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SYSTEMIC FUNGICIDES AS A DRENCH APPLICATION TO CONTROL WHITE ROOT DISEASE OF RUBBER

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ABSTRACT

White root disease caused by Rigidoporus microporus *(Fr) Overeem is the most serious disease of* Hevea *in Sri Lanka.*

The most effective method of controlling the disease is by adopting cultural practices recommended by the Rubber Research Institute of Sri Lanka, prior to replanting.

The chemical treatment procedure is quite effective if the diseased trees are detected at an early stage. However the conventional 'collar protectant' treatment is very tedious and labour intensive. Therefore to overcome this fungicide drenching method was tested.

Although the fungicides, Bayleton (triadimefon), Bayfidan (triadimenol), Folicur (tebuconazole) and Contaf (hexaconazole) are equally effective in controlling the disease, when the cost involved are considered, it is not economical to use Bayleton and Bayfidan. Therefore the fungicides, Folicur (tebuconazole) and Contaf (hexaconazole) can be used effectively and economically to reduce the white root disease incidence in young rubber plantations.

Key words: drenching, fungicides, *Hevea, Rigidoporus microporus*, white root disease

INTRODUCTION

White root disease caused by *Rigidoporus microporus* (Fr.) Overeem [Syn: *R. lignosus*, (Klotzsch) Imazeki,] is the predominant root disease of rubber in Sri Lanka. Root diseases are an important economic factor in the rubber industry since they kill the trees irrespective of age or vigor. Losses resulting from root diseases during the early stages of planting will incur extra expenditure owing to the need of resupplying the young plants. In mature plantings, the losses will lead to a reduction in trees per hectare which would reduce the crop.

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Use of systemic fungicides against white root

After a series of experiments Peries & Liyanage (1973) recommended that the best method of controlling white root disease is by adopting certain cultural practices prior to replanting. The present method of control include pre and post-planting measures which comprise of the following,

(1) From the 3rd month onwards until about the 5th year, the clearings should be inspected quarterly, especially in the demarcated 'Fomes' patches, for early detection of white root diseased plants.

(2) Diseased trees which could be saved should be treated with a collar protectant dressing containing a suitable fungicide (Phenolic compounds).

(3) This chemical treatment should be continued in each direction in the row until a healthy tree is detected.

(4) It is also important to excavate the food base within the row.

Chemical treatment method described above is quite effective if the trees are detected at an early stage of infection. However this method of treatment is quite tedious and labour intensive. Therefore to overcome these problems a fungicide drenching method was reported by Tran in 1985.

In Africa after detection and marking of the diseased trees and their two direct neighbours, the trees are treated by pouring 2 litres of 0.5% Calixin (tridemorph) emulsion into a furrow around the base of the marked trees twice a year at a 6 monthly interval (Tran, 1985). This has given very successful results and as a result Calixin was recommended by Institut de Recherches Sur le Caoutchouc of Ivory Coast (Tran, 1985) for countries in Africa, such as Ivory Coast, Cameroon and Gabon for the control of white root disease of rubber trees.

Tan (1990) has reported a fungicide drenching experiment with Bayleton 25 wp (triadimefon), Bayfidan 250 EC (tridimenol), Tilt 250 EC (propiconazole), Anvil 50 SC (hexaconazole), Folicur 250 EC (tebuconazole), Systane 12E (myclobutanil) and Calixin 750 EC (tridemorph). Drenching was carried out by marking a furrow approximately 22 cm wide radius and 5 cm deep around the collar of the tree. Two applications of fungicide drenching were carried out at a 5 month interval. Assessments were carried out 13 months after the first drenching. Bayfidan 20 ml, Bayleton 20 g and Tilt 40 ml per tree were found to be the most effective fungicides for drenching. Least effective was the Calixin. Tan (1990) also reported that Anvil 25 ml, Folicur 20 ml and Systane 20 ml in a litre of water appeared to be promising on young budded plants when observed 4 months after drenching. In Ivory Coast the effectiveness of the fungicides tridemorph at the rate of 7.5 g ai/tree, Cyproconazole at the rate of 0.5g a.i/tree and triadimenol in granular form at a rate of 0.5g a.i/tree were tested (Gohet *et al.*, 1991). The chemicals were applied twice, 6 months apart. Results obtained after a year of application of the fungicides showed that these 3 fungicides were also equally effective as Anvil, Folicur and Systane.

