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BIOLOGICAL CONTROL OF *RIGIDOPORUS MICROPORUS*, THE CAUSE OF WHITE ROOT DISEASE IN RUBBER

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ABSTRACT

White root disease caused by *Rigidoporus microporus* is abundant in rubber plantations of Sri Lanka. Control of the disease by applying systemic fungicides is expensive, pollutes the environment and causes health hazards. *Trichoderma* isolates obtained from rubber growing areas were screened and found antagonistic against *R. microporus*. Research confirmed the ability of *Trichoderma harzianum* to control the pathogen *in vitro*. *T. harzianum* (T3) isolate produced a relatively higher number of phialospores. T3 isolate stored in T10 medium (T310) consisting of rice bran and farm manure preserved more than 50% of T3 spores up to 4 months in dry form. T310 was antagonistic to the pathogen.

Key words: *Hevea brasiliensis*, white root disease, *Rigidoporus microporus,* biocontrol, *Trichoderma harzianum* .

INTRODUCTION

White root disease is the most destructive root disease in rubber plantations of Sri Lanka and many other rubber growing countries. The causative agent *Rigidoporus microporus* (Fr.) Overeem persists on dead or live root debris for a long time, while causing new infections on healthy plants. During the past 15 years, several active ingredients have been tested against the white root disease in the world. In most instances, collar protectants containing fungicides and sulphur amendments (Peries, 1969), systemic active ingredients such as propiconazole, hexaconazole (Lam and Chiu, 1993), and other triazoles (Lim *et al*., 1990; Gohet *et. al*., 1991), triadimenol, PCNB, triadimefon (Jollands, 1983; Ng and Yap, 1990), pentachlorophenol (PCP) (Jayasinghe *et al*., 1995) and phenol (Jayaratne e*t al*., 1997) were found to be effective against the fungus. The net cost of a single application of hexaconazole is about SLRs. 20-40 per single application per tree and perhaps repeated application a would be necessary after 2-3 months if the trees do not recover. Though expensive, the fungicides, hexaconazole or tebuconazole, appeared to be highly effective in our trials. However, the success of application of the fungicide would be higher if the infection is mild. Therefore, the most effective way is to identify the infection at a very early stage and treat accordingly.

One way to minimize the amount of fungicide required for a single application is integration of such application with chemical resistant antagonists, which are capable of suppressing the weakened pathogen in soil. However, results from integrated applications of systemic fungicides along with *Trichoderma koningii* were not consistent (Hashim and Chew, 1997). In addition, weakening effect of some chemicals such as furfuraldehyde on *R. microporus* (Jayasuriya and Deacon, 1996; Jayasuriya *et al*., 1996a) has been discussed and this effect was considered as a trigger for biological control (Katan *et al*., 1992). Therefore, controlling the disease using biological preparations could be important in terms of cost, environmental concerns and health hazards. Possibility of using antagonistic organisms to control *R. microporus* has been discussed previously (Jayasuriya, 1996; 1997; 1998). Research has been undertaken to explore the efficacy of antagonistic organisms against *R microporus* (Jayasuriya and Deacon, 1995; Jayasuriya *et al*., 1996b; Jayasuriya, 1997) and for other fungi causing diseases in agricultural crops (Schoeman *et al*., 1996; Yang *et al*., 1992) including pine in Europe (Rishbeth, 1963). In the majority of incidences *Trichoderma* spp. were highly effective against many pathogens of agricultural crops. The objective of the

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investigation is to explore the efficacy of the naturally existing antagonistic soil microorganisms against the causal agent of white root disease of rubber and utilize them in an effective disease controlling system. This paper further discusses the possibility of using an alternative and economical method to control the white root disease in immature rubber plants using *T. harzianum* Rifai together with effective formulation of the antagonist using simple techniques.

