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Effects of Integrating Trichoderma *and Fungicides on Control of White Root Disease of* Hevea *Rubber*

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The effects of integration of fungicide with Trichoderma koningii *on the control of white root disease of* Hevea *rubber were investigated. Control of white root disease with* Trichoderma *was erratic and not persistent. Similarly, the effects of* Trichoderma *on infected nursery plants drenched once with fungicides were also inconsistent. The addition of* Trichoderma *to plants in field plantings, which had been twice drenched with fungicides, did not improve control. In fact, addition of* Trichoderma *reduced control by propiconazole. Triadimefon and propiconazole were effective for the control of white root disease. The population of* Trichoderma *in soil following fungicide treatment was determined and its relationship with disease control discussed.*

White root disease of *Hevea* rubber, which is caused by the fungus, *Rigidoporus lignosus* Klotzsch, is still causing severe economic losses despite the existence of various recommendations and procedures to control the disease. Painting protective fungicides after exposing the tap and part of lateral roots is a procedure long adopted to manage root diseases of rubber[1]. As labour becomes more scarce and expensive, this procedure is difficult to be implemented. Subsequently, drenching of some fungicides was found effective and in Malaysia triadimefon and propiconazole were recommended[2,3]. Unfortunately drenching with these fungicides is effective only for young trees with mild infection. The cost of treatment is also expensive especially when a repeat drench is required.

Drenching of fungicide is popular, as the procedure is easy and fast. However, there is a need to reduce the cost of treatment and to improve control of more severe infection. Previously, the increase in population of *Trichoderma* following amendment of the soil around the plant with sulphur was associated to the mechanism of control of white root disease by sulphur since sulphur is not fungicidal to *R. lignosus*[4–6]. It has been shown that certain species of *Trichoderma* were antagonistic to various fungi[6] inclusive of *R. lignosus*[7,8]. *Trichoderma* has also been used in the field to control white root disease[9].

In the case of *Armillaria mellea* (Vahl.) Quel., *Trichoderma* was able to antagonise the pathogen which has been exposed to

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fumigants[10] Integration of biological control with fungicides was effective against several diseases[11-13] This paper reports on the effects of combinations of *Trichoderma* and fungicide treatments on white root disease.

MATERIALS AND METHODS

Laboratory Studies

Isolation of Trichoderma *from soil in a rubber area.* Soil samples were taken from around diseased plants and plants which two years previously, had been drenched with either tridemorph or propiconazole The soil samples were plated on a *Trichoderma* selective medium[14] and incubated at room temperature in the dark. The colony of *Trichoderma* was isolated (and purified if necessary) and subsequently maintained on malt extract agar medium (MEA)

Evaluation of antagonism of Trichoderma *to* R lignosus. The antagonism of *Trichoderma* to *R lignosus* was tested by the paired dual culture technique. The degree of antagonism was visually assessed as described by Bell *et al.*[6]

The production of inhibitors by one isolate of *Trichoderma* was also determined. *Trichoderma* was inoculated on cellophane sheet placed on the surface of MEA in Petri plates When the edge of the colony of *Trichoderma* nearly reached the edge of the plate, the cellophane sheet with the colony of *Trichoderma* was removed The medium was then inoculated with *R lignosus*. The radial growth of *R lignosus* was periodically monitored.

Toxicity of fungicides to Trichoderma. The fungicides were added to MEA before autoclaving. The agar medium in Petri plates was then inoculated with a mycelial disk from a five-day-old culture of *Trichoderma* and was then incubated at room temperature. The diameter of the colony of *Trichoderma* was then measured. The fungicides evaluated were triadimefon (*Bayleton*), tridemorph (*Calixin*), propiconazole (*Tilt*), penconazole (*Topas*) and cyproconazole (*Alto*).

