure compounds or as standardized extracts provide an enormous bioresource of potential use as antifungal compounds due to their antimicrobial activities[4-6].

Rahman *et al*.[2] reported that *Jatropha curcas* has the antifungal activity against anthracnose of papaya. Methanol extracts of *Echinops* sp, *Thymus serrultus* and *Ocimum lamifolium* have best antifungal activity against *C. gloeosporioides*[7]. Prasad and Anamika[8] reported that the extracts of *L. camara* were found to be most effective for the control of *C. gloeosporioides*.

Thus, the objectives of this study were to investigated the antifungal activity of extracts obtained from locally available plants, namely, Golden shower (*Cassia fistula*), Lantana (*Lantana camera*), Moringa (*Moringa oleifera*), Thibbatu (*Solanum torvum*), Maduruthala (*Ocimum tenuiflorum*) and Edaru (*Ricinus communis*) against causal agent of anthracnose in papaya and to identify most effective plant extracts to formulate botanical fungicide against *C. gloeosporioides*.

MATERIALS AND METHODS

Experiments were carried out during a period of 20 weeks from January-May, 2018 in the Division of Microbiology at Plant Virus Indexing Centre, Homagama, Sri Lanka.

Isolation and identification of *C. gloeosporioides*: The causal agent of the anthracnose of Papaya fruits was isolated by using the standard procedure. A pure culture of the pathogen was prepared and culture was maintained in PDA culture tubes at 4°C and used as stock culture throughout the study. The pathogen was identified using macroscopic and microscopic features of fungi.

Plant leaf extracts preparation: Fresh healthy leaves of six different plants, namely *Cassia fistula* (Golden shower), *Lantana camara* (Lantana), *Moringa oleifera* (Drumstick), *Ocimum tenuiflorum* (Maduruthala), *Ricinus communis* (Edaru) and *Solanum torvum* (Thibbatu) were air-dried, milled to a fine powder and stored in glass jars until use. The stock extracts were obtained by soaking 10 g of air-dried and milled plant material in 100 mL of methanol (10% w/v) at room temperature (25°C) for 24 h with occasional shaking. Then, the mixtures were filtered through two layers of cheesecloth to obtained leaf extract. The extracts were subjected to centrifugation for 10-15 min at 4000 rpm, again it was filtered through Whatman No. 1 filter paper and finally, the supernatants were collected. Then supernatants pour into the sterilized breaker and put into the incubator at 40°C for evaporating the methanol. About 50 mg of the evaporated methanol extracts of each plant were weighed, dissolved in 1 mL of the methanol and then tested for antifungal activities.

***In vitro* evaluation of antifungal activity of plant extracts:** The inhibitory effect of plant extract against *C. gloeosporioides* causing papaya anthracnose was done by agar incorporation method[9]. The cultures were incubated for 7 days in the dark under room temperature. The diameter of the fungal colony was measured daily. The inhibitory activity to the radial growth (IR) was determined according to the following equation[10]:

$$IR\ (\%) = \frac{dc - dt}{dc} \times 100$$

Where:
IR = Inhibitory activity to the radial growth
dc = Average increase in mycelia growth in control plates
dt = Average increase in mycelia growth in treated plates

Spore production inhibition assay: Based on the methodology described by Siqueira, Jr. *et al.*[11], all tested Petri dishes described above were maintained for three additional days under the same experimental conditions for spore production. At the end of the total incubation period (10 days), five agar discs (5 mm diameter) of mycelia were removed from each tested plate, including control. The agar discs were immersed in 5 mL potato dextrose agar broth and stirred until spores were separated. Samples (50 µL) were examined under a light microscope to determine the number of spores mL^{-1} from each treatment using a hemocytometer. Fungal sporulation with regard to the concentrations of extracts, compared to control is presented as percentage inhibition.

