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DEVELOPMENT OF ENVIRONMENT FRIENDLY CONTROL METHOD TO MINIMIZE FRUIT ROT DISEASES OF GUAVA *(Psidium guajava)* THROUGH PLANT EXTRACTS

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ABSTRACT

An experiment was conducted at the Fruit Crops Research and Development Centre, Horana to study the fungi associated with the fruits of guava and evaluate several control measures. The most common pathogenic fungi isolated were *Colletotrichum gloeosporioides*, *Pestalotiopsis psidii*, *Botryodiplodia theobromae* and *Phomopsis psidii*. Pathogenicity of fungi was confirmed and the symptoms of each pathogen were recorded as anthracnose, canker, soft rot and styler end rot respectively. The treatments consisted of a control, three plant extracts, cinnamon oil and recommended levels of three fungicides. The efficacy of leaf extracts; *Cinnamomum zeylanicum* (cinnamon), *Mimosa pudica* (nidikumba), neem seed extract and cinnamon oil and selected fungicides in suppressing the growth of pathogenic fungi were evaluated *in vitro*. Cinnamon leaf extract showed the highest reduction of growth of all four fruit rot pathogens. Neem seed extract reduced the growth of mycelium of anthracnose and canker pathogens. Mimosa leaf extract had moderate effect on the reduction of some pathogenic mycelial growth while the cinnamon oil had little effect. Chlorothalonil reduced the growth of anthracnose, styler end rot and soft rot pathogens while carbendazim controlled the styler end rot pathogen (more than 80% reduction of growth of mycelium). None of the fungicides controlled the canker pathogen. Thiovit had shown the moderate effect on reducing the mycelial growth. Cinnamon leaf extract can be used to control all four fruit rot diseases compared to fungicides. Neem seed extract can be used to control anthracnose and canker diseases. The plant extracts do not produce health hazards to human and in addition it is environment friendly. Therefore, it can be successfully applied to reduce fruit spoilage of guava.

KEYWORDS: Fungicides, Fruit rot diseases of guava, Plant extracts.

INTRODUCTION

Guava (*Psidium guajava*), which belongs to the Family Myrtaceae is indigenous to tropical America. It is widely distributed in the tropics and subtropics. The fruit contains high levels of vitamin C (approximately 200 mg 100/g) and is utilized as fresh or processed products. There are five popular varieties grown in Sri Lanka, "Horana red", "Horana white", "Pubudu", "Kanthi" and "Bangkok giant".

There are many pathogens which cause diseases to guava, mainly fungal pathogens. Guava wilt is the most important disease (Dwivedi, 1990). The fruit rot diseases are also important as they cause serious crop losses. In Sri Lanka fruit rot diseases are caused by several fungi. Many post-harvest fruit rot diseases initiate at the pre-harvest stage on the tree, while

many post-harvest fruit rots occur on mature or ripening fruits prior to harvest, at harvest, and in transit and storage.

Cultural, chemical, biological methods and growing resistant varieties are practiced as disease control methods. The most common method used in controlling fruit rot diseases is the application of synthetic fungicides. Although fungicides are effective in disease control, they are harmful to consumers since they accumulate in the product. This can cause negative effects such as development of pathogen resistance, harm on environment friendly organisms and difficulties in the disposal of unused fungicides (Sivakumar *et al.*, 2001). Therefore, alternative measures like effective plant extracts should be identified to control pathogens and also to develop environment friendly disease control method.

MATERIALS AND METHODS

Isolation and identification of causal organisms

Guava fruits with symptoms indicating fruit rots were obtained from approximately 58 trees grown in the Fruit Crops Research and Development Centre, Horana, including six varieties "Horana red", "Horana white", "Pubudu", "Kanthi", "Bangkok giant" and "Sapida".

The affected areas of fruit rots were surface sterilized with 70% ethanol. Small pieces (2 mm diameter) of tissue, five from each fruit were aseptically removed from the advancing edge of the rot, 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 obtain pure cultures for identification purposes. Identification of pathogens were carried out according to the descriptions of CAB International Mycological Institute, U.K. (Mordue, 1969).