Population of indigenous Trichoderma *in fungicide drenched soil.* The experiment was carried out on about ten-year-old seedlings growing in a nursery at the RRIM Experiment Station, Sungai Buloh. As the plants were closely planted (1.2 m × 1.2 m), their sizes were equivalent to about four- to five-year-old plants growing in normal field planting Plants infected by *R lignosus* were selected by inspecting their collars for the presence of rhizomorphs after exposing part of the tree collars with a wooden spade to avoid injuring the roots. Ten randomly distributed plants were drenched with each fungicide by pouring one liter of aqueous solution of the fungicide into a shallow furrow dug around the tree collar. Sulphur powder was sprinkled around the plants and forked into the soil. Dazomet granules were applied into five holes dug in the soil around the plants and the treated soil around the plants was covered with plastic sheets. Soil samples were taken at periodic intervals (1, 2, 4 and 6 months) from three plants per treatment Using a soil auger, the top 15 cm of soil was taken from three spots within 30 cm radius around the plants and the soil samples were pooled.

The population of *Trichoderma* in the soil was determined by the soil dilution plating technique using the *Trichoderma* selective medium of Elad *et al.*[14]

Survival of Trichoderma *preparation in fungicide treated soil.* Ten infected plants from the same nursery were treated with fungicides. For each fungicide treatment, five plants were also treated with *Supergro*; an organic fertiliser made from palm-oil-mill-effluent. *Trichoderma* cultures prepared as described below were applied to all the plants a day after the fungicide applications. Soil samples were taken from randomly selected plants and the population of *Trichoderma* was determined as described above.

Preparation of Trichoderma *for field application.* The rice bran-sawdust medium used for culturing *Trichoderma* was prepared by mixing 100 g of rice bran, 100 g of sawdust and 100 ml water in an autoclavable plastic bag, which was later sealed. The bags were autoclaved for 15 min and when cooled were inoculated by injecting 1 ml of a spore suspension of *Trichoderma*. The bags were covered with black cloth and were incubated at room temperature for about two weeks before being used.

The medium containing *Trichoderma* was used in the nursery and field trials by spreading the medium evenly in a shallow furrow dug around a rubber plant. The furrow was immediately covered with soil. Unless otherwise stated, *Trichoderma* was applied two months after fungicide drenching.

Experiment I: Control of Root Disease in Nursery Trials

The plants used in the nursery trials were the seedlings in the RRIM nursery at Sungai Buloh. The soil is sandy Sungai Buloh series. Infected plants were identified by carrying out collar inspection as described previously. The effects of treatments were assessed by determining the number of dead and living plants every six months.

Effects of Trichoderma. The effectiveness of *Trichoderma* cultures on control of root disease was assessed in two trials whereby in each trial, ten infected plants were treated with *Trichoderma* cultures and another ten plants were used as untreated control.

Effects of Trichoderma *and fungicides.* In a preliminary trial, the effects of time of application of *Trichoderma* on the control of root disease with fungicides was evaluated. In this trial, ten infected plants were selected and drenched with fungicides. *Trichoderma* cultures were applied either on the same day, one or two months after fungicide application.

In four subsequent trials, the *Trichoderma* cultures were applied at two months after fungicide applications. In each trial, ten plants were treated with fungicides while another ten plants were treated with fungicides and *Trichoderma*. The fungicides used in these trials were tridemorph, triadimefon and propiconazole.

Effects of number of application of fungicide and Trichoderma. In one trial, the effects of one or two applications of fungicides and *Trichoderma* were compared. The second application of fungicides and *Trichoderma* was given six months after the first application. In each trial, ten plants were treated with fungicides only and another ten plants were treated with fungicides and *Trichoderma*.

Experiment II. Control of Root Disease in Field Trials

The trials were carried out at several smallholdings at Labu, Negeri Sembilan. The

plants were about two years old at the commencement of the trials. Plants with light to moderately severe infection were identified by collar inspection as described above. Infected plants were drenched with aqueous solutions of fungicides (triadimefon or propiconazole) at one liter/tree. The concentrations of the fungicides were as stated in the text and tables. *Trichoderma* preparations were applied two months after the initial application of fungicide. A repeat application of fungicides was given one year after the first fungicide application.