Studies carried out in Malaysia (Chin & Sheng, 1993) showed that soil drenching with hexaconazole (Anvil 5 SC) at 0.5 g a.i/plant in 2 litres of water onto one year old rubber plants followed by a repeat application four months later was also effective in controlling the disease.

A series of experiments were carried out to find economically viable systemic fungicides to be used as drench applications to control the white root disease in field plants as the conventional "Collar protectant" application method is very tedious and labour intensive.

MATERIALS AND METHODS

Organism

The white root disease causing fungus *Rigidoporus microporus* isolated from Delwalakanda estate, Avissawella and maintained on malt agar was used throughout the study.

In the second part of the experiment 4 strains were selected using their biological characters (colony morphology and growth characters) from 10 isolates, collected from different rubber growing areas of the island. The differences among the strains were confirmed by comparing DNA banding patterns using PCR studies (Nanayakkara, 2000). These four strains were from Madeniya (Warakapola), Arapolakanda (Neboda), Nakiyadeniya (Galle) and Kuruwita (Kuruwita, Ratnapura) estates.

Artificially inoculated nursery plants and naturally infected field plants in different estates situated in different agro-climatic regions were used to determine the curative effects of the effective fungicides selected from *in vitro* tests.

Six month old nursery plants (budgrafted and planted at a distance of 2'×4') were inoculated with, artificially infected 25 g of small root pieces which were grown in 250 ml coñical flasks under sterile conditions. The root pieces in these flasks were of 1-1.5 cm diameter and 8-10 cm long and were inoculated with 10, 2 cm diameter agar discs taken from 7 day old cultures of *R. microporus* growing on malt agar. These flasks were incubated at room temperature for 8 weeks. Thereafter, inoculation of plants were done by placing the infected root pieces around the root system at 25-30 cm depth by digging the soil taking care not to damage the root system.

Fungicides

The fungicides used were, Bayleton (triadimefon), Bayfidan (tridimenol), Anvil (hexaconazole), Tilt (propiconazole), Folicur (tebuconazole), Contaf (hexaconazole) and Daconil (chlorothalonil).

Fungicide screening methods

Poisoned food technique (PFT)

Mycelial growth inhibition was evaluated by the poisoned food technique described by Schmitz, 1930. Seven dosage rates, *i.e.* 50, 100, 200, 400, 800, 1600 and 3200 µg/ml of each fungicide were used. Appropriate quantities of fungicides were added into sterilized malt agar media at 45±2°C to get the required concentration.

Ten ml of each amended medium was then poured into a petri dish using a parrot. Each plate was thereafter inoculated at the centre with a mycelial disc 5 mm in diameter, taken from the periphery of actively growing 7-day old cultures, and incubated at 28 ± 2°C for 10 days. Five replicates were used for each concentration. In controls no fungicides were added to the medium. The colony diameter was measured at 10^{th} day and the percent inhibition of growth in each treatment was calculated with respect to the growth of the colony by using the equation of Vincent, 1927.

$$I = \frac{100\,(C-T)}{C}$$

Where I = percentage inhibition of mycelial growth
C = growth in controls
T = growth in treatment

Soil fungicide screening method (SFST)