***In vitro* evaluation of antifungal activity of plant extracts:** Extracts that showed antifungal activity was further tested for their effect against papaya anthracnose on harvested fruit. Aqueous extracts of *L. camera* and *O. tenuiflorum* were evaluated at concentrations of 10 and 20% (w/v). Papaya fruits were surface-sterilized by dipping in 1% sodium hypochlorite solution for 10 min rinsed in sterilized distilled water and dried. Then, plant extracts were sprayed in each Papaya fruits separately, while the control fruits were sprayed with sterile distilled water and dried. After treatment, papaya fruits were evenly sprayed with a spore suspension of *C. gloeosporioides*. Conidial suspension of *C. gloeosporioides* was prepared from 10 day old culture on PBD media and adjusted to 10^5 spores mL^{-1} using hemocytometer. Fruits were stored in plastic trays at room temperature (25-28°C) and after a 7 day storage period. Individual fruit was used as replicates. For each treatment have three fruits. The severity of infection, Total soluble solids (TSS) content, pH and fruit mass loss were evaluated for *in vivo* experiments.

Disease severity index (DSI) of disease was calculated by using the standard equation[12]:

$$DSI = \frac{\text{Sum of individual disease rating}}{\text{No. of samples}} \times \frac{100}{\text{Maximum disease grade}}$$

Fruit mass loss was calculated by using the equation:

$$\text{Weight loss (g per 100 g)} = \frac{\text{Sum of individual disease rating}}{\text{No. of samples}} \times \frac{100}{\text{Maximum disease grade}}$$

Determination of quality of papaya fruit: The TSS was measured using a hand refractometer with a range of 0-33 Brix. The TSS was determined by placing 1-2 drops of clear juice on the prism. The pH value of the Papaya juice was measured with a pH meter.

Experimental design: A complete randomized experimental design was used for the experiments with three replication of following five treatments.

Data analysis: Data were statistically analyzed using statistical analysis software (SAS) packages. Duncan's multiple range test was used to determine the significance of the treatment effects at $p<0.05$ level. The Microsoft Excel (2010) computer software package was used to prepare all the graphs.

RESULTS

Identification of the pathogen: The causal organism of papaya anthracnose was identified as *C. gloeosporioides* based on macroscopic and microscopic features. The fungal colony from 10 days old culture on potato dextrose agar was white to dull white with smooth margins. The mycelium was hyaline, superficial, septate and branched. The aerial mycelium was white to dull white. Conidia of *C. gloeosporioides* were hyaline, smooth and thin-walled, cylindrical and straight. The average length of conidia from the culture measured 5-6 µm. Similarly, Tasiwal *et al.*[13] reported that the conidia of *C. gloeosporioides* were cylindrical, hyaline and single-celled with oil globules in the center and size of the conidia varied from 9-20×3-7.5 µm. Sharma[11] reported that the ten days old culture on potato dextrose agar was white to dull white with smooth margins. After 9-10 days, pinkish ooze could be seen in the culture. Conidia were single-celled measuring 10-20 µm long and 4.8-6.7 µm wide.

***In vitro* evaluation of antifungal activity of plant extracts:** The inhibitory effect on mycelium growth by plant extracts against *C. gloeosporioides* was observed in *L. camara* (0.65 cm) which was significantly superior over all the plant extracts tested. Next best was *O. tenuiflorum* (3.63 cm) and *M. oleifera* (3.87 cm). Least mycelia growth reduction was observed in *R. communis* (5.63 cm) and *Cassia fistula* (5.48 cm) (Fig. 1). The data, effect of plant species used on radial mycelium growth and conidia formation against of *C. gloeosporioides in vitro* is presented in Table 1. The data revealed that among the 6 plant extracts tested against *C. gloeosporioides*, *L. camara* (T2) was significantly superior over all other plant extracts. It was showed maximum inhibition of mycelial growth and conidia formation (90.71 and