The effects of fungicide and *Trichoderma* treatments were assessed every six months by determining whether the foliage of treated trees visually show the yellowing symptoms of being infected by *R. lignosus*, or the tree was dead.

Five trials were conducted in the field experiment. The number of plants used per trial varied (based on availability of infected plants) however, not less than ten plants were used per treatment per trial.

RESULTS

Population of *Trichoderma* Antagonistic to White Root Disease

About 37% of the *Trichoderma* isolates obtained from rubber soil showed good growth on malt extract medium and when paired with *R. lignosus*, these isolates overgrew *R. lignosus*. These isolates were considered as antagonistic to *R. lignosus*. About 26% of the isolates were less antagonistic. One antagonistic isolate was chosen for subsequent field trials and this isolate also produced inhibitors, which inhibited growth of *R. lignosus* in culture medium. This isolate was identified as *T. koningii* Oudem by the International Mycological Institute (IMI 363006).

Toxicity of Fungicides to *Trichoderma*

Triadimefon was less toxic to *Trichoderma* than tridemorph, propiconazole, penconazole and cyproconazole. At 10 mg/l, triadimefon inhibited radial growth by 10.6% while the % inhibition by the other fungicides was 71.6% for penconazole and 81.2%, 91.2% and 86.4% for tridemorph, propiconazole and cyproconazole, respectively. At 100 mg/l, triadimefon inhibited radial growth by 46.6% while the other fungicides totally inhibited growth.

Effects of Fungicides on Population of Indigenous *Trichoderma*

The population of indigenous *Trichoderma* was influenced by fungicide drenching. The population of *Trichoderma* in soils drenched with tridemorph and propiconazole was lower than in the untreated soil (*Table 1*). However, the differences were only significant for propiconazole at three and four months. Within the first four months, the population of *Trichoderma* in soils drenched with triadimefon was slightly lower in the first month, higher in the second and third month and lower in the fourth month as compared to the control. These differences were not significant. Dazomet and sulphur promoted population of *Trichoderma* but these differences were also not significant.

Survival of *Trichoderma* Cultures in Fungicide Treated Soil

In the trial whereby *Trichoderma* cultured in rice bran was applied to soil which had been drenched with various fungicides, the

TABLE 1. POPULATION OF INDIGENOUS *TRICHODERMA* IN SOILS DRENCHED WITH FUNGICIDES

Treatments (Quantity product/ plant)	Population of *Trichoderma* (cfu/g soils) at 1 mth	2 mth	3 mth	4 mth
No fungicide	502.2 (2.09)^{abcde}	344.4 (2.37)^{abcde}	273.3 (1.67)^{abcde}	720.0 (2.79)^{abc}
Tridemorph (10 ml)	455.5 (2.25)^{abcde}	193.3 (2.25)^{abcde}	191.1 (0.92)^{de}	215.6 (2.25)^{abcde}
Triadimefon (20 g)	413.3 (2.24)^{abcde}	900.0 (2.86)^{abc}	571.1 (2.51)^{abcde}	135.6 (1.54)^{abcde}
Propiconazole (20 ml)	206.7 (2.10)^{abcde}	262.2 (2.33)^{abcde}	182.2 (1.29)^{bcde}	46.7 (0.72)^{de}
Dazomet (20 g)	444.4 (1.04)^{bcde}	426.7 (1.83)^{abcde}	5 682.2 (3.00)^{ab}	4 911.0 (1.39)^{bcde}
Sulphur (200 g)	931.0 (2.68)^{abc}	474.1 (2.63)^{abc}	5 135.6 (3.34)^{a}	911.1 (2.84)^{abc}

Numbers in brackets are data transformed to log (x + 1)
Numbers with same letters are not significantly different at $p<0.05$ (LSD = 1.8195)

population of *Trichoderma* within the first six months after fungicide treatment is shown in *Table 2*. Within the first four months after fungicide treatment, the population of *Trichoderma* in soils treated with *Trichoderma* was significantly higher than in soils not treated with *Trichoderma*. In the majority of the treatments, the highest population of *Trichoderma* was recorded about two months after treatments and decreased thereafter and by the sixth month, the difference was not significant. Generally, the difference in the population of *Trichoderma* in soils treated with the organic fertiliser as compared to untreated soils was not significant. The mean populations were significantly different between treatments at $p<0.01$ (LSD = 0.313). The interaction between treatments, time and organic fertiliser was also significant.

