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Identification of the Pathogen and Fungicidal Screening for Control of New Leaf Disorder in Cashew (*Anacardium occidentale* L.) Nursery Plants

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ABSTRACT

Cashew (*Anacardium occidentale* L.) is a tree nut which can even survive in the driest areas of the country. It has been identified as a low input, high income generating crop. Requirement of the high quality planting materials is increasing. Therefore, disease management is very important in cashew nurseries. An experiment was conducted to identify the pathogen and select a suitable fungicide to control a new leaf disorder recorded in cashew nursery. Identification procedures confirmed that a *Pestalotiopsis* species is causing the disorder. Two bio fungicides, commercial preparations of *Trichoderma viride* and *Trichoderma harzianum* and two synthetic fungicides, Chlorothalonil and Thiophanate methyl that were screened against the identified *Pestalotiopsis* species were effective in controlling while Thiophanate methyl and *Trichoderma viride* are superior than others.

KEYWORDS: *Anacardium occidentale*, Bio fungicides, Cashew, Leaf disorder, *Pestalotiopsis* species

INTRODUCTION

Cashew (*Anacardium occidentale* L.) tree is a tropical evergreen plant that belongs to family Anacardiaceae. The commercial cashew nut native to coastal Brazil is grown as a plantation crop. Among the tree nuts, cashew nut ranks third in international trade with 20% of the market (Mandal, 2000).

It has gained the status of one of the most suitable crop to be cultivated in areas with wide range of agro ecological conditions including the North and Eastern provinces of Sri Lanka. Kurunegala, Puttalam, Hambanthota and Gampaha are the major cashew producing districts in Sri Lanka (Jayasekera *et al.*, 2000). It also has been identified as a low input, high income generating fruit crop which can survive even in the driest parts of the other areas of the country (Indulgoda *et al.*, 2006).

Therefore, the demand for cashew planting material is rising continuously. However the Sri Lanka Cashew Corporation and other private sector planting material producers are still unable to meet the demand (Jayasekera and Kodikara, 2003).

In the nurseries young cashew plants are provided with optimum conditions to promote vigorous and healthy growth. As a result, there is a possibility of high pest and disease attacks. High plant density is also another reason for rapid spreading of diseases. Root rot, damping off, leaf curling, color rot and dieback of scion are some of the major diseases in cashew nurseries (Mubarak and Ranaweera, 2001).

Currently, synthetic fungicides are applied to suppress the fungi populations and reduce the spread of fungi. However, the over use of synthetic fungicides has resulted in resistance. Environmental residues are also a concern because consumers demand for chemical-free food. Thus, many countries are trying to reduce their use of chemicals. Biological control is an alternative control method that utilizes bio agents. While being beneficial to the environment cost effective bio agents provide long term, sustainable management of fungus (Jayasinghe and Ranaweera, 2015).

Antagonists of phytopathogenic fungi have been used to control plant diseases, and 90% of such applications have been carried out with different strains of the fungus *Trichoderma*. Most of these strains are classified as imperfect fungi since they have no known sexual stage. The most common biological control agents (BCAs) of the *Trichoderma* genus are strains of *T.virens*, *T. viride* and *T. harzianum* (Benitez *et al.*, 2004).

The success of *Trichoderma* strains as BCAs is due to their high reproductive capacity, ability to in the utilize nutrients from different sources, capacity to modify rhizosphere, strong aggressiveness against phytopathogenic fungi, and efficiency in promoting plant growth and defense mechanisms (Benitez *et al.*, 2004).

Bio controls results either from competition for nutrients and space or as a result of the ability of *Trichoderma* BCAs to produce and/or resist metabolites that impede spore germination (fungistasis), kill the cells (antibiosis) or modify the rhizosphere. Bio control may also result from a direct interaction between pathogen itself and BCA, as in mycoparasitism, which involves physical contact and synthesis of hydrolytic enzymes,

toxic compounds and/or antibiotics that act synergistically with the enzymes (Benitez *et al.*, 2004).

In 2015, a new unidentified leaf disorder was recorded with significant incidence level at the cashew nursery of Kamandoluwa Plantation of Sri Lanka Cashew Corporation. Initially leaves showed small brown colour spots of 0.5-2 mm diameter with yellow colour margin on whole leaf area. Consequently leaf became yellow colour and finally shed. Identification and management of the disorder is of paramount importance for the supply of good quality planting material from nursery. Therefore, an experiment was carried out with objectives to identify the causal organism of the disease and to screen a potential fungicide and bio fungicide to control the disease.