This technique was first described by Zentmeyer in 1955. Corden & Young (1962), and Sharma and Mohanan (1991) modified the method slightly and used it to test the lethal effect of the fungicides on microsclerotia. Air-dried sieved (25 mesh) soil of pH 4.6 was used throughout the study. Ten grams of soil was autoclaved (2h at 15 psi) and placed in sterile tubes (20 mm diameter and 140 mm long). A mycelial disc (8 mm diameter) obtained from close to the centre of a 12 day old culture of the fungus on malt agar at 28± 2°C was transferred to the soil surface in the tubes. Another 10 g of sterile soil was then placed over the mycelial disc. Afterwards, 10 ml of the desired aqueous fungicide solution (concentrations used were 50, 100, 200, 400, 800, 1600, 3200 or 6400 µg/ml) was gently poured over the soil surface. Each concentration had 5 replicates and in control 10 ml of sterile distilled water was used instead of the fungicide. The mouths of the tubes were then covered with aluminium foil, and incubated for 24 h at 28±2°C. At the end of the incubation period tubes were emptied and the mycelial discs were washed with sterile distilled water to remove any adhering soil particles. The discs were then placed with the mycelial surface down, on to the surface of fresh malt agar plates, and incubated for 10 days at 28± 2°C. Colony diameter was measured at the end of the incubation period. Percent inhibition of the mycelial growth in each treatment was calculated using the equation given for the Poisoned food technique.

Soil fungicide drenching method

Artificially infected nursery plants and naturally infected field plants aged 1-2 years were treated with the test fungicides at the stage the infection causes slight yellowing of the foliage. The appearance of symptoms in artificially infected nursery plants were seen approximately after 6-9 months from inoculating the plants as described in page 3. The fungicides were used as follows:

Fungicide	ml/g – Product/plant	g.a.i./plant
Bayleton	10-20g	2.5-5.0
Bayfidan	10-20 ml	2.5-5.0
Anvil	10-20 ml	0.5-1.0
Folicur	10-20 ml	2.5-5.0

The treatment consisted of carefully loosening the soil and forming a funnel-like furrow around the base of the plant, and then pouring the chemicals into the furrow along the tree trunk. A second application of the fungicides were carried out 4 months after the 1st application. The experimental details are given in Table 1.

Evaluation of the effect of the fungicides was carried out at 3 monthly intervals by inspecting the collar region and the foliage of the treated plants. In all experiments pre-treatment assessments were carried out and visual assessment rating was given in the following manner.

Disease score	Symptoms
0	Collar free of infection
1	Collar slightly affected with no foliar symptoms
2	Collar half circumference rotted with slight yellowing of the foliage
3	Collar badly rotted with severe foliar symptoms
4	Collar completely rotted with completely wilted leaves
D	Dead plants

RESULTS AND DISCUSSION

In this study EC 100 (100% inhibition of the mycelia) is considered as the effective dosage of the fungicides. The efficacy of the fungicides (% inhibition of the fungus) varied with the concentration and the type of fungicide used. Of the six fungicides tested Tilt, Folicur, Contaf, Bayfidan were effective at concentrations of

800, 800, 300 and 3200 ppm respectively when soil fungicide screening method was used (Table 2). However when PFT was used Tilt, Folicur, Contaf and Bayfidan were effective at concentrations of 200, 50, 50 and 900 ppm respectively (Table 3).

Table 1. *Details of field experiments*

Location (Estate/Div)	Fungicide	Inspection interval
Arappolakanda	Anvil	2 months
Padukka	Beyleton	2 months
(Menerigama Div)	Bayfidan	2 months
Age 1 year	Calixin	2 months
Padukka	Beyleton	1 month (Up to 4th assessment)
(Main Div)		2 months (From 5th assessment)
	Bayfidan	1 month (Up to 4th assessment)
		2 months (From 5th assessment)
Madeniya Age 1 ½ years	Folicur	3 months
Peenkanda	Penta chloro phenol	3 months
(Parawatta Div)	Bayleton	3 months
Age 1 year	Bayfidan	3 months
Dartonfield	Folicur	3 months
(Gallewatta Div)		
Malaboda	Folicur	2 months
Yatadola	Bayleton	3 months
	Bayfidan	3 months
	Penta chloro phenol	3 months
Dewalakanda	Folicur	2 months
Age 1 year		
Madeniya*	Bayleton	3 months
(Panana Div)	Bayfidan	3 months
	Penta chloro phenol	3 months
Dartonfield*	Bayleton	3 months
	Bayfidan	3 months
	Penta chloro phenol	3 months
	Anvil	3 months

***Artificially inoculated plants were used.**
Age of plants were 2 years unless otherwise stated.

Table 2. *Percentage inhibition of mycelial growth by fungicides.*

The method used was **SFST.** The results are mean of 5 replicates.