Symptoms and pathogenicity of fungal isolates

In order to confirm that the isolated fungal strains from guava fruit rot diseases produce the same type of symptoms, when reinoculated on to healthy guava fruits, healthy matured fruits of guava were used to inoculate each isolated fungal species. 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.

Drops (approximately 20 ml) of conidial suspension of each causal organism were placed on three matured healthy fruits. Five inoculation

sites were made on each fruit with five controls made up of drops of sterilized distilled water.

The inoculated fruits were kept on plastic trays on the wet tissues, covered with transparent polythene to maintain the humid conditions necessary for germination of conidia. The plastic trays and polythene were removed after 3 days of incubation and the fruits were then maintained under normal laboratory conditions 28°C±2°C until symptoms developed.

Infection levels of different fruit rot diseases on different guava varieties

Diseased guava fruit were collected from the orchards of Fruit Crops Research and Development Centre, Horana. Diseased samples were taken from different guava varieties "Horana red", "Horana white", "Pubudu", "Kanthi", "Bangkok giant", "Sapida" and "strawberry guava". Percentage of disease incidence of anthracnose, canker, styler end rot and soft rot in different guava varieties were investigated.

Screening of fungicides against the pathogens causing fruit rot diseases of guava *in vitro* condition

Effect of two contact fungicides, chorothalonil 75% (Daconil) and sulphur wettable powder (Thiovit), and one systemic fungicide, carbendazim (Bavistin) on the growth of four pathogens causing anthracnose, fruit canker, soft watery rot and styler end rot were tested *in vitro* with five replicates. The fungicide concentrations were 2 g/l of carbendazim, 2 ml/l of chlorothalonil and 4 g/l of wettable sulphur. Thirty nine grams potato dextrose agar (oxoide commercially grade) was added to each one litre fungicide solutions and the amended medium was autoclaved at 121°C and 1.09 kg/cm^2 pressure for 15 minutes. Agar pieces from four different pathogenic fungi (using 0.5 cm diameter cork borer) grown on PDA were transferred to the fungicidal amended medium in petri-dishes and incubated at 25°C. The test fungi were also grown without fungicides on PDA under similar conditions. Colony diameter was measured at 2, 4, 6 and 8 day intervals.

Effect of different plant extracts on growth of four pathogenic fungi causing anthracnose, fruit canker, soft watery rot and styler end rot in *in vitro* condition

Effect of *Cinnamomum zeylanicum* oil, leaf extract of *Mimosa pudica, Cinnamomum zeylanicum* and seed extract of *Azadiracta indica* on the growth of four pathogenic fungi causing anthracnose, fruit canker, soft watery rot and styler end rot were tested *in vitro* with five replicates.

The plant extracts were obtained by macerating 250 g fresh leaves in 1000 ml distilled water in an electric blender at a speed of 21,000 rpm for two minutes. Hundred ppm cinnamon oil was added to 1 litre of distilled water and 200 g/l neem seeds were crushed to prepare the neem solution. Thirty nine gram PDA was added to 1litre of filtered extracts and amended medium was autoclaved at 121°C and 1.09 kg/cm^2 pressure for 15 minutes.

Agar pieces from four different pathogenic fungi (using 0.5 cm diameter cork borer) grown on PDA were transferred to the fungicidal amended medium in petri-dishes and the plates were incubated at 25°C. The test fungi were also grown without plant extracts on PDA under similar conditions. Colony diameter was measured at 2, 4, 6 and 8 day intervals. The percentage reduction in growth due to the effect of different plant crude extracts was calculated according to the formula,

$$\text{Percentage reduction in growth} = 100 \times \frac{\text{Diam .C - Diam .F}}{\text{Diam .C}}$$

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.