MATERIALS AND METHODS

Isolation of *Trichoderma* from soils

Soil samples were collected from 15-20 cm depths of rubber growing soils in Sri Lanka. A single composite sample contained soils collected from many plantations in the same area and they were bulked together and air-dried on a laboratory bench for a few days at room temperature (27°±2°C) to minimize the bacterial population and make the fungal isolation easy. After air-drying, soils were passed through a mesh (0.5 mm) and stored at room temperature until used. Aliquots (*ca* 5 g) of sieved soil from samples were introduced on to Potato Dextrose Agar (PDA) or V-8 juice agar (200 ml V-8 juice, 800 ml of distilled water, 1 g of glucose, 20 g of agar, 6 ml of 0.1N NaOH) and the plates were incubated at room temperature for 2-3 days under normal light/dark conditions. Fungal colonies that appeared to be *Trichoderma* spp. were purified by repeated sub-culturing. Some isolates were authenticated by CABI, UK, while the rest were identified according to Rifai (1969).

Spore production by *Trichoderma* spp.

Phialospore production is a distinguished characteristic in the proliferation of *Trichoderma* spp. on introduced targets. Therefore, spore production of the isolates was compared by growing on PDA. Petri-plates (9 cm) having 15 ml of PDA were inoculated in the center with 5 mm agar plugs removed from actively growing *Trichoderma* cultures and the plates were incubated up to 7 days at room temperature under normal light/dark conditions. The total numbers of phialospores produced on agar plates were quantified. One quarter of the colony from each plate was cut along with underlying agar and transferred into a 50 ml centrifuge tube with approximately 20 ml distilled water and homogenized at 12,000 rpm using a homogenizer. Thereafter, the resultant was made up to 25 ml and 1 ml from the suspension was serially diluted. The spore concentration was determined using a haemocytometer. The total number of spores on a single plate was quantified. There were six replicates for each isolate.

Screening for antagonism against *R. microporus*

Trichoderma isolates were screened against *R. microporus* on dual culture plates. *R. microporus* was inoculated first and 3 days later *Trichoderma* was inoculated since its growth rate is high. The growth of the two organisms on single agar plates was assessed according to Jayasuriya (1997) over a period of 6 days after inoculation of *Trichoderma* isolates and overgrowth of *Trichoderma* on *R. microporus* was measured as the total extension (mm) of *Trichoderma* on the agar plate towards *R. microporus*. Four replicate plates were employed for assessing each isolate and four measurements were obtained from each plate. The total number of measurements obtained from each isolates was 16.

Preparation of artificial storage base for *T. harzianum*

The most difficult challenge in successful biological control is to effectively establish the biological control agent at the target site. Based on preliminary tests conducted in the department, cheap substrates such as sun-dried farm manure, rice bran, sawdust and red yellow podzolic and clay soils were mixed in different proportions to formulate 20 basal substrates indicated as T_1-T_{20} (Table 1). Ten grams of each substrate in glass containers were autoclaved for 30 min at 121°C and allowed to cool down to room temperature.

A mycelial mat with spores obtained from T3, which was grown for 7 days in 100 ml of 3% molasses (15 g of molasses, 2.5 g of yeast in 500 ml of distilled water and autoclaved) in 250 ml conical flasks (*Ca* 2.6×10^{11} spores), was oven dried at 35°C. Thereafter, the dried fungal mat was ground using a sterile mortar and pestle and mixed with each sterile basal substrate preparations and the preparation was stored under normal light/dark conditions at room temperature. Twelve replicates were employed for each substrate.

Determination of the viability of T3 in different media

Viability of *T. harzianum* isolate T3 (IMI393247) spores stored in the above 20 basal substrates was assessed over a period of 4 months

Table 1. Basal mixture combinations and their composition as % of each ingredient.

