Evaluation of the antagonistic effect of different plant species on white root disease causing fungus: *Rigidoporus microporus*

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Abstract

Antagonistic effect of some locally available plant spp. on Rigidoporus microporus *(Fr.) Overeem, which causes white root disease was tested in vitro and in the field. As in vitro tests, Poisoned Food Technique (PFT) and Soil Fungicide Screening Test (SFST) were used. In the field study, the plant species were established in plots and were inoculated with* Rigidoporus microporus. *The innoculum potential of the soil of each plot was assessed after a certain period of time. Among the nine species used, several species showed antagonistic action over* Rigidoporus microporus *at in vitro tests, while* Alpinia galanga *L. (Galangale) showed the most significant inhibitory effect. In the field trial, the plot with* Alpinia galanga *showed a significantly low innoculum potential.* Alpinia galanga *has the potential to be developed as an antagonistic agent against the white root disease.*

Key words: antagonistic plants, *Rigidoporus microporus*

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Introduction

White root disease is the most destructive root disease in rubber plantations of Sri Lanka and in many other rubber growing countries. It spreads in a devastating rate and causes unproductive bare patches in mature plantations and death of young plants in immature plantations. The currently recommended management practices are mainly directed at preventive approaches based on the removal of the infectious source. As an integrated practice, the drench application of two systemic fungicides tebuconazole and hexaconazole are currently recommended. However, fungicides have been known to have a negative effect on human health, cause environmental pollution and leave residues in the agricultural soil (Soytong *et al.,* 2005; Haggag and Mohamed*,* 2007). Pesticides which are readily metabolized or having broad spectrum of activity can endanger non-target pests or other components of the ecosystem. Moreover, several plant pathogenic fungi have developed resistance to

chemical fungicides (Benítez *et al.,* 2004; Kim and Hwang, 2007). Therefore, the importance of biological control of pests and disease stands un-controversial. Controlling plant diseases via biological means could be important in terms of cost and environmental concerns and health hazards. Research has been undertaken to explore the efficacy of antagonistic fungi against *R. microporus* while, *Trichoderma* species proved to be the most effective against *R. lignosus* (Jayasuriya and Thennakoon, 2007). Based on the antibiotic content, several plant species have been identified which release antibiotic exudates and cause changes in biochemical and physical properties of the soil (Situmorang *et al.,* 2010). The effectiveness of planting Galangale *(Alpinia galanga)*, Turmeric *(Curcuma domestica)*, Snake plant *(Sansevieria trifasciata)* and Cathedral bells *(Kalanchoe pinnata)* around three months old rubber have been reported to have a potential to protect the rubber plants from the infection of the fungus (Situmorang *et al.,* 2010). However, the prospects of using the extracts of the potential antagonistic plants against *R. lignosus* have not been studied.

The objective of the present study was to investigate antagonistic effect of the extracts of some locally-available plant species on *Rigidoporus microporus in vitro* and to test the antagonistic action of these plant species in the field.

Materials and Methods
The potential antagonistic plant species were selected based on literature and among them, nine plant species were used for the study (Plate 1).

In vitro *antagonistic effect*
The fungus *Rigidoporus microporus* was grown on Malt Extract Agar (MEA) medium at room temperature in 9cm diameter petri dishes. The test was performed in two laboratory methods; Poisoned Food Technique (PFT) described by Schmitz in 1930 and Soil Fungicide Screening Test (SFST) described by Zentmeyer in 1955.

Poisoned Food Technique (PFT)
This experiment was carried out in a completely randomized design with four replicates. Freshly uprooted underground parts were washed thoroughly and the extracts were prepared by grinding 100g of each fresh sample in 100ml of sterilized distilled water (100% extract concentration). Thereafter, the ground materials were squeezed in cheese cloths. The extract amended MEA were prepared by dissolving 5g of commercial MEA in 100ml of the filtered extracts before autoclaving. Sterilized distilled water was substituted for the extract in the control experiment.

Plate 1. The potential antagonistic plant species used in the study

(a) *Curcuma domestica* L. (Turmeric) (b) *Curcuma xanthorrhiza* L. (Wild ginger)
(c) *Zingiber officinale* L. (Ginger) (d) *Alpinia galanga* L. (Galangale)
(e) *Elettaria cardamomum* L. (Cardamum) (f) *Sansevieria trifasciata* L. (Snake plant)
(g) *Maranta arundinacea* L. (Arrowroot) (h) *Pedilanthus tithymaloides* L. (Slipper flower) (i)
Kalanchoe pinnata L. (Cathedral bells)

The effect of the extracts at the concentration of 100% was evaluated on the mycelial growth of the pathogen. Approximately 10ml of the extract amended MEA was dispensed into the each petri dish. Each plate was inoculated at the centre of the dish with a mycelial disc of 10mm in diameter, taken from the periphery of actively growing 5-day-old culture of the pathogen. Colony diameters were measured starting from the third day and thereafter in three days intervals. At each reading, the actual radius was obtained after subtracting 05mm from each radius reading. The mean of the four-radius readings perpendicular to each other was taken as the mycelial radius of each plate. Percent inhibitions of growth in each of the treatment were calculated with respect to the control by the equation given by Vincent (1927).

