Proceedings of 12th Agricultural Research Symposium (2013) 145-149

White Root Disease of *Hevea brasiliensis*, its Morphological Differences and Method of Control *(in-vitro)* in North Western Province

A.M.N. SUJEEWA[1], D.B. KELANIYANGODA[1] AND A. NUGAWELA[2]

[1]*Department of Horticulture and Landscape Gardening, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP)*

[2]*Department of Plantation Management, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP)*

ABSTRACT

Rubber industry plays a significant role in Sri Lanka's economy. One of the biotic constrains for the improvement of productivity is diseases. White Root Disease (WRD) is widespread in all rubber growing countries and this syndrome is becoming a matter of serious concern. This experiment was conducted to study morphology of *Rigidoporus microporus* extracted from five different areas in North Western Province (NWP) and to find out most effective fungicide and its best level of dosage to control pathogen *in-vitro*. The isolation of causative fungus was done by infected root. Field soil did not contain the pathogen. The results revealed that there is no morphological variability of the pathogen collected from different locations within the NWP. Tebuconazole, Bitertanol, Carbendazim and Hexaconazole were tested with recommended and sub dosage levels. Bitertanol was not successful in controlling the pathogen at their recommended dosage level and cost is high Hexaconazole. However, the results of the study suggest that Carbenderzim can be effectively used as an alternative chemical for Tebuconazole and half doses of both Tebuconazole and Carbendazim can control pathogen effectively under *in vitro* condition.

KEYWORDS: Morphology, *Rigidoporus microporus*, Rubber, White root disease,

INTRODUCTION

Rubber (*Hevea brasiliensis*) which belongs to the family Euphorbiaceae is the most economically important member of the genus *Hevea*. Currently more than 935 million hectares distributed in about 40 countries are involved in production of the natural rubber. Annual rubber production in the world is about 7.5 million of tons (Verheye, 2010).

The introduction of rubber tree to Sri Lanka from its native Amazonian habitat was in 1876. Rubber is the third largest plantation crop grown in Sri Lanka and the economic product of the tree is latex. Rubber industry plays a significant role in Sri Lanka's economy. In 2011 total rubber production was about 157.9 million kg from an extent of 127,000 ha. Export volume of raw rubber is 42.6 million kg (Central Bank Annual Report, 2011).

Rubber cultivations are affected by a number of diseases. Among them, there are several economically important foliar, stem and root diseases. These diseases are one of major constraints for the development of the industry. White Root Disease (WRD) caused by *Rigidoporus microporus* is the most serious root disease of rubber due to its fast spreading nature. In affected trees the foliage first discolour from deep-green to yellowish-brown and then appear as leathery and downward curved leaves and finally drop. Premature flowering and fruit production, branches die back and white mycelia on roots are also common symptoms. In latter stages of the disease bracket like fructifications appear on the collar region of the tree.

This disease was first reported from Singapore (Ridley, 1904) and then from Sri Lanka in 1905 (Petch, 1921). Now it is found in all rubber planting areas throughout the world, for example Indonesia, Malaysia, Sri Lanka, Thailand, West and Central Africa. It was also reported in several other crops and fruit trees. Presently around 5-10% of the cultivated lands in Sri Lanka are affected and under bare patches due to white root disease (Jayasinghe *et al.*, 1995).

Rigidoporus microporus persists on dead or alive root debris for a long time. It forms many white, flattened mycelial strands which grows and extends rapidly through the soil in the absence of any woody substrate (Nandris *et al.*, 1987) and highly moist condition. The root of healthy rubber tree can be infected by contact with a disease source, such as rhizomorphs, infected root, dead stump or wood debris (Nandris *et al.*, 1987; Guyot and Flori, 2002). In plantation, infected trees are separated by using trenches to prevent the spread of disease into adjacent plant. Field sanitation and removal of all affected root

materials have been suggested as the most important preventive measures.

There is no WRD resistant clone of rubber available and its control is very difficult. Since 1930s many chemicals had been tested and recommended for the control of the disease (Napper, 1939; John, 1958; Riggenbach, 1961; Peries *et al.,* 1963; Peries, 1965; Fox, 1966; Gohet *et al.,* 1991). However, considering the economy of the industry, the controlling of the disease by using these chemicals is expensive. So the focus of work in this investigation was to explore most effective chemical treatment and concentration and alternative chemicals that might be as cheap as possible for control.