Fungicide	Concentrations *a.i* ppm										
	Control	50 ppm	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	800 ppm	1600 ppm	3200 ppm	6400 ppm
Bayfidan	0 ±0.00	-	-	-	-	-	-	84.77 ±1.79	94.85 ±0.80	100 ±0.00	100 ±0.00
Bayleton	0 ±0.00	-	-	-	-	-	-	48.38 ±9.32	64.6 ±0.54	69.6 ±2.19	83.98 ±0.96
Anvil	0 ±0.00	59.77 ±1.64	68.59 ±1.24	73.88 ±0.51	-	77.18 ±0.54	-	80.24 ±1.05	81.65 ±0.66	82.12 ±0.51	82.82 ±0.22
Tilt	0 ±0.00	4.47 ±0.24	11.06 ±1.38	18.59 ±1.83	-	57.53 ±2.87	-	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00
Folicur	0 ±0.00	6.59 ±0.92	22.82 ±3.48	56.24 ±0.61	-	72.35 ±1.52	-	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00
Contaf	0 ±0.00	69.88 ±4.17	71.77 ±1.77	83.18 ±0.14	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00

± - Std. Error of the mean
(-) Not determined

Table 3. *Percentage inhibition of mycelial growth by fungicides*

The method used was **PFT**. The results are mean of 5 replicates.

Fungicide	Concentrations *a.i.* – ppm														
	Control	25 ppm	50 ppm	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm	700 ppm	800 ppm	900 ppm	1600 ppm	3200 ppm	6400 ppm
Bayfidan	0 ±0.00	-	-	81.75 ±0.31	82.13 ±0.25	86.25 ±0.00	87.50 ±0.00	88.75 ±0.00	-	-	-	100 ±0.00	-	-	-
Bayleton	0 ±0.00	-	-	69.13 ±0.15	78.50 ±0.51	79.75 ±0.83	83.38 ±0.15	83.63 ±0.23	85.13 ±0.13	86.38 ±0.23	92.50 ±0.00	92.50 ±0.00	-	-	-
Anvil	0 ±0.00	-	100 ±0.00	100 ±0.00	100 ±0.00	-	100 ±0.00	-	-	-	100 ±0.00	-	100 ±0.00	100 ±0.00	100 ±0.00
Tilt	0 ±0.00	-	Show Thin ring	Show Thin ring	100 0.00	-	100 ±0.00	-	-	-	100 ±0.00	-	100 ±0.00	100 ±0.00	100 ±0.00
Folicur	0 ±0.00	78.25 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	-	100 ±0.00	-	-	-	100 ±0.00	-	100 ±0.00	100 ±0.00	100 ±0.00
Contaf	0 ±0.00	84.38 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	-	100 ±0.00	-	-	-	100 ±0.00	-	100 ±0.00	100 ±0.00	100 ±0.00

± - Std. Error of the mean

(-) Not determined

Contaf (hexaconazole) and Folicur (tebuconazole) were found to be effective at low concentrations (50 g/ml) against all 4 isolates of the fungus in the Poison food technique (Tables 4.1- 4.4). However, in SFST, Contaf showed EC 100 at a concentration of 200 μg/ml, while Folicur required 800 μg/ml. (Table 5).

Table. 4.1. *Percent inhibition over control of different isolates at different concentrations of Bayleton, by using PFT*

	Concentration *a.i.* - ppm								
Isolate	25	50	75	100	200	400	800	1600	3200
AK	-	-	-	78.77	81.18	85.92	83.01	85.49	86.48
				A	A	A	A	B	C
KE	-	-	-	67.22	76.11	77.44	81.55	80.66	81.44
				B	B	B	A	B	B
KW	-	-	-	62.00	68.11	74.11	78.99	80.33	78.55
				C	C	B	B	A	A
NKD	-	-	-	66.11	76.11	77.64	78.70	79.99	81.76
				B	B	B	B	B	C

Figures with same capital letters in each column are not significantly different at the P = 0.05 level according to Duncun's Multiple Range Test.