MATERIALS AND METHODS

Experimental Site

The study was carried out at the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila from December 2015 to May 2016.

Collection of Samples

Cashew nursery plants that were affected by new disorder were collected from cashew nursery of Kamandoluwa Plantation of Sri Lanka Cashew Corporation and kept under normal nursery condition.

Identification of the Pathogen by Visual Observation

Disease symptoms on plant leaves were observed visually by naked eye and through stereoscope microscope for their preliminary identification by symptoms.

Isolation of Pathogen on PDA Medium

Infected plant leaves were washed under running tap water. Then leaves were surface sterilized by 5% sodium hypochlorite solution, and washed three times with sterile distilled water.

Potato dextrose agar (PDA) medium that was used as the culturing medium to isolate fungal pathogen from infected plant leaves, were prepared and autoclaved at 121 °C and 15 psi for 20 minutes (Johnston and Booth, 1983). All glassware and other isolation instruments were sterilized at 170 °C for two hours. Petri dishes with PDA were inoculated by placing four 3×3 mm size infected leaf laminar pieces with symptom and surrounding area in a laminar flow cabinet. Inoculated plates were incubated for seven days in an incubator at 25 °C.

Confirmation of Pathogen

Koch's postulations were carried out for the confirmation of pathogenicity (Johnston and Booth, 1983). Spore suspension of pathogen was prepared by scraping the pathogen colony with the addition of distilled water and sieving through double layered gauze. The spore suspension was diluted up to 1×10^6 spores mL^{-1}. Then three drops of tween 20 was added for 500 mL of inoculum. Uniformly grown cashew seedlings were artificially inoculated with the isolated pathogen culture by rubbing the spore suspension of the pathogen on leaf. Inoculated plants were kept under humid and shaded condition for three days.

Fungicide Screening

Two synthetic fungicides and two bio fungicides were selected to evaluate the effect on identified pathogen. They are Chlorothalonil 500 g/l (Daconil), Thiophanate methyl 70% (w/w) (Topsin), *Trichoderma harzianum* 1.15% (w/w) (sample of commercial product) and *Trichoderma viride* 1.15% (w/w) (sample of Blister Blast). Sterilized distilled water was used as the control (Table 1).

Table 1. Tested synthetic and bio-fungicides for controlling fungal infection in cashew

T	Fungicide	Concentration
1	Daconil (Chlorothalonil)	3 ml/l
2	*Trichoderma harzianum*	7.8125 g/l
3	Topsin (Thiophanate methyl)	0.6 g/l
4	*Trichoderma viride*	7.8125 g/l
5	Control	-

T- Treatment

In vitro Screening of Synthetic Fungicides

The poison food technique was used for *in-vitro* screening to determine the effectiveness of synthetic fungicides in controlling identified pathogenic fungi (James *et al.*, 1995). Required concentrations of the fungicides for different treatments were prepared by dissolving the requisite quantity of each fungicide separately in sterile distilled water. One milliliter of each fungicide was mixed with 20 mL of PDA solution separately at 45 °C. Fungicide amended PDA medium was poured aseptically into 9 cm diameter sterile petri dish and allowed to cool and solidify.

Seven day old actively growing pure culture of pathogen was cut into 0.5 cm diameter discs by using a sterilized cork borer. A single disc was placed aseptically at the center of each petri dish containing fungicide amended PDA medium.

In vitro Screening of Bio Fungicides

Potato Dextrose Agar medium (20 mL) was poured into 9 cm diameter sterilized petri

dish and kept for solidifying. Required concentrations of bio preparations for different treatments were prepared by dissolving the requisite quantity of each bio preparation separately in sterile distilled water. One milliliter of each bio fungicide solution was poured separately on the solidified PDA dishes and spread uniformly using a sterilized spreader.

Seven day old actively growing pure culture of pathogen was cut into 0.5 cm diameter discs by using a sterilized cork borer. A single disc was placed aseptically at the center of each petri dish containing bio fungicide.

Mycelia disk of pathogen on PDA medium without any synthetic fungicide or bio fungicide was used as the control. The prepared PDA dishes were incubated at 25 °C for eight days. The experiment was conducted in Randomized Complete Block Design (RCBD) with four replications for each treatment. Replicate size was four.