RESULTS

Isolation and identification of causal organisms

The fungi isolated from fruit rots of guava are shown in the Table 1. Total of 300 isolations were made from 60 rotted guava fruits. Most common groups of fungi isolated were *Colletotrichum gloeosporioides, Pestalotiopsis psidii, Botryodiplodia theobromae, Phomopsis psidii, Fusarium spp* and *Aspergillus spp.*

Pathogencity and symptom development

Pathogencity of *Colletotrichum gloeosporioides*, *Pestalotiopsis psidii*, *Botryodiplodia theobromae, Phomopsis psidii*, was confirmed and the symptoms of each pathogen were described and tabulated in Table 2. The experiment failed to confirm pathogenicity of *Fusarium spp.* and *Aspergillus spp.*. Predominant fruit rot disease causal agents and the symptoms when re-inoculated to the guava fruits were described as follows. Commonly, in the field condition these fungal infections occur concurrently. Pathogen identification was done according to the CMI descriptions (Mordue, 1969).

Table 1. Symptoms of each pathogen when re-inoculated to the guava fruits.

Pathogenic Fungus	*Symptoms showed in the re-inoculated guava fruits*
1. *Colletotrichum gloeosporioides*	anthracnose (black lesions with salmon pink conidia in the lesion) (Fig. 5)
2. *Pestalotiopsis psidii*	fruit canker (Fig. 6)
3. *Botryodiplodia theobromae*	soft watery rot (Fig. 7)
4. *Phomopsis psidii*	styler end rot (Fig. 8)

Infection levels of different fruit rot diseases on different guava varieties

Table 2. Disease incidence level of different guava fruit rot diseases in different guava varieties.

Variety	*Disease incidence (%)*			
	Anthracnose	*Canker*	*Styler end rot*	*Soft rot*
1. Horana red	10.18	18.85	20.00	26.12
2.Horana white	18.05	47.16	67.62	48.17
3. Pubudu	20.62	37.83	40.15	76.03
4. Kanthi	11.97	36.18	31.91	30.97
5. Bangkok giant	40.17	76.67	60.68	80.72
6. Sapida	60.86	00.00	07.26	36.41
7. Strawberry guava	60.12	62.80	30.46	36.64
P	0.01	0.01	0.01	0.01
LSD	19.07	26.31	28.72	30.12
CV%	18.51	24.02	17.92	21.51

According to the results obtained, all the guava varieties (Horana red, Horana white, Pubudu, Kanthi, Bankok giant, and strawberry guava) were susceptible to all four diseases in different levels. Only the Sapida variety had shown the resistance character to fruit canker disease but was susceptible to anthracnose and soft rot disease.

Screening of fungicides and plant extracts to control different fruit rot pathogens

Effect of different plant extracts and fungicides on the growth of anthracnose fungus, canker casing fungus, styler end rot fungus and soft rot fungus during the incubation period is shown in Figures 1, 2, 3 and 4. The percentage reduction in growth of the different pathogens due to the effect of different plant crude extracts was calculated and shown in Table 3.