The percentage of inhibition over control: $I = \{(C - T) / C\} \times 100$
Where, I = Percentage Inhibition over control
C = Growth of pathogen in control
T = Growth of pathogen in treatment

Analysis of variance was done for percentage inhibition over control using the statistical software SPSS ver. 20. Gabriel's procedure was used to compare the means of inhibition over control values of the different species.

Soil Fungicide Screening Test (SFST)
15g of top soil was autoclaved and placed in sterile boiling tubes. The fungus was grown on MEA medium at room temperature. A mycelial disc (12mm diameter) obtained from the edge of 5-day old culture was transferred to the soil surface of each tube. Another 15g of sterile soil was then placed over the mycelial disc. Afterwards, 10ml of the desired extract solution was gently poured over the mud surface. In the control experiment 10ml of sterilized distilled water was used instead of the extract. The open ends of the tubes were covered with aluminium foil and the tubes were incubated for 24 hours at room temperature. At the end of the incubation period, tubes were emptied and the mycelial discs were washed with sterile distilled water to remove any adhering mud particle. The discs were then placed with the mycelial surface down, on the surface of 10ml of MEA in a petri dish, and incubated till the colony had grown. After the incubation period, the colony diameter was measured and the percentage inhibition was calculated as mentioned for PFT method.

Antagonistic effect under field conditions
This experiment was carried out in a completely randomized design with four replicates. The different plant species were established in plots (2m x 3m), each species consisting of four replicates. Within the plot, the plants were established in rows (with a between-row spacing of 30 cm and a within-row spacing of 20 cm) as to cover the whole block area. Another four plots were kept as control plots, where rubber seedlings were planted

instead of the potential antagonistic species. In Turmeric, Wild ginger, Ginger and Arrowroot, rhizomes were used as the planting materials, while in Galangale, Snake plant and Slipper flower, the planting materials used were plants. Stem cuttings were used for Cathedral bells. Two months after the establishment of the planting materials, gap filling was carried out.

Inoculation procedure
Each plot was inoculated with root pieces which are naturally-infected with *Rigidoporus microporus*. Root pieces (1.0 – 1.5 cm thick) with rhizomorphs on the surface were collected from the white root disease-infected rubber trees and were cut into pieces of about 5cm. Then the root pieces were covered with litter and were kept under shade for seven days to allow the fungus to multiply. Between the plants, the top soil layer of the plots was forked and the infected root pieces were incorporated into the soil as to touch the roots of the plants. In order to maintain the viability of the fungus, litter was also mixed with soil. The inoculation procedure was initiated at three months of plant establishment and was repeated twice in two months intervals.

Measurement of the innoculum potential
The rapid upward movement of the fungus in the presence of mulch such as *Gliricidia* is shown to be useful for early detection of the white root disease

(Fernando *et al.,* 2011). This method was used to quantify the innoculum potential of the plots. Six months after the establishment, fifteen Gliricidia poles were established in each plot. Twelve months after the first inoculation, the collar area of each pole was observed for the presence/absence of the rhizomorphs of the fungus *Rigidoporus microporus*. In each plot, the number of infected poles was recorded versus the total number of poles. The infected percentage of each plot was calculated as below, and was considered to correspond to the innoculum potential of the soil in each plot.

The Infection Percentage of each plot
IP% = (I/T) x 100
Where, I = Number of infected poles in the relevant plot
T= Total number of poles in the relevant plot
Analysis of variance was done for the infection percentage using the statistical software, SAS version 9.2. Subsequently, mean separation was done with Duncan's Multiple Range Test (DMRT).

Results and Discussion
In vitro *antagonistic effect*
The percentage inhibition levels over the control, shown by the extracts of the different plant species are summarized in the Tables 1 and 2.