Control of *R. lignosus* by Fungicides and *Trichoderma* in Nursery Trials

In the two trials on nursery plants, the *Trichoderma* isolate used had mild and non-persistent effect on control of white root disease. A summary of the results of the two trials indicated that the percentage number of trees treated with *Trichoderma* which were still alive at 12 and 24 months were 60% and 35%, respectively compared to 35% and 10%, respectively in the untreated control.

In the preliminary trial to ascertain a suitable period to apply *Trichoderma* after drenching of fungicide, the application of *Trichoderma* at one or two months after drenching of triadimefon or propiconazole was more effective in controlling the disease than applying *Trichoderma* immediately after fungicide drenching (*Table 3*). The number of plants living was higher in treatments where *Trichoderma* was applied at one or two months after fungicide treatments as compared to treatments where *Trichoderma* was applied on the same day with the fungicide. The difference was more obvious with propiconazole.

The survival of closely planted nursery plants following fungicide and *Trichoderma*

TABLE 2. SURVIVAL OF *TRICHODERMA* APPLIED TO SOIL DRENCHED WITH FUNGICIDES

Treatments (Product/plant)	*Trichoderma* population (cfu/g soil × 10^3) at									
	0 mth		1 mth		2 mth		4 mth		6 mth	
	P*	A*	P	A	P	A	P	A	P	A
Tridemorph (10 ml)	0.28	0.21	29.22	221.89	57.18	77.76	3.14	0.89	7.67	2.76
	$(0.11)^{d-l}$	$(0.07)^{o-p}$	$(1.24)^{c-j}$	$(1.39)^{b-l}$	$(1.66)^{a-d}$	$(1.17)^{e-k}$	$(0.45)^{k-p}$	$(0.27)^{d-g}$	$(0.84)^{f-n}$	$(0.44)^{k-p}$
Triadimefon (20 g)	0.08	0.16	33.00	4.34	118.00	136.00	28.22	1.57	4.73	7.69
	$(0.03)^{p}$	$(0.06)^{o-p}$	$(1.49)^{a-f}$	$(0.70)^{n-p}$	$(2.03)^{ab}$	$(2.09)^{ab}$	$(1.45)^{a-g}$	$(0.38)^{l-p}$	$(0.65)^{j-p}$	$(0.80)^{f-o}$
Propiconazole (20 ml)	0.46	0.07	12.24	9.25	290.00	42.78	17.78	1.06	2.91	9.87
	$(0.15)^{n-p}$	$(0.03)^{p}$	$(1.02)^{d-l}$	$(0.72)^{g-p}$	$(1.90)^{a-c}$	$(1.42)^{b-h}$	$(1.10)^{d-l}$	$(0.26)^{m-p}$	$(0.56)^{j-p}$	$(0.96)^{d-m}$
Dazomet (20 g)	0.46	0.22	54.73	6.18	94.44	22.92	10.00	2.93	7.16	10.22
	$(0.13)^{n-p}$	$(0.09)^{o-p}$	$(1.51)^{a-f}$	$(0.78)^{f-p}$	$(1.91)^{a-c}$	$(1.43)^{b-h}$	$(1.03)^{d-l}$	$(0.49)^{k-p}$	$(0.90)^{e-m}$	$(0.91)^{e-m}$
No fungicide	0.22	0.30	45.44	99.11	484.22	21.40	17.13	3.36	8.87	6.33
	$(0.07)^{o-p}$	$(0.11)^{n-p}$	$(1.64)^{a-e}$	$(1.64)^{a-e}$	$(2.20)^{a}$	$(1.13)^{d-k}$	$(1.02)^{d-l}$	$(0.74)^{g-p}$	$(0.99)^{d-m}$	$(0.80)^{f-o}$