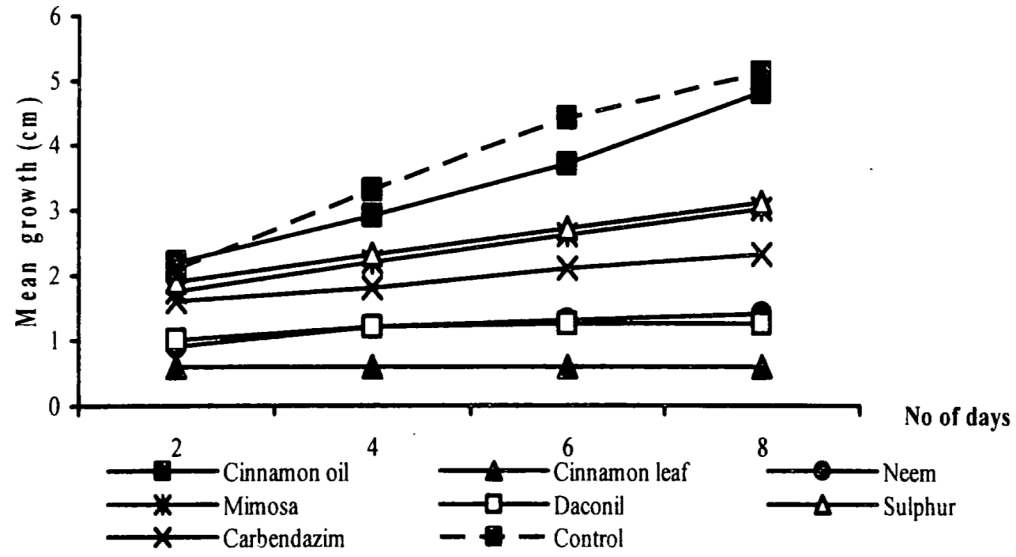

Figure 1. Effect of plant extracts and fungicides on the growth of *Colletotrichum gloeosporioides.* (anthracnose fungus).

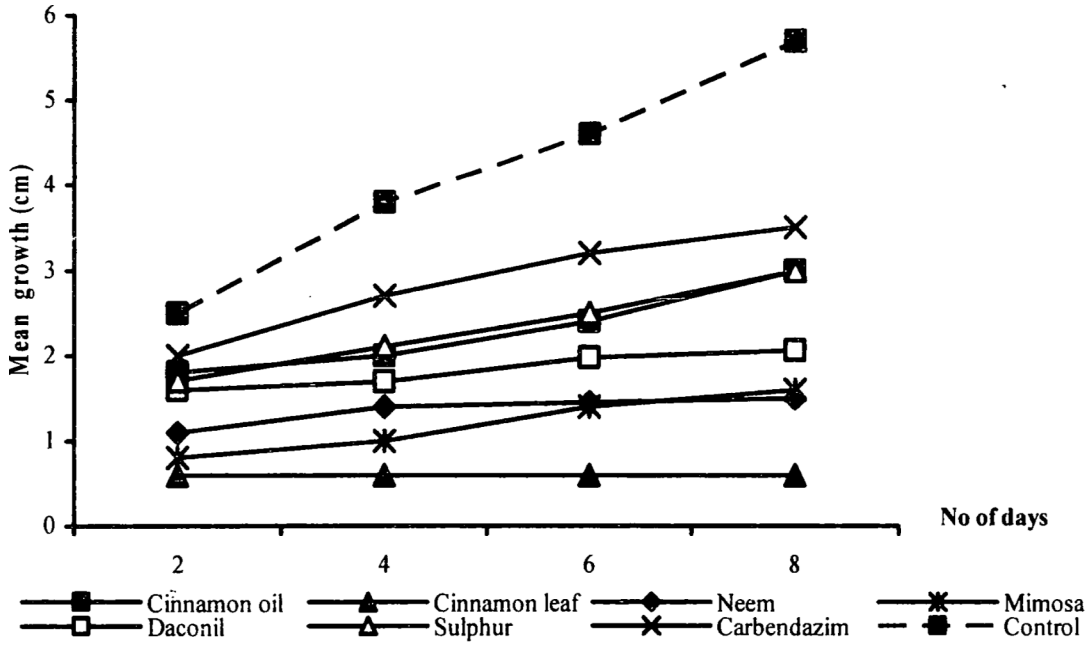

Figure 2. Effect of plant extracts and fungicides on the growth of *Pestalotiopsis spp.* (canker causing fungus).