Plates were observed and diameters of the newly developed fungi colonies were measured daily from fourth day along the intersecting lines and the mean of three measurements were recorded for each plate in each replicate using a ruler.

The maximum growth of mycelium in control was recorded on seventh day after culturing. Therefore, on the seventh day colony diameter of pathogen was recorded in all treatments and percentage of growth inhibition was calculated by using the formula given by Kaiser (2005).

$$\text{Percentage Inhibition} = \frac{C\text{-}T}{C} \times 100\%$$

Where;
C-Colony diameter of the control (cm)
T-Colony diameter of the treated plate (cm)

Inhibition percentage was calculated and data was analyzed by the SAS system 9.2.

RESULTS AND DISCUSSION

Identification of Fungi

After sub culturing for two times, one fungal type was isolated on the PDA media. Characteristics of the fungi were observed using light microscope (VIOLA MC30). The fungal colony was white colour with thick cotton like texture. Colony margins were nearly curve. Seven days after culturing, sporulation started (Figure 1). The spores were fusiform, five celled, with three dark colour median cells and hyaline end cells (Figure 2), and with two or three apical appendages arising from the apical cell (Figure 3). According to the colony, mycelium and spore characteristics of isolated fungi was identified as *Pestalotiopsis* spp. according to the guidelines of identification keys (Agrios, 1997; Barnett and Hunter, 1995).

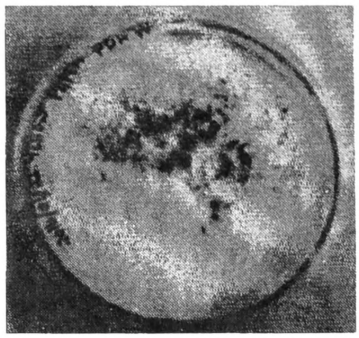

Figure 1. White colour colony of *Petalotiopsis* spp. on potato dextrose agar medium

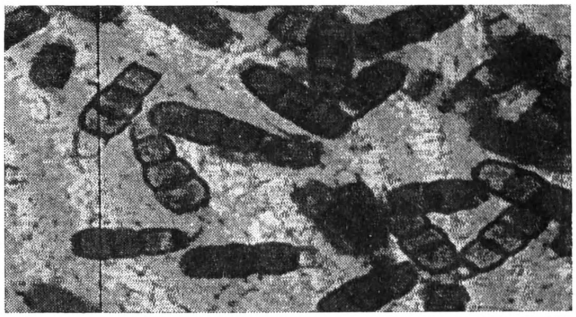

Figure 2. Light microscope images (×40) (LABOMED LX400) of spores of *Pestalotiopsis* spp.

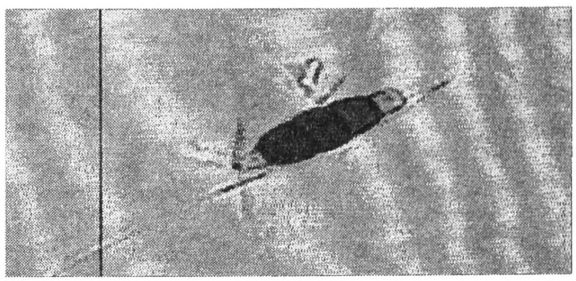

Figure 3. Light microscope image (×40) (LABOMED LX400) of a single spore of *Pestalotiopsis* spp.

Most *Pestalotiopsis* species are plant pathogens and most are facultative saprophytes and endophytes (Hu *et al.*, 2007), degraders of plant materials or grow on decaying wild fruits.

Pestalotiopsis spp. cause grey blight disease in tea (*Camellia sinensis*), coconut (*Cocos nucifera)* and som (*Persea bombycina*) plants, leaf spot in eucalyptus (*Eucalyptus globulus*) and fruit rots in some tropical fruit species. Symptoms caused by *Pestalotiopsis* spp. in some other plants are having similar pattern of the development as in cashew plants (Figure 4), i.e. leaf blight in mangosteen (*Garcinia mangostana*) with the lesions commence as small brown spots and gradually expand with straw colour and a dark brown margin. Young infected leaves may be distorted

and become blighted; symptoms develop as slightly depressed, brown lesions (Ploetz, 2003), leaf spot in blueberry (*Vaccinium corymbosum*) nursery plants are having 0.4-0.8 cm diameter spots with brown margins that enlarge and coalesce (Luan, 2008). Pathogenicity of the identified pathogen culture was confirmed by Koch's postulations.