Table 1. *Percentage inhibition of the fungus over the control shown by different plant species at PFT*

Plant species	Percentage inhibition over the control		
	Day3	Day6	Day9
Curcuma domestica L. (Turmeric)	96.5	73.8	57.5
Curcuma xanthorrhiza L. (Wild ginger)	99.7	75.0	60.0
Zingiber officinale L. (Ginger)	72.7	43.3	0
Alpinia galanga L. (Galangale)	100.0	100.0	100.0
Elettaria cardamomum L. (Cardamum)	18.2	0	0
Sansevieria trifasciata L. (Snake plant)	54.3	24.1	0
Maranta arundinacea L. (Arrowroot)	-1.8	0	0
Pedilanthus tithymaloides L. (Slipper flower)	12.7	0	0
Kalanchoe pinnata L. (Cathedral bells)	34.5	0	0

Table 2. *Percentage inhibition of the fungus over the control shown by different plant species at SFST*

Plant species	Percentage inhibition over the control	
	Day2	Day4
Curcuma domestica L. (Turmeric)	10.4	-39.3
Curcuma xanthorrhiza L. (Wild ginger)	-7.7	-68.8
Zingiber officinale L. (Ginger)	100.0	84.0
Alpinia galanga L. (Galangale)	92.6	59.8
Sansevieria trifasciata L. (Snake plant)	-14.2	-52.4
Maranta arundinacea L. (Arrowroot)	-23.3	-275.0
Pedilanthus tithymaloides L. (Slipper flower)	05.5	-60.6
Kalanchoe pinnata L. (Cathedral bells)	-24.9	-60.0

According to the results of the PFT trial, at the probability level of 0.05, *Curcuma domestica* L. (Turmeric), *Curcuma xanthorrhiza* L. (Wild ginger), *Zingiber officinale* L. (Ginger), *Alpinia galanga* L. (Galangale) and *Sansevieria trifasciata* L. (Snake plant) show a significant effect on fungal growth at the first two observations. However, at the last reading, only *Alpinia galanga* L. (Galangale) showed a significant effect over the growth of fungus. This species totally inhibits the growth of the fungus and therefore it is the most significantly effective plant extract over the other species. According to the results of the SFST trial, at the probability level of 0.05, *Zingiber officinale* L. (Ginger) and *Alpinia galanga* L. (Galangale) showed a significant effect on fungal growth at both observations.

Antagonistic effect under field conditions

The Infection Percentages (IP%) shown by different plant species are summarized in the Table 3. According to the results, *Alpinia galanga* L. (Galangale) showed a significant effect on inhibiting the fungus.

According to the overall results, the potential of using these plant species; especially *Alpinia galanga* L. (Galangale) over *Rigidoporus microporus* is highlighted. These results can be extended into the practical application in different ways. The prospects of using botanical extracts as a soil treatment either at planting or before planting could be looked for as a preventive approach. Moreover, the rubber plants at early infection could be sprayed or drenched with these extracts as curative organic fungicides. Furthermore, it would be possible to plant these species as to get an antagonistic action over *Rigidoporus microporus* in the field.

In order to be used as antagonistic plants, the agronomic aspects of these species have to be taken into consideration. Among these species, Galangale is locally considered as a wild species which has no economic importance. Due to its significantly high regeneration capacity, the availability of the rhizome is very high. Moreover, it can thrive in marginal environmental and soil conditions and thus the continuous presence in the location is proven. Ginger and turmeric are income-generating agricultural crops which may contribute to enhance the profitability of the industry when grown as intercrops. Snake plant is a decorative plant species with an economic value.

Table 3. *The infection percentages shown by different plant species*

Species	Infection percentage (IP%)*
Control *(Hevea brasiliensis)*	34.479 [a]
Kalanchoe pinnata (Cathedral bells)	31.013 [a]
Curcuma domestica L. (Turmeric)	29.140 [a]
Curcuma xanthorrhiza L. (Wild ginger)	28.013 [a]
Maranta arundinacea L. (Arrowroot)	26.068 [a]
Pedilanthus tithymaloides (Slipper flower)	25.655 [a]
Sansevieria trifasciata L. (Snake plant)	20.556 [ba]
Zingiber officinale L. (Ginger)	18.056 [ba]
Alpinia galanga L. (Galangale)	4.762 [b]

*According to DMRT, the values with the same letter are not statistically significant at probability level of 0.05

The antagonistic plants would be having direct or indirect effects on the development of white root fungus in the soil. Primary factor which is affecting directly on the hite root fungus is the exudates released from the roots, while secondary factors are the indirect effects on soil biochemical-physical properties (Situmorang *et al.,* 2010). The use of antagonistic plants can be integrated with the other preventive methods to increase the effectiveness in controlling white root disease (Situmorang *et al.,* 2010).

With the results of the study, it can be concluded that there are several species which can be potentially used as antagonistic plant species over *Rigidoporus microporus* while *Alpinia galanga* L. (Galangale) showing the most significant inhibitory effect in both *in vitro* and field tests. This is a positive trend which may be helpful in combating the menace of this destructive rubber root disease in an economically viable and environmental-friendly manner.

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