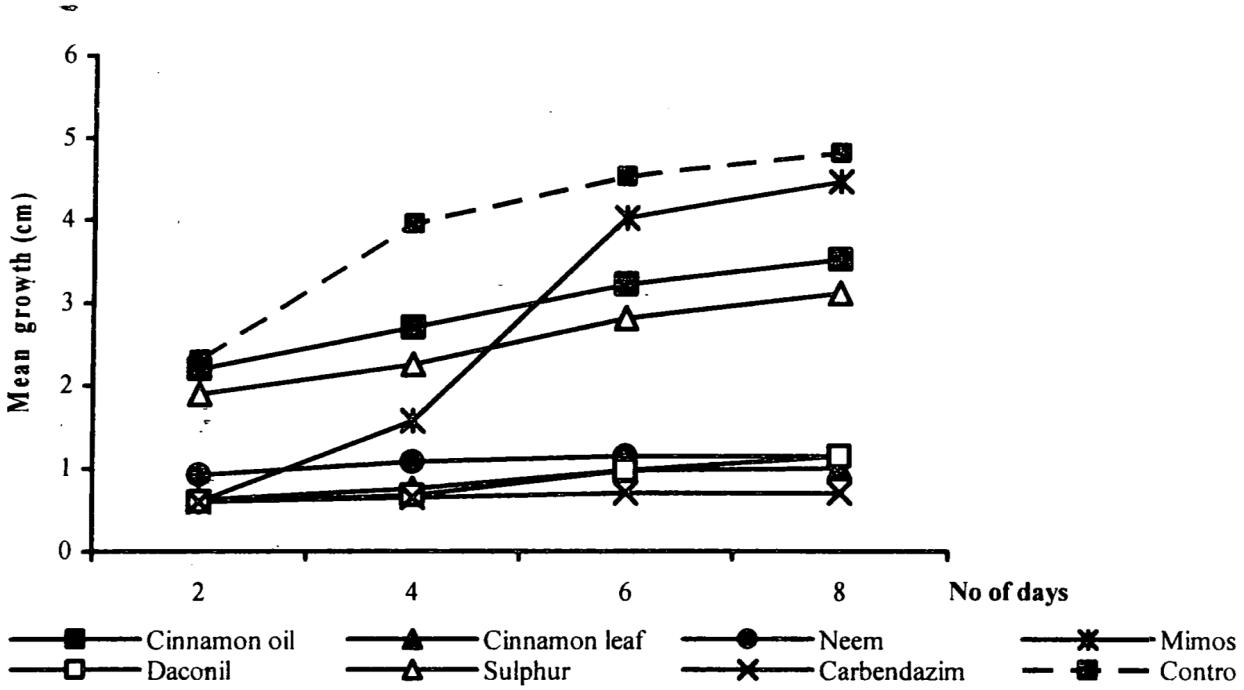

Figure 3. Effect of plant extracts and fungicides on the growth of *Phomopsis spp* (styler end rot fungus).

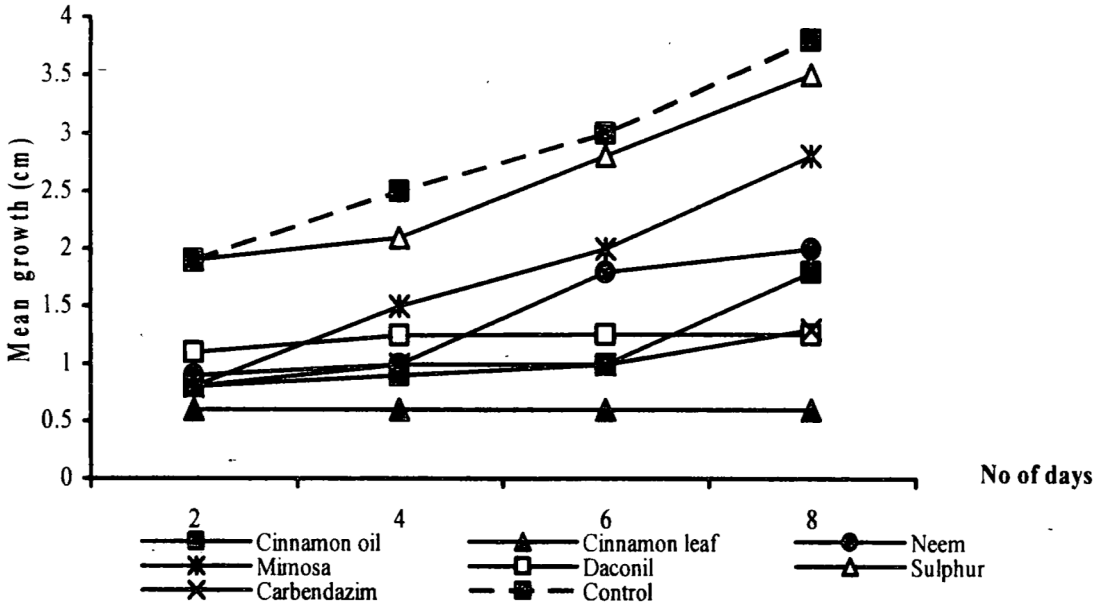

Figure 4. Effect of plant extracts and fungicides on the growth of *Botryodiplodia theobromae* (soft rot fungus).