As described by Oghenekaro *et al.,* (2012) morphology of the Nigerian isolates was different from the South American isolates of WRD. According to that, this study attempted to find out whether there are any morphological differences of isolates in North Western Province (NWP) with ecological and geographical variance.

MATERIALS AND METHODS

Location

The study was carried out at the Pathology laboratory from January to April 2013 in the Faculty of Agriculture and Plantation Management of Wayamba University.

Collection of Samples

Roots and soil samples were randomly taken from diseased patches in the field upto a depth of 15-20 cm in North Western Province (NWP). Five estates including Muwankanda estate in Mawathagama, Eadella estate in Polgahawela, Weniwella estate in Alauwa, Hathbawa estate in Rambukkana and Udapola estate in Godawela were selected according to convenient sampling from one year plant.

Isolation of the Causal Organism using Soil Samples

Initially 10 g from sampled soil was diluted in to 90 ml of sterilized distilled water (10^{-2} dilution). After that it was shaken for 30 min at 165 rpm. Then 1 ml was pipetted in to 9 ml of sterilized distilled water (10^{-3} dilution). Potato Dextrose Agar media (PDA) was autoclaved under 121°C and 15 psi pressure for 20 minutes. Glassware and other equipment were sterilized by dry air oven at 160°C for 2 hr (Dugger, 1998). Finally 0.05 ml from the diluted solution was pipetted into agar plates in laminar flow, spread by a spreader and incubated at room temperature for (28- 30°C) for 5-6 days under normal light and dark conditions.

Isolation using Root Parts

Root parts were washed three times with distilled water and small portion of the root was directly placed on (PDA) media and incubated above mentioned condition.

Developed colonies were observed under light microscope and pathogen was identified by using branching pattern of hyphae, shape of the hyphae and colony colour. Fungal colonies were purified by repeated sub-culturing and pure cultures were maintained.

Morphological Characterization

The variability of this isolates were studied using morphological characters, such as length of hyphae, branching pattern of hyphae, shape and interseptate length of the hyphae.

Efficacy of Fungicides (in-vitro)

First a trial test was done with recommended dosage to select effective fungicide to control WRD. Further study was carried out to check the economical level of dosage.

Trial Test

Five main treatments with recommended dosage were applied and cost was calculated for each treatment.

Table 1. Fungicides with recommended dosage

Treatments	Recommended dosage (ml,g/L)
T_1-Tebuconazole	0.35
T_2-Bitertanol	1.00
T_3-Carbendazim	0.70
T_4-Hexaconazole	2.00
T_5-Control	-

Note: Throughout this study fungicide application and cost calculation was done for one year age rubber plants.

Economical Assessment

The best chemicals selected from the trial test were subjected for further testing with two low sub dosages.

Koch's Postulates

Inoculums were prepared and incubated at room temperature. Then the five months rubber trees were planted in pots containing sterilized mixed soil (soil : sand : compost; 8 : 8 : 2) and inoculum was placed into planting pot next to the root system as described by

Kaewchai, and Soytong, (2010). Inoculated rubber trees were maintained in nursery and observed for disease symptoms.

Data Collection and Analysis

Colony diameters of cultures were used as the parameter for evaluating treatment effect. Least production of mycelia or minimum colony diameter was selected as the most effective chemical.

The data obtained from the study were statistically analyzed with complete randomized design (CRD) using Minitab 15.0 software.

RESULTS AND DISCUSSION

Isolation of Pathogen using Soil Sample

Cultures which were isolated from field soil did not contain *Rigidoporus microporus*. As described by Nandris *et al.*, (1987) *Rigidoporus microporus* persists on dead or alive root debris for a long time and they grow and extend rapidly through the soil in the absence of any woody substrate. Field soil contains dead and alive root debris and that pathogen is associated with roots not in the soil. Due to that reason, pathogen was unable to be isolated by using field soils.

Isolation using Root Parts

In root cultures some dark, brown and white colonies were observed at the first stage. As described by Kaewchai and Soytong, (2010) colonies having white and flattened myceliums on PDA after six days of culturing were identified as *Rigidoporus microporus*. Pure cultures were obtained separately for above selected five areas by sub culturing.

Morphological Characterization

Length of hyphae, branching pattern of hyphae, shape of the hyphae and inter septate length were not different in all five isolates. Pathogen was extracted from different locations within IL3, IM1c and IL1a agro ecological zones. Although the areas were geographically different, the result proved the similar morphology of the pathogen. It appears that pathogen due to long time exposure under the different agro ecological condition resulted with the absence of morphological variations within the province.