Basal substrate	Basal substrate combinations and code numbers									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
	Composition of basal substrates (%)									
Clay soil	25	50	50	50	75	25	25	-	25	-
Red Yellow Podzolic Soil	25	50	-	-	25	75	-	25	-	-
Farm manure	25	-	50	-	-	-	75	75	-	50
Saw dust	25	-	-	50	-	-	-	-	75	-
Rice bran	-	-	-	-	-	-	-	-	-	50
	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20
	Composition of basal substrates (%)									
Farm manure	75	25	50	25	75	25	25	12.5	33.3	10
Saw dust	-	-	25	25	-	-	50	75	33.3	80
Rice bran	25	75	25	50	25	75	25	12.5	33.3	10

to determine the shelf life of T3 spores in each basal substrate. The percentage viability of spores was checked by diluting 0.1 g samples of stored material with sterilized distilled water 10^2 times and then with molten PDA in Petri-plate (just before solidifying) 10 times making the total dilution to 10^3. The plates were incubated under normal light/dark conditions at room temperature for 12 h and the number of colonies emerged was counted using a colony counter. Formation of *Trichoderma* colonies on agar indicated the viability of individual spores. The test was carried out at monthly intervals up to 4 months. Three replicates from each substrate were used at each time for assessing the viability of spores.

Preparation of T310 medium

T_{10} mixture as 500 g packets in heat resistant polyethylene bags (12"×12") were sterilized at 121°C for 45 min. A 7 day-old *T. harzianum* (T3) mycelial mat along with spores grown in 100 ml of 3% molasses was filtered through a filter paper and homogenized in 100 ml of 3% sterilized molasses using a homogenizer (Ika Ultra-Turax) at high speed. Thereafter, the homogenate including mycelial fragments was aseptically inoculated to the sterilized T_{10} basal mixture. The bag was re-sealed and the mixture was thoroughly mixed ensuring homogeneity and incubated at room temperature for 7-10 days in a dark chamber. The medium was considered to be active and ready for application, when *T. harzianum* has properly colonized and produced abundant phialospores, which could be seen as dark clusters on the outer surface. Before application, contents in the bag were thoroughly mixed for homogeneous distribution of viable phialospores in the medium.

Persistence of *Trichoderma harzianum* (T3) in soil

To check the viability of T3 in rubber soils, 500 g of the active T310 mixture as prepared above was added to the topsoil layer around the base of a one year old healthy rubber plant and mixed thoroughly. The initial number of T3 spores in 10 g of soil was determined just after mixing T310 with soil, using soil serial dilution method and thereafter, the same was measured up to 24 weeks with intervals as described above.

Effect of T310 preparation on infected roots

Cement pots (10 kg) were half-filled with top soil (red-yellow podzolic) obtained from a rubber land adjoining the institute. Approximately 250 g of active T310 preparation was spread on the surface of the filled soil. Four rubber root pieces each of 8-20 cm length and 2-3 cm thickness obtained from white root disease infected trees were placed on top of this layer. The rest of the T310 preparation was added on to the root pieces and covered with a soil layer of 2-3 cm. Root pieces having infection scores +1, +2 or +3 ($^+3$ = infected was on 75% of the surface of the root piece, $^+2$ = infected is on 50% of the surface of the root piece, $^+1$ = infected is on 25% of the

surface of the root piece) were placed separately in separate pots for easy assessment. This experiment was repeated twice and the results were combined.

After 6 weeks, root pieces were retrieved from pots and the viability of mycelia on each and every root piece was determined. They were immediately wrapped separately in moistened filter papers and stored for 7 days in a dark chamber at room temperature. After this period, root pieces were exposed and the mycelia were considered dead (or the infection has ceased), if no mycelia were grown out onto the filter paper.

Efficiency of white root disease control by T310 *in vivo*

One year old rubber plants planted at 4'×4' were artificially inoculated with naturally infected rubber root pieces (about 1-2" thick and 4" long) during the wet period. Three such pieces were buried within the topsoil close to the taproot of each plant ensuring a good contact. After 3-4 months, roots were checked for infections and the infections were rated using the, score; $^{+}$1= mild infection, when 1/4th of the collar circumference was infected and covered by mycelia, $^{+}$2= medium infection, when ½ of the collar circumference was infected and covered by the mycelia, $^{+}$3= severe infection, when 3/4th of the collar circumference was infected and covered by the mycelia.