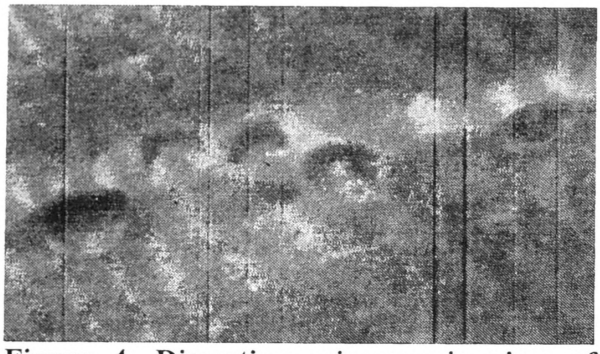

Figure 4. Dissecting microscopic view of disease symptom

In vitro Screening of Synthetic Fungicides and Bio Preparations

When inhibition percentages of fungal colonies were compared, all treatments were significantly different from each other except treatment three and four. Seven days after inoculation, 100% fungal colony inhibition was observed on the plates with Thiophanate methyl and *Trichoderma viride*. Statistical analysis showed that Thiophanate methyl and *Trichoderma viride* are very effective on suppressing isolated *Pestalotiopsis* spp. on PDA medium. Lowest inhibition percentage was recorded in Chlorothalonil treated plates (Table 2).

Table 2. Mean inhibition percentage of fungal colonies on PDA medium

Treatment	Mean
Chlorothalonil	59.3056^{c}
T. harzianum	72.7225^{b}
Thiophanate methyl	100.0000^{a}
T.viride	100.0000^{a}

Treatment No. five was not subjected to SAS analysis as there was zero level inhibition recorded. Means with the same letter are not significantly different at 0.05 levels. Mean-83.0070R^2-0.9850. Co-efficient of Variance -2.8521.

In bio fungicides treated plates, both antagonistic fungal mycelium and pathogenic fungal mycelium grew at initial stage. Fourth day after culturing, *T. viride* grew over the pathogen mycelium and reduced the diameter of pathogen culture gradually. At seventh day after culturing, pathogen culture was completely inhibited by *T. viride*. In *T. harzianum* treated plates, pathogen colony diameter was not increasing and constant from the fourth day. Although the pathogen colony gradually increased their diameter in Chlorothalonil treated plates, it was less than control (Figure 5).

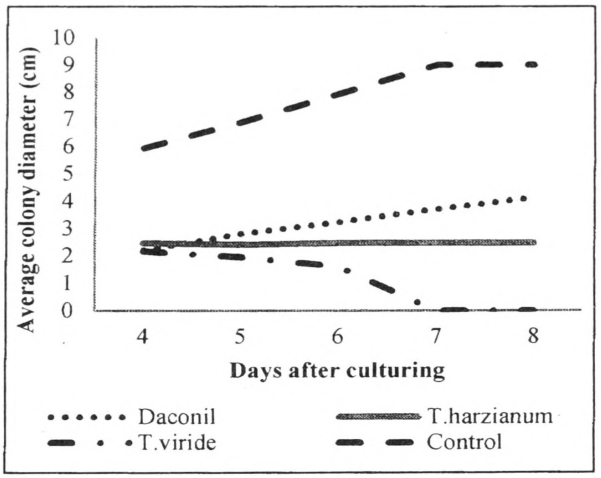

Figure 5. Average colony diameter of pathogenic fungi (cm) against days after culturing

CONCLUSIONS

According to the results of the experiment, cashew nursery disease recorded at Kamandoluwa Plantations in 2015 was caused by *Pestalotiopsis* spp. As a facultative saprophyte, it can be managed by maintaining higher sanitation level in nursery. Removal of affected plant parts and destroyal by burning, maintenance of a weed free environment, avoidance of building high relative humidity by improving ventilation, use of a potting mixture with good drainage, use of reliable non contaminated water are some phytosanitation methods that can be suggested as preventive measures of disease. During the periods of favourable conditions for the disease development, Thiophanate methyl can be used to control the pathogen and control the epidemics. Chlorothalonil also can be used for alternative application; to prevent the development of resistance by the pathogen to Thiophanate methyl due to its prolonged repeated applications. Both *T. viride* and *T. harzianum* can be applied as an effective environmental friendly method of disease control. However, further detailed studies are required for the development of application methodology of fungicides in nursery and plantation conditions.

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