Efficacy of Fungicides (in-vitro)
Fungicide with Recommended Dosage

Table 2. Effect of fungicides with DOA recommended dosage on pathogen growth

Treatments	Dosage (ml,g/L)	Avg. Colony Diameter (cm)
T_1-Tebuconazole	0.35	0.00
T_2-Bitertanol	1.00	2.75[a]
T_3-Carbendazim	0.70	0.00
T_4-Hexaconazole	2.00	0.00
T_5-Control	-	11.00[b]

Average colony diameters followed by the different letters are significantly different at 0.05 levels.

As shown in Table 3, after five days of incubation, Tebuconazole, Carbendazim and Hexaconazole had suppressed the pathogen completely. Anyhow, Bitertanol had only control the pathogen upto a diameter of 2.75 cm when compared with diameter of the control which is 11 cm and it is significantly different. Therefore, the fungicides except for Bitertanol were successful in controlling the pathogen at their recommended dosage level.

Economical Assessment

Table 3. Estimated cost of fungicides application with recommended dosage.

Treatments	Dosage (ml,g/L)	Cost/ha (Rs)
T_1-Tebuconazole	0.35	1207.50
T_3-Carbendazim	0.70	1295.00
T_4-Hexaconazole	2.00	3700.00

Note: Plant per hectare = 500

Hexaconazole shows a comparatively high price relative to the other two fungicides (Table 4). Prices of Tebuconazole and Carbendazim are relatively the same. Therefore, the latter two fungicides are cheaper than Hexaconazole. Currently Tebuconazole is used practically in the field. But Carbendazim also can be recommended to be used as an alternative to reduce the chance of building up resistivity.

Practically used dosage of Tebuconazole by the farmers is 10 ml/L (DOA recommendation for rubber plant) even though

recommendation for rubber plant) even though the recommendation is 0.35 ml/L for other crops. The high dosage level has enhanced the cost and the environmental pollution as well. Therefore, it is necessary to study the effectiveness and the minimum concentrations of Tebuconazole and Carbendazim.

Fungicide with Sub Dosage Levels

Table 4. Effect of fungicides with sub dosage on pathogen growth

Treatments	Dosage (ml, g/L)	Avg. Colony Diameter (cm)
T_1-Tebuconazole	0.250	0.00
T_2-Tebuconazole	0.175	0.00
T_3-Carbendazim	0.500	0.00
T_4-Carbendazim	0.350	0.00
T_5-Control	-	11.00

Results showed that in both fungicide concentrations lower than the recommended dosage can control the pathogen effectively under *in-vitro* condition (Table 5). Therefore, low dosages can be used which will lead to low cost and less environmental pollution during disease management.

Economical Assessment

An economical assessment to find the cost effective treatment out of Tebuconazole and Carbendazim was done.

Table 5. Estimated cost of fungicides application with sub dosages

Treatments	Dosage (ml, g/L)	Cost/ha (Rs)
T_1-Tebuconazole	0.250	862.50
T_2-Tebuconazole	0.175	603.75
T_3-Carbendazim	0.500	925.00
T_4-Carbendazim	0.350	647.50

Note: Plants per hectare = 500

The cost can be cut down upto Rs 603.75 by using the low dosage (0.175 ml/L) of Tebuconazole whereas it costs Rs 1207.50 when using the recommended full dose (0.35 ml/L). The cost can be cut down to Rs 647.50 with low dosage of Carbendazim (0.35g/L) whereas it is Rs 1295.00 when using the full dose (0.7 g/L).

This study was done in *in-vitro* conditions. Therefore to confirm the findings of the efficacy of fungicides, further experiments should be carried out under field condition.

Koch's Postulates

Further a period of about 70 days in needed to gather the results. Hence the result is not possible to reports in this publication.

CONCLUSIONS

The results reveal that the pathogen cannot be isolated by using field soils whereas it could be isolated only from infected roots. The fungal morphology did not change in the isolates collected from different part of NWP. The study also shows that both Tebuconazole and Carbendazim can be used at concentration lower than the recommended to effectively control the pathogen under *in-vitro* conditions. These findings will help to reduce cost and environmental pollution during the disease management. Further, Carbendazim can be used as an alternative for reducing the risk of building up of resistivity condition.

ACKNOWLEDGEMENTS

Authors wish to express their sincere appreciation to Mr. A.S.A. Salgadoe. Finally the help given by Mr. R.M.A. Padmasiri, Technical Officer and the staff of the Department of Horticulture and Landscape Gardening is greatly appreciated.

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