Eight plants with $^{+}$1, 28 plants with $^{+}$2 and 27 plants with $^{+}$3 infection scores were treated with 500 g of T310 preparation per plant. The preparation was mixed with the top soil around infected roots of the collar region, ensuring close contact. To protect from direct sun light and to maintain adequate humidity, soil was covered with a mulch layer. There were eighteen control plants including 11 plants with $^{+}$2 and 7 plants with $^{+}$3 infection scores, which were not treated. All treated plants were at fully randomized locations situated in 3 beds at closed proximity. The experiment was repeated three times and replicates from all three experiments were combined in making the assessments.

Assessments on the success rates of the treatments have been taken after 10, 30, 60 and 90 days. The post-treatment disease assessments were made using the same ratings as before. An infection was considered as cured if the infection score was less than what was before the treatment or, if the infection was completely ceased and mycelia were dead and turned yellow.

Data analysis

Data obtained from replicates were averaged and the Standard Error of Means (SEM) values were calculated.

RESULTS

***Trichoderma* isolates and spore production on agar**

Table 2 gives the *Trichoderma* isolates obtained from different rubber growing areas of Sri Lanka. T3 isolate was authenticated by the Commonwealth Mycological Institute, UK as *Trichoderma harzianum* Rifai (IMI 393247).

Screening for antagonism against *R. microporus*

All *in vitro* test results confirmed that *Trichoderma* spp. were highly antagonistic against *R. microporus* on agar and all isolates rapidly overgrew *R. microporus* colonies and marked differences were observed in data on overgrowth of the *R. microporus* cultures by *Trichoderma* isolates (Table 2).

Viability of *T. harzianum* (T3) spores in different media

The number of viable spores in T_6, T_{10}, T_{18}, T_{19} and T_{15} formula declined during 1-4 months storage period in that respective order. However, the viability of T3 spores in T_6 medium was dropped markedly by the second month than that of in T_{10} (Table 3). Therefore, T_{10} medium was selected as the best medium for growing and storing T3 spores under laboratory conditions.

Persistence of *T. harzianum* colony forming units (cfus) in soil

Table 4 shows the number of cfu of *T. harzianum* in soil over a period of 24 weeks, when T310 was added to soil. The highest number of cfu was detected after 6 weeks and thereafter, the population dropped gradually up to 20 weeks and became stabilized around the initial level after 24 weeks.

Effect of T310 on infected roots

The recovery rate of root pieces which had $^{+}$3 infection score was 23.6%, while no recoveries were recorded from the controls. About 58% of root pieces with $^{+}$2 initial infection score had recovered during the same period, while no recoveries of similar root pieces were in control pots. The highest recovery rate of 72% was recorded from root pieces which were initially having $^{+}$1 infection score and none from similar root pieces in control pots (Table 5).

Table 2. *Trichoderma* isolates obtained from different areas of Sri Lanka and their spore production capacities and overgrowth on *R. microporus* cultures.

Location	Isolate code	Number of Spore produced per plate ($\times 10^8$)**	Overgrowth on *R. microporus* (mm) after 6 days*
Agalawatta	T1	15.2± 2.20	60±0
	T2	1.6± 0.68	58±0.8
	T3	18.7± 1.40	60±0
	T4	1.0± 0.68	60±0.8
Rathnapura	TR	17.2± 0.79	57±0.7
Kuruwita	TKur	26.8± 0.72	59±0
Kiriella	TK	3.9± 0.89	58±0
Mahaoya	TM1	2.1± 0.31	60±0
	TM2	2.6± 0.45	60±0
	TM3	32.0± 1.20	60±0
	TM4	9.1± 1.00	60±0
Atale	TA1	30.8± 1.90	60±0
	TA2	28.7± 1.60	60±0
Sapumalkanda	TS1N	3.3± 0.50	60±0
	TS2N	5.8± 0.70	60±0
	TS3N	19.6± 0.58	60±0
	TSF1	1.0± 0.34	60±0
	TSF2	30.0± 4.20	60±0

Table 3. Viability of *T. harzianum* (T3) phialospores in basal mixture combinations at room temperature.