Table 3. Effect of plant extracts and fungicides on the percentage reduction in growth of each pathogen after 8 day interval.

Treatments (plant extracts and fungicides)	*Mean percentage reduction in growth of each fruit rot pathogen*			
	Colletotrichum gloeosporioides	*Pestalotiopsis psidii*	*Phomopsis psidii*	*Botryodiplodia theobromae*
1. cinnamon oil	6.67	52.94	30.95	62.05
2. cinnamon leaf extract	100	100	90.47	100
3. neem seed extract	80.02	84.31	87.14	56.25
4. Mimosa leaf extract	77.71	83.85	8.20	31.25
5. chlorothalonil	85.33	63.70	86.90	80.00
6. Sulphur	44.44	53.92	40.47	9.37
7. carbendazim	66.66	64.70	97.61	79.78
8. Control	0.00	0.00	0.00	0.00
P	0.01	0.01	0.01	0.01
LSD	21.12	18.68	13.41	22.52
CV%	8.60	9.82	7.12	8.80

DISCUSSION

According to the symptoms observed, the causal agents were identified as, *Colletotrichum gloeosporioides* causing anthracnose, *Pestalotiopsis psidii* causing fruit canker, *Phomopsis psidii* causing styler end rot and *Botryodiplodia theobromae* causing soft watery rot.

Similar pathogens have been recorded as causal agents associated with the above symptoms in India, Malaysia and Nigeria (Adisa, 1985; Madhukar and Reddy, 1989; Lim and Khoo, 1990). According to the results obtained from the screening of plant extracts and fungicides on the growth of anthracnose fungus, the most efficient treatments which had shown above 80% reduction of growth of pathogen were cinnamon leaf extract, chlorothalonil and neem seed extract (Table 3 and Fig. 1).

The most effective treatments which had shown above 80% reduction of growth of *Pestalotiopsis spp.* (canker pathogen) were cinnamon leaf extract, neem and mimosa. None of the fungicides efficiently controlled the pathogenic growth (Table 3 and Fig. 2).

The growth of *Phomopsis* spp. (styler end rot pathogen) can be reduced by using two fungicides carbendazim, and chlorothalonil as well as two plant extracts, cinnamon leaf extract and neem seed extract (Table 3 and Fig. 3).

Figure 5. Symptoms of anthracnose.

Figure 6. Symptoms of canker.

Figure 7. Symptoms of soft rot.

Figure 8. Symptoms of styler end rot.

The most effective treatments that can reduce the growth of *Botryodiplodia* spp. (soft rot pathogen) were cinnamon leaf extract, chlorothalonil and carbendazim (Table 3 and Fig. 4). Similar results were obtained from the fungicide screening tests in other countries (Rawal and Ullasa, 1988; Butt *et al.*, 1995).

CONCLUSIONS

It appears that, cinnamon leaf extract had shown the highest reduction of growth of all four fruit rot pathogens (90-100 % reduction of mycelial growth). Neem seed extract efficiently reduced the growth of mycelium of anthracnose and cancker pathogens (more than 80% reduction of mycelial growth). Mimosa leaf extract had a moderate effect on the mycelial growth of some pathogenic fungi while cinnamon oil had only a little effect on reducing the mycelial growth.

Chlorothalonil effectively reduced the growth of anthracnose, styler end rot and soft rot pathogens while carbendazim effectively controlled the styler end rot pathogen (more than 80% reduction of growth of mycelium). None of the fungicides effectively controlled the cancker pathogen. Neem seed extract can be used to control anthracnose and cancker diseases. The plant extracts do not produce health hazards to human beings and in addition they are environmental friendly. Therefore, they can be successfully applied to reduce fruit spoilage of guava.

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