*Organic fertiliser Supergro® applied (P) or not applied (A)

Numbers in brackets are data transformed to log (x + 1)

Numbers with same letters are not significantly different at $p< 0.05$ ($LSD_{0.05=0.748}$)

TABLE 3. INFLUENCE OF TIME OF *TRICHODERMA* AMENDMENT ON CONTROL OF WHITE ROOT DISEASE

Treatment	Tridemorph (10 ml/plant) Months				Triadimefon (20 g/plant) Months				Propiconazole (10 ml/plant) Months				Propiconazole (5 ml/plant) Months				Total mean
	12	24	36	Mean	12	24	36	Mean	12	24	36	Mean	12	24	36	Mean	
No *Trichoderma*	40	0	0	13.3[a]	50	30	30	36.7[a]	90	70	40	66.7[a]	70	30	0	33.3[a]	37.5[a]
Simultaneously	30	0	0	10.0[a]	80	40	30	50.0[ab]	20	10	0	10.0[b]	30	0	0	10.0[b]	20.0[a]
One month after fungicide drenching	40	20	20	26.7[b]	80	80	80	80.0[c]	70	40	40	50.0[a]	80	20	10	36.7[a]	48.4[b]
Two months after fungicide drenching	20	0	0	6.7[a]	90	50	50	63.3[bc]	90	90	40	73.3[a]	60	30	20	36.7[a]	45.0[ab]
LSD 0.05				11.04				24.24				24.24				22.83	9.79

Mean with same letters are not significantly different at $p < 0.05$

treatments varied with the type of fungicides, the trials and period after treatments (*Table 4*) The difference in percentage of living trees was not significant between fungicides However better disease control was achieved with triadimefon and propiconazole as compared to tridemorph at 12 months and control was about similar at 24 and 36 months. Similarly, the difference in disease control between fungicide alone and fungicide plus *Trichoderma* was also not significant The effectiveness of *Trichoderma* plus tridemorph or triadimefon varied between trials. At 12 months after treatment, *Trichoderma* plus tridemorph had higher control than tridemorph only in one trial but not in the other three trials. Similarly, mixture of *Trichoderma* and triadimefon had better control than triadimefon alone in two trials The application of *Trichoderma* reduced root disease control by propiconazole in all the four trials In addition, the effectiveness of control decreased significantly ($p<0.05$) with time as indicated by the lower number of living trees at 36 months compared to 12 months

In the trial to determine the effects of multiple application of fungicides and *Trichoderma*, two application of tridemorph at six month interval failed to control root disease. However control was improved when *Trichoderma* was applied once or twice following two applications of tridemorph (*Table 5*) Two applications of triadimefon and *Trichoderma* produced better control than the other treatments. In the case of propiconazole, the application of *Trichoderma* reduced control by the fungicide

Control of *R. lignosus* by Fungicides and *Trichoderma* in Field Plantings

Triadimefon and propiconazole were effective in controlling white root disease in field planted plants. The percentage number of living trees following triadimefon and propiconazole treatments was 89 3% and 92 7% respectively, at 36 months (*Table 6*) The inclusion of *Trichoderma* did not improve control In fact, disease control was significantly lower in propiconazole plus *Trichoderma* treatment as compared to propiconazole alone at 36 months

The addition of *Trichoderma* was however beneficial in reducing the presence of rhizomorphs of *R lignosus* on the tree collars The number of trees with rhizomorphs still occurring at their collars and tap roots were lower for trees treated with fungicide and *Trichoderma* as compared with fungicide only (*Table 7*) However the difference was not significant in most instances

DISCUSSION

Increase in population of *Trichoderma* spp. in soils following chemical amendment has been associated to the control of several pathogens of rubber roots such as *R lignosus*[15], *Ganoderma philippii*[16] and *Armillaria mellea*[10]. Lysis of mycelia of *R. lignosus* occurred when it touches mycelia of *Trichoderma*[7,8]

The current practice to control white root disease is to drench with fungicides Normally, at least two drenches are given and this makes the treatment expensive Moreover drenching of fungicides often fail to cure rubber trees severely infected by root disease[2] Whether *Trichoderma* can contribute to the control of white root disease following chemical drenching is yet to be seen.