Table. 4.2. *Percent inhibition over control of different isolates at different concentrations of Bayfidan by using PFT*

	Concentration *a.i.* - ppm								
Isolate	25	50	75	100	200	400	800	1600	3200
AK	-	-	-	100	100	100	100	100	100
				A	A	A	A	A	A
KE	-	-	-	82.56	80.58	87.07	92.74	100	100
				B	D	B	B	A	A
KW	-	-	-	84.44	84.66	91.88	100	100	100
				B	C	B	A	A	A
NKD	-	-	-	82.81	89.51	92.18	100	100	100
				B	B	B	A	A	A

Figures with same capital letters in each column are not significantly different at the P= 0.05 level according to Duncun's Multiple Range Test.

Table. 4.3. *Percent inhibition over control of different isolates at different concentrations of Folicur by using PFT*

	Concentration *a.i.* - ppm								
Isolate	25	50	75	100	200	400	800	1600	3200
AK	100.0 A	100	100	100	100	100	100	100	100
KE	83.75 B	100	100	100	100	100	100	100	100
KW	70.37 C	100	100	100	100	100	100	100	100
NKD	73.41 C	100	100	100	100	100	100	100	100

Figures with same capital letters in each column are not significantly different at the P= 0.05 level according to Duncun's Multiple Range Test .

Table. 4.4. *Percent inhibition over control of different isolates at different concentrations of Contaf by using PFT*

	Concentration *a.i.* – ppm								
Isolate	25	50	75	100	200	400	800	1600	3200
AK	100 A	100	100	100	100	100	100	100	100
KE	88.27 B	100	100	100	100	100	100	100	100
KW	86.17 C	100	100	100	100	100	100	100	100
NKD	85.95 C	100	100	100	100	100	100	100	100

Figures with same capital letters in each column are not significantly different at the P= 0.05 level according to Duncun's Multiple Range Test .

This study reveals that with SFST a much higher concentration was required to reach EC 100 compared to PFT. The main reason for this is that toxicity of the fungicides are diluted in the soil environment due to various factors such as soil physical and chemical properties and microbial activity. However, SFST closely simulate field conditions and therefore greater weightage should be given to these results.

Table. 5. *Percent inhibition over control of different concentrations of fungicides by using SFST*

	Concentration *a.i.* - ppm						
Fungicide	50	100	200	400	800	1600	3200
Contaf	70.28 A	72.28 A	100 A	100 A	100 A	100 A	100 A
Foicur	7.45 B	23.11 B	56.45 B	72.50 B	100 A	100 A	100 A

Figures with same latter in each column are not significantly different from each other's at the P=0.05 level according to Duncun's Multple Range Test.

Due to the above reasons the field testing of fungicides were done at the fairly high concentrations of 2.5-5.0 g of ai per plant. The percentage recovery varied from 30 to 98% depending on the fungicide used and the pre-treatment assessment (Table 6). According to this investigation the fungicides Bayleton, Bayfidan, Anvil and Folicur showed promising high recovery percentages.

The success of chemical control of white root disease in rubber plants depends on many other factors such as the stage of infection at the time of fungicide application, type and rate of fungicide applied. However, as reported earlier (Ng & Yap 1990; Tan 1990; Chan *et al.*, 1991) early detection of the disease is critical for the treatment success.

Although the fungicides, Bayleton, Bayfidan, Folicur and Contaf are equally effective in controlling white root disease in rubber plantations, when the cost involved are considered (Table 7), it is not economical to use the two fungicides Bayleton and Bayfidan. Folicur and Contaf can be used economically and effectively to reduce the white root disease incidences in rubber plantations.