Basal mixtures	Initial number	Number of viable spores ($\times 10^5$) in 0.1 g of substrates after			
		1 month	2 month	3 month	4 month
T1	2.5×10^{11}	*2.2±0.18	1.8 ±0.064	0.4±0.16	-
T2		8.8±0.09	8.5±0.14	4.6±0.27	1.9 ±0.05
T3		5.2±0.16	3.7±0.53	1.2±0.07	0.47±0.09
T4		9.0±0	7.9±0.63	6.2±0.27	2.5 ±0.27
T5		10.0 ±0.14	8.3±0.19	6.2±0.27	2.5 ±0.27
T6		1.0×10^{10}±0	6.2±0.12	1.3±0.067	-
T7		4.5±0.3	4.2±0.3	2.5±0.2	-
T8		4.3±0.27	3.9±0.64	0.75±0.24	0.3±0
T9		8.0±0	5.0±0.6	3.3±0.11	1.3±0.07
T10		1.0×10^{10}±0	1×10^{10}±0	8.3±0.18	5.3±0.27
T11		4.0±0.37	2.6±0.47	2.0±0.09	1.2±0.16
T12		2.3±0.3	0.4±0.13	0.2±0.07	0.2±0.08
T13		4.9±0.6	4.1±0.4	0.9±0.2	0.2±0.03
T14		2.7±0.31	2.6±0.26	1.2±0.13	0.4±0.16
T15		13.0 ±0.2	11 ±0.3	9.0±0.66	5.3±0.27
T16		5.8±0.16	3.0±0.19	3.0±0.05	2.7±0.03
T17		2.0±0.44	1.7±0.5	1.3±0.2	0.5±0.3
T18		12.0 ±0.7	9.6±0.1	8.4±0	7.7±0.7
T19		13.0 ±0	12 ±0.2	0.1±0.9	7.3±0.5
T20		3.7±0.8	1.3±0.1	0.8±0.1	0.6±0.1

*Values are averages of 4 replicates± SEM, values with 10^{10} power are indicated accordingly and all other values are of 10^5 power.

Table 4. Viability of T310 preparation in soil up to 24 weeks (1× 10^5 spores per g of soil).

	Viability of T320 spores X 10^5					
	Initial level	6 weeks	12 weeks	16 weeks	20 weeks	24 weeks
Control soil	*3.16±0.3	3.6±0.54	4.1±0.53	3.25±0.35	3.5±0.35	2.1±0.16
T310 added soil	52±0.2	48±0.3	7.1±0.81	5.25±0.4	4.2±0.23	2.7±0.06

*Values are averages of 3 replicates± SEM.

Table 5. Effect of T310 formulations on naturally infected root pieces buried in pots.

Infection Rate/Control	Number		% Recovery	
	Control	Treated	Control	Treated
$^+3$	11	55	-	23.6
$^+2$	12	34	-	58
$^+1$	1	25	-	72

$^+3$ = infection was on 75% of the surface of the root piece, $^+2$ = infection is on 50% of the surface of the root piece, $^+1$ = infection is on 25% of the surface of the root piece. Results are combination of data from two trials

Table 6. Success rates of infected plants after treatment with T310.

Treat-ments	Plants Qty	Initial rating	After 10 days		After 30 days		After 60 days		After 90 days	
			cured	still infected	cured	still infected	cured	still infected	cured	still infected
Control	-	$^+1$	-	-	-	-	-	-	-	-
	11	$^+2$	20	80	10	90	40	60	40	60
	7	$^+3$	90 ($^+2$)	10 ($^+3$)	33	66	44	56	44	56
T310	8	$^+1$	87.5	12.5 ($^+1$)	90	10	100	-	100	-
	28	$^+2$	80	20	80.6	19.3	96.7	3.2	100	-
	27	$^+3$	70	30 ($^+1$)	93.1	6.9	100	-	100	-

Values are presented as percentages out of the total number of root pieces employed from each score. Values in parentheses indicate the existed infection scores at the time of assessments.