In this study, about 50% of the nursery plants treated with only *Trichoderma* survived at

TABLE 4. EFFECT OF FUNGICIDES AND *TRICHODERMA* ON SURVIVAL OF INFECTED NURSERY PLANTS

Treatments	Experiment	Survival of plants (%) 12 months +T	12 months –T*	24 months +T	24 months –T	36 months +T	36 months –T
Tridemorph (10 ml/plant)	1	80	40	80	20	80	10
	2	20	40	0	0	0	0
	3	10	0	10	0	10	0
	4	40	20	30	0	10	0
	Mean	37.5 (1.47)$^{a-c}$	25.0 (1.14)$^{a-d}$	30 (1.11)$^{a-d}$	5.0 (0.33)^{ef}	25 (1.00)$^{b-f}$	2.5 (0.26)^{f}
Triadimefon (20 g/plant)	1	90	60	70	0	50	10
	2	90	50	50	30	50	30
	3	10	10	0	0	0	0
	4	40	50	20	30	10	0
	Mean	57.5 (1.64)^{ab}	42.5 (1.56)^{ab}	35.0 (1.22)$^{a-d}$	15 (0.75)$^{c-f}$	27.5 (1.11)$^{a-e}$	10 (0.63)$^{d-f}$
Propiconazole (20 ml/plant)	1	60	90	10	70	0	70
	2	80	90	70	90	40	40
	3	70	80	30	80	30	20
	4	30	40	20	20	10	20
	Mean	60.0 (1.76)^{ab}	75 (1.86)^{a}	35.5 (1.43)$^{a-e}$	65 (1.76)^{ab}	20.0 (1.04)$^{b-f}$	37.5 (1.53)$^{a-e}$
		40.00 (1.04)^{y}		35.8 (1.11)^{xy}		24.6 (1.46)^{x}	

* +T, *Trichoderma* applied; –T, *Trichoderma* not applied

Numbers in brackets are data transformed to log (x + 1)

Numbers with same letters are not significantly different at $p < 0.05$

TABLE 5. EFFECT OF NUMBER OF APPLICATIONS OF FUNGICIDE AND *TRICHODERMA* ON CONTROL OF WHITE ROOT DISEASE

Treatment	Survival of plants (%)												
	Tridemorph (10 ml/plant) Months				Triadimefon (20 g/plant) Months				Propiconazole (20 ml/plant) Months				Total mean
	15	24	36	Mean	15	24	36	Mean	15	24	36	Mean	
One application	0	0	0	0.0[a]	10	0	0	3.3[a]	80	80	20	60.0[a]	21.1[a]
One application + *Trichoderma* (1)*	10	10	10	10.0[a]	10	0	0	3.3[a]	70	30	30	43.4[a]	18.8[a]
Two applications	20	10	0	10.0[a]	40	30	20	30.0[b]	70	60	0	43.4[a]	27.8[a]
Two applications + *Trichoderma* (1)*	70	50	0	40.0[b]	40	40	30	36.7[b]	50	50	50	50.0[a]	42.2[b]
Two applications of fungicide + *Trichoderma* (2)*	80	80	60	73.3[c]	90	90	60	80.0[c]	30	30	20	26.7[a]	59.7[c]
LSD 0.05				28.0				12.4				36.1	13.7

**Trichoderma*, one (1) or two applications (2)
Twenty plants per treatment
Means with same alphabets are not significantly different at $p < 0.05$