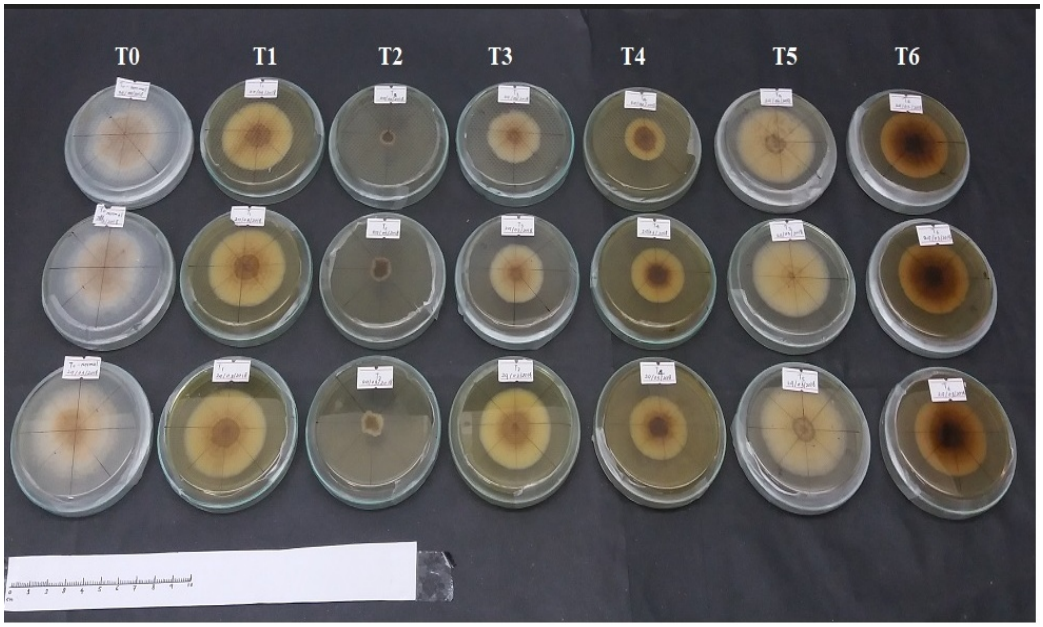

Fig. 1: Mycelium growth inhibition for different concentrations of leaf extract of *Cassia fistula* (T_1), *Lantana camara* (T_2), *Moringa oleifera* (T_3), *Ocimum tenuiflorum* (T_4), *Ricinus communis* (T_5) and *Solanum torvum* (T_6) against *Colletotrichum gloeosporioides*

Table 1: Effect of plant species used on radial mycelium growth and conidia formation against *Colletotricum gloeosporioides in vitro*

Treatments	Plant type	Mycelium growth inhibition (%)	Conidia formation inhibition (%)
T0	Control (no plant extract)	0.0^{e}	0.0^{d}
T1	Golden shower (*Cassia fistula*)	17.48^{d}	17.48^{c}
T2	Gadapana (*Lantana camera*)	90.71^{a}	70.85^{a}
T3	Moringa (*Moringa oleifera*)	35.76$^{c\,d}$	22.65^{bc}
T4	Maduruthala (*Ocimum tenuiflorum*)	45.71^{b}	20.17^{bc}
T5	Edaru (*Ricinus communis*)	19.63^{d}	15.63^{c}
T6	Thibbatu (*Solanum torvum*)	22.04^{d}	10.32^{c}

Means followed by different letters within a column differ significantly at p<0.05

Table 2: Effect of two different concentration of *L. camara* and *Ocimum tenuiflorum* extracts of plants on severity of anthracnose disease and quality parameter of papaya

Treatments	Severity score	DSI	Weight loss (g per 100 g)	TSS	pH
Control (T1)	4.6	93.33^{a}	16.07^{a}	11.60^{a}	5.81^{a}
Lantana camera-10% (T2)	2.3	46.67^{bc}	5.83^{c}	10.23^{bc}	5.53^{b}
Lantana camera-20% (T3)	1.0	20.00^{c}	3.31^{c}	10.60^{b}	5.54^{b}
Ocimum tenuiflorum-10% (T4)	4.3	86.67^{a}	11.15^{b}	10.00^{c}	5.58^{b}
Ocimum tenuiflorum-20% (T5)	2.6	53.33^{b}	9.87^{b}	10.37^{bc}	5.53^{b}

Means followed by different letters within a column differ significantly at p<0.05

70.35 %) followed by *O. tenuiflorum* (45.71%). Next best was *M. oleifera* extract (44.76%). Rest of the botanicals gave comparatively low levels growth of inhibition of both mycelium growth and conidia formation.

***In vivo* evaluation of antifungal activity of plant extracts**

Effect of plant extracts on disease severity of anthracnose of papaya: Disease severity index (DSI) at 7 days after postharvest treatment at two different concentrations (10 and 20%) of *Lantana* and *Ocimum* is presented in Table 2 and Fig. 2. Among two plant extracts evaluated *in vivo*, *Lantana* 20% concentration showed the lowest disease severity index followed by *L. camara* (46.67%) at 10% and *Ocimum* (53.33%) at 20%. Maximum DSI was observed in control (93.33%) (Table 2).