In soil drenching treatments a follow-up application after a 4-6 month period was necessary. Chan *et al* (1991) showed that the re-infection could occur in some treated trees 6-10 months after fungicide treatment. Hexaconazole has a half-life of approximately two months in tropical soils and residuces up to 15% remained in soil after six months (Atreya, 1990). Tebuconazoles also have a similar half-life. The effectiveness of these fungicides will thus be reduced in 2-4 months. Therefore it is possible to initiate re-infection of the roots after 3-4 months provided the 'food bases' are large enough to survive this period before being fully decayed. A second application of hexaconazole or tebuconazole after 4-6 months from initial application is therefore important to achieve an effective control. At the same time it is much more appropriate to remove from the surrounding vicinity any large "food baseses" that can last for more than 6 months. This practice will ensure that the cured plants will not be re-infected at a later stage.

Table 6. *Efficacy of fungicides tested (Recovery percentage) under field conditions*

Fungicide	Site	Age (years)	Concentration ml/litre/tree	Pretreatment assessment (No. of trees treated with disease score) *				Percentage recovery
				1	2	3	4	
Anvil (hexaconazol)	Arappolakanda	2	20	1	1	6	4	37%
	Dartonfield	2 (Artificially inoculated plants)	20	7	4	2	1	73%
	Peenkanda	2	20	8	6	3	-	75%
Bayleton (triadimefon)	Menerigama	1	20	0	4	5	4	30%
	Menerigama	2	20	2	1	5	1	55%
	Madeniya	2	20	0	1	5	1	93%
	Peenkanda	1	20	2	8	6	1	81%
	Dartonfield	2	20	1	5	17	1	83%
	Yatadola	2	20	1	3	2	3	33%

(Table 6 contd.)

Fungicide	Site	Age (years)	Concentration (ml/litre/tree)	Pretreatment assessment (No. of trees treated with disease score) *				Percentage recovery
				1	2	3	4	
Bayfidan (triadimenol)	Menerigama	1	20	5	1	6	1	76%
	Menerigama	2	20	0	1	5	1	43%
	Madeniya	2	20	0	8	8	1	75%
	Peenkanda	1	20	0	10	6	0	94%
	Dartonfield	2	20	-	2	21	2	76%
	Yatadola	2	20	2	5	7	9	42%
Folicur	Malaboda	2	20	11	5	2	0	94%
	Dartonfield	2	20	4	3	3	0	90%
	Devalakanda	1	20	8	13	14	4	77%
	Madeniya	1½	10	10	23	8	1	98%

(Table 6 contd.)

Fungicide	Site	Age (years)	Concentration ml/litre/tree	Pretreatment assessment (No. of trees treated with disease score) *				Percentage recovery
				1	2	3	4	
Penta choloro Phenol (as a collar protectant)	Madeniya	2	-	0	10	7	0	41%
	Peenkanda	1	-	0	8	6	0	57%
	Daronfield	2	-	1	8	9	2	35%
	Yatadola	2	-	1	0	7	0	50%

*Disease score:

1 – Collar slightly affected with no foliar symptoms
2 – Collar half circumference rotted with light foliar symptoms
3 – Collar badly rotted with severe foliar symptoms
4 – Collar completely rotted with fully wilted leaves

Table 7. *Economics involved in the use of chemical treatments*

Fungicide	Cost/litre or 1 Kg	Chemical cost / tree (2 applications)	Labour cost /tree	Total cost/tree
Mason' s collar protectant (Phenol)	Rs.110.00	Rs.22.00 (One application only)	Rs.55.00	Rs.77.00
Bayleton (triadimefon)	Rs.5800.00	Rs.232.00	Negligible	Rs.232.00
Bayfidan (triadimenol)	Rs.6250.00	Rs.250.00	Negligible	Rs.250.00
Anvil (hexaconazol)	Not available			
Tilt (Propiconazol)	Rs.3750.00	Rs.150.00	Negligible	Rs.150.00
Folicur (tebuconazole)	Rs.4200.00	Rs.168.00	Negligible	Rs.168.00
Contaf (hexaconazole)	Rs.2550.00	Rs.102.00	Negligible	Rs.102.00

It can be concluded that fungicides Folicur and Contaf can be used to control white root disease effectively, provided that the infection is detected at a relatively early stage in young (1-2 year old) plants.

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