Effect of T310 on young infected plants

Treated plants with $^+1$ infection score completely recovered after 60 days. However, there were no plants in control, which were assigned $^+1$ score for a comparison. The complete recovery of plants with $^+2$ and $^+3$ infection scores was delayed and achieved only after 90 days. Control plants with $^+2$ and $^+3$ infection scores

continued at that level even after 60 days, but the number of plants still having infection was reduced to 40% and 44% respectively. Therefore, the actual percentage of the recovered plants with $^{+}2$ and $^{+}3$ infection scores after 90 days was 60% and 56% respectively (Table 6).

DISCUSSION

Trichoderma isolates obtained from soils of rubber growing areas in this study had variable characteristics, especially on phialospore production which is an important criterion for selecting an antagonist as a biological control agent. Shelf life is also an important factor when biological agents are used for long term experiments. The shelf life of T3 in T_{10} was satisfactory even after 4 months under room temperature. During the first two months period, the viability of T3 phialospores continued to be at a higher level than that of the other isolates. This is an important character of a promising biological control agent which could be stored for a longer period.

Proliferation of the introduced organism in the target sites is also important since, a long term antagonistic effect is required. The greatest barrier to overcome is the antagonistic buffering effects imposed by the resident organisms against the introduced organism causing a rapid deterioration of the introduced organism. Therefore, special emphases were given to those media which were capable of proliferating T3 in soil after the introduction to the target sites. The combinations of nutrient mixtures had variable properties but the T_{10} was more effective in storing and providing adequate nutrients for quick proliferation and longer survival in soil at an effective level. Results showed the survival of the T3 in soil up to 20 weeks when introduced as T310 mixture even at a higher density than in control soils. This is quite a good indication and a difficult to achieve target.

The extent to which *R. microporus* was controlled in infected plants was the prime interest of the investigation. The assessments have shown the effectiveness of the T310 mixture to eliminate the pathogen from the infected sites when the plants were severely infected at an intensity of $^{+}3$. In control plants there was a tendency to naturally decline the infection score, which was due to long droughts and other natural causes which were beyond our control. It was impossible to overcome such natural constraints during this investigation. Therefore, the actual recovery rate due to the treatment was rectified as 60% of infected plants with $^{+}2$ infection score and 56% of infected plants with $^{+}3$ infection score by subtracting the recovery rate of control plants with same scores at 90 days. Assuring these facts, T310 formulation would probably be successful in the field for controlling infections caused by *R. microporus* in immature rubber plants depending on the severity of the infection and the age. However, a large scale field trial shall be established to investigate the *in vivo* effect of the T310 to be used as a biological control formulation for controlling the white root disease in young rubber. It is also worthwhile to explore the possibility of applying this preparation for controlling root diseases of other agricultural crops.

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REFERENCES

Gohet, E., Canh, T. Van, Louanchi, M. and Despreaux, D. (1991). New developments in chemical control of white root disease of *Hevea brasiliensis* in Africa. *Crop Protection* **10**: 234-238.

Hashim, I., and Chew, B.H. (1997). Effects of integrating *Trichoderma* and fungicides on control of white root disease of *Hevea brasiliensis*. *Journal of Natural Rubber Research* **12**: 43-57.

Jayaratne, R., Jayasinghe, C.K. and Wettasinghe, P.C. (1997). Economical and less hazardous fungicide for management of white root disease. *Journal of the Rubber Research Institute of Sri Lanka* **80**: 20-29.

Jayasinghe, C.K., Jayasuriya, K.E. and Fernando, T.H.P.S. (1995). Pentachlorophenol- Effective and economical fungicide for the management of white root disease caused by *Rigidoporus lignosus* in Sri Lanka. *Journal of the Rubber Research Institute of Sri Lanka* **75**: 61-70.

Jayasuriya, K.E. (1996). Biological control of plant pathogens and possible biocontrol approaches against *Rigidoporus lignosus*, the cause of white root disease of rubber. *Bulletin of the Rubber Research Institute of Sri Lanka* **33**: 1-12.