Effect of plant extracts fruit quality parameter of papaya: Weight mass loss at 7 days after post-harvest treatment at

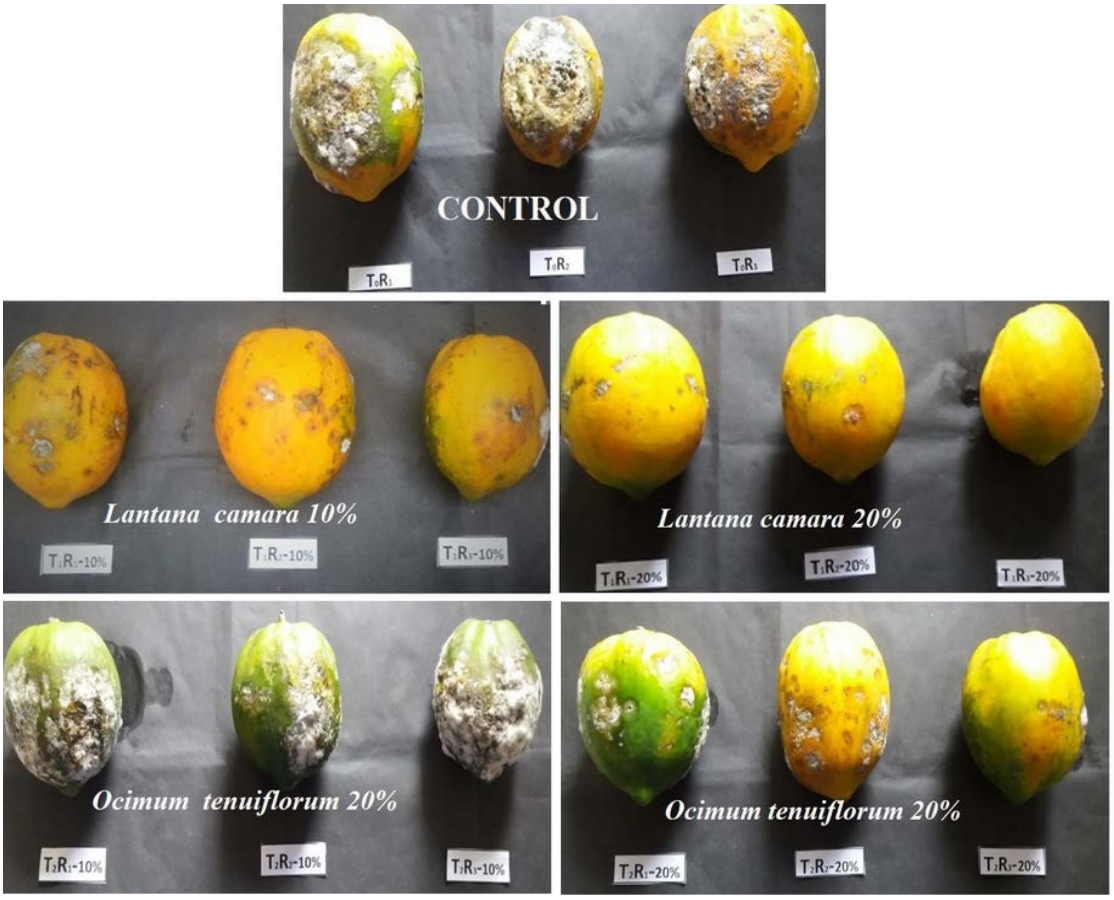

Fig. 2: Effect of plant extracts, *Lantana camara* and *Ocimum tenuiflorum* on anthracnose disease development in papaya fruit

two different concentrations (10 and 20%) is presented in Table 2. Data showed the significantly differ over control, among two plant extracts evaluated *in vivo*, *Lantana camara* at 20% concentration kept lowest weight loss (3.33%) followed by *L. camara* (5.83%) at 10% and *O. tenuiflorum* (9.87%) at 20%. Maximum weight loss was observed in control (16.07%). There was a high positive correlation between weight loss and DSI.

Total soluble solid of treated papaya fruit significantly ($p = 0.05$) differed from those of the control. There was a significant difference in the TSS of Papaya fruit as a result of treatment of the fruits with plant extracts (Table 2), the maximum TSS value (10.60 and 10.370 Brix.) was exhibited in fruits treated with extracts of *L. camara* of 20% and *O. tenuiflorum* at 20% concentration, respectively. The highest pH was recorded in untreated control (5.81) and fruit treated with the extract of *L. camara* and *O. tenuiflorum* at all concentration, ranges from 5.53-5.58 with no significant ($p = 0.0659$) difference among them.