Jayasuriya, K.E. (1997). Use of potentially antagonistic fungi to control some *Hevea* pathogens *in vitro*. **In**: Symposium on Agronomy Aspects of the Cultivation of Natural Rubber (*Hevea brasiliensis*), Beruwela, Sri Lanka, 1996 (Ed). International Rubber Research and Development Board (IRRDB) Brickendonbury, UK, 109-117.

Jayasuriya, K.E. (1998). Environmental friendly disease control systems. *Bulletin of the Rubber Research Institute of Sri Lanka* **37**: 40-46.

Jayasuriya, K.E. and Deacon, J.W. (1995). *In vitro* interactions between *Rigidoporus lignosus*, the cause of white root disease of rubber and some potentially antagonistic fungi. *Journal of the Rubber Research Institute of Sri Lanka* **76**: 36-54.

Jayasuriya, K.E. and Deacon, J.W. (1996). A possible role for 2-Furaldehyde in the biological control of *Rigidoporus lignosus*, the cause of white root disease of rubber. *Journal of the Rubber Research Institute of Sri Lanka* **77**: 15-27.

Jayasuriya, K.E., Deacon, J.W. and Fernando, T.H.P.S. (1996a). Weakening effect of 2-Furaldehyde on biological control of *Rigidoporus lignosus*, the cause of white root disease of rubber. *Journal of the Rubber Research Institute of Sri Lanka* **77**: 54-65.

Jayasuriya, K.E., Deacon, J.W. and Fernando, T.H.P.S. (1996b). *In vitro* antagonism caused by some species of fungi on *Rigidoporus lignosus*. *Journal of the Rubber Research Institute of Sri Lanka* **78**: 89-101.

Jollands, P. (1983). Laboratory investigations on fungicides and biological agents to control three diseases of rubber and oil palm and their potential applications. *Tropical Pest Management* **29**: 33-38.

Katan, J., Ginzburg, C. and Freeman, S. (1992). The weakening effect as a trigger for biological control and criteria for its evaluation. In: Tjamos, *et al*. (Eds) *Biological Control of Plant Diseases*, Plenum Press, New York, Pp. 55-61.

Lam, C.H. and Chiu, S.B. (1993). Hexaconazole (Anvil 5SC), a cost effective fungicide for controlling white root disease in immature rubber. *Planter* **69**: 465-474.

Lim, T.K., Hamm, R.T. and Mohamad, R.B. (1990). Persistency and volatile behaviour of selected chemicals in treated soil against three basidiomycetous root disease pathogens. *Tropical Pest Management* **36**: 23-26.

Ng, K.T. and Yap, T.H. (1990). The effect of triadimefon and triadimenol for controlling white root disease of rubber. *Proceedings of 3rd International Conference on Plant Protection in the tropics, Malaysia.* Volume 2: 31-35.

Peries, O.S. (1969). Economics of control of the white root disease (*Fomes lignosus*) of *Hevea brasiliensis* in Ceylon. In: Toussoun, T.A., Bega, R.V. and Nelson, P.A. (Eds), *Root Diseases and Soil-Born Pathogens*. *Second International Symposium on Factors Determining the Behaviour of Plant Pathogens in Soil*, Imperial College, London. University of California Press.

Rifai, M.A. (1969). A Revision of the Genus *Trichoderma*. Commonwealth Mycological Institute, Kew, Surrey, England.

Rishbeth, J. (1963). Stump protection against *Fomes annosus* III. Inoculation with *Peniophora gigantea*. *Annals of Applied Biology* **52**: 63-77.

Schoeman, M.W., Webber, J.F. and Dickinson, D.J. (1996). The effect of diffusible metabolites of *Trichoderma harzianum* on *in vitro* interactions between basidiomycetes isolates at two different temperature regimes. *Mycological Research* **100**: 1454 -1458.

Yang, D., Plante, F., Bernier, L., Piche, Y., Dessureault, M., Laflamme, G. and Ouellette, G.B. (1992). Evaluation of a fungal antagonist, *Phaeotheca dimorphospora*, for biological control of tree diseases. *Canadian Journal of Botany* **71**: 426-433.