DISCUSSION

Due to the drawback of the use of synthetic chemicals to control post-harvest disease of fruits, there is a huge demand for alternative methods. The utilization of natural products, especially the plant extracts has been shown to be effective against many plant pathogens and considered to be safe for consumers and environments[14-15]. However, in Sri Lanka, only a few reports are available on the exploration of the antifungal potential of some plant species. In this study, the antifungal nature of the methanol extracts of six different locally available plant species was evaluated against *C. gloeosporioides* both in *in vitro* and *in vivo* conditions. The study revealed that leaf extract of *L. camara* inhibited radial mycelium growth, conidia formation of *C. gloeosporioides* and anthracnose disease development in papaya remarkably. The finding is in agreement with the findings of Prasad and Anamika[4], who confirmed the antimicrobial activity of leaf extracts of *L. camara* in

controlling *C. gloeosporioides.* Antimicrobial activity of leaf extract from *L. camara* could be attributed to its chemical constituents as reported by Singh and Srivastava[16].

The highest inhibition of conidial formation was obtained only with the extract of *L. camara* and none of the other extracts did not show significant differences in inhibition of conidial formation. It is difficult to point out the real reason for different results. However, in the previous findings of some researchers revealed that the same isolates of *C. gloeosporioides* showed differences in mycelial growth and spore germination due to the great genetic variability of this fungus[17-18].

Other than disease suppression extending the storage life of perishables are the most important properties, that should accomplish with any kind plant-based extract utilize in post-harvest disease management. Therefore in this study, it have evaluated the quality parameter of the papaya fruits treated with different plant extract. This study showed that application of plant leaf extracts reduced the physiological loss and prolonging the shelf life of papaya fruits by checking the growth of microbes that are responsible for rotting and reduce metabolic rate of the fruits, which cause the loss in weight through respiration. Bautista-Banos *et al.*[19] reported that desiccation and decay are the two major causes of the termination of the commercial life span of fruits, which can be the result of various post harvest disease and other physiological disorders. Also, Gamagae *et al.*[20] clearly stated that, increased in incidence and severity of the disease resulted in softening and rotting of fruit tissue which in turn leading to the reduction in the marketability of the fruits. A similar result was observed in the present study.

The high TSS content of papaya fruits could be due to the high level of anthracnose severity, which could have accelerated ripening, thus, resulting in incensement of sugar level of the fruits. The low TSS may be the attribute of the low ripening process as a result of low level of anthracnose infection on the fruits[21]. According to the study of Alemu *et al.*[21] reported that there was a significant difference in the TSS of mango fruit as a result of treatment of the fruits with plant extracts, the maximum TSS value was exhibited in fruits treated with extracts of *L. camara*. A high level of TSS was also recorded in the untreated control and plant extracts at the lower concentration. The low value of TSS in the fruit caused to delaying early senescence of the fruit. The reason for increased TSS content during storage is mainly due to the conversion of starch into soluble sugar with advances in ripening[22]. A high rate of disease incidence and severity caused to higher rates of respiration which would raise pH of the fruit juice as ripening advances[23].

Bautista-Banos *et al.*[19] reported that fruits in the untreated control ripened quickly and this led to the reduction of acidity in fruit. It's caused to increase in the pH and total soluble solid contents of papaya fruits. Earlier, Abbasi *et al.*[24] demonstrated that the ascorbic acid and TA of mango fruit first increase then decrease, while pH and the TSS values increase during senescence.

Abbasi *et al.*[24] suggested that the change in pH is associated with the effect of treatment on the respiration and metabolic activity of the fruits. In this study, it seems that fruits with a high rate of disease incidence and severity had higher rates of respiration which would raise pH of the fruit juice as ripening.

CONCLUSION

Post-harvest treatment of papaya fruits with various plant extracts can suppress the development of anthracnose. Leaf extract of *L. camara* was inhibited growth of *C. gloeosporioides* as well as anthracnose development on artificially inoculated papaya fruits and could be a potential source of sustainable environmentally-friendly botanical fungicides to control papaya anthracnose.

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