TABLE 6. EFFECT OF *TRICHODERMA* ON CONTROL OF WHITE ROOT DISEASE BY FUNGICIDE IN FIELD PLANTING

Treatment	Experiment	Survival of plants (%)			
		24 months		36 months	
		Trichoderma		*Trichoderma*	
		+T*	−T	+T	−T
Triadimefon	1	88.9	100.0	88.9	100.0
(20 g/plant)	2	80.0	100.0	80.0	100.0
	3	85.7	75.0	71.4	75.0
	4	75.0	100.0	75.0	100.0
	5	100.0	71.4	100.0	71.4
	Mean	85.9 (1.94)^{ab}	89.3 (1.95)^{ab}	83.1 (1.95)^{ab}	89.3 (1.95)^{ab}
Propiconazole	1	100.0	100.0	83.3	80.0
(10 ml/plant)	2	75.0	100.0	75.0	100.0
	3	66.7	83.3	50.0	83.3
	4	75.0	100.0	75.0	100.0
	5	83.3	100.0	83.3	100.0
	Mean	80.0 (1.90)^{ab}	96.7 (1.99)^{a}	73.3 (1.86)^{c}	92.7 (1.97)^{ab}

Numbers in brackets are data transformed to (log x + 1)
Means with same letters are not significantly different at p = 0.05 (LSD = 0.0698)
*See *Figure 5*

TABLE 7 EFFECT OF *TRICHODERMA* AND FUNGICIDES ON RHIZOMORPHS OF *R LIGNOSUS*

Treatment	Experiment	Number of trees (%) with rhizomorph 12 months +T	12 months −T*	18 months +T	18 months −T	24 months +T	24 months −T	36 months +T	36 months −T
Triadimefon	1	44 4	55 6	22 2	44 4	22 2	22 2	11 1	22 2
	2	0 0	0 0	50 0	25 0	25 0	0 0	0 0	25 0
	3	42 9	40 0	14 3	60 0	28 6	40 0	14 3	40 0
	4	0 0	0 0	50 0	20 0	0 0	0 0	0 0	0 0
	5	0 0	28 6	0 0	28 6	0 0	28 6	0 0	28 6
	6	–	50 0	44 4	33 3	55 6	66 7	55 6	66 7
	Mean	21 6 (0 823)[b]	29 0 (1 091)[ab]	30 2 (1 270)[ab]	35 2 (1 531)[a]	21 9 (1 001)[ab]	26 3 (1 047)[ab]	13 5 (0 670)[b]	30 4 (1 283)[ab]
Propiconazole	1	16 7	33 3	33 3	66 6	0 0	33 3	16 7	50 0
	2	33 3	0 0	0 0	0 0	0 0	0 0	0 0	0 0
	3	16 7	16 7	50 0	16 7	50 0	16 7	66 7	16 7
	4	50 0	0 0	25 0	0 0	25 0	0 0	25 0	0 0
	5	100 0	0 0	0 0	0 0	50 0	0 0	0 0	0 0
	6	40 0	62 5	20 0	62 5	0 0	62 5	0 0	62 5
	Mean	42 8 (1 559)[a]	18 8 (0 764)[b]	21 4 (0 997)[ab]	24 3 (0 813)[b]	20 8 (0 805)[b]	18 8 (0 769)[b]	18 1 (0 749)[b]	21 5 (0 793)[b]

*See *Figure 5*

Numbers with same letters are not significantly different at $p < 0\ 05$

12 months after treatment, a percentage comparable to one drench of fungicides. Unfortunately the effect was not persistent as most of the treated trees died at 24 months or later This indicates that *Trichoderma* isolate used could only delay the advancement of death of the trees but could not cure the disease. The better effect of *Trichoderma* at 12 months or earlier could be related to the population of *Trichoderma*. *Trichoderma* population was high especially within the first four months after soil application of *Trichoderma* cultures and the population decreased thereafter (*Table 2*). This indicates that the ability to maintain high population of *Trichoderma* is important in white root disease control. In fact better control of white root disease occurred when *Trichoderma* was applied twice in combinations with tridemorph or triadimefon as compared to two applications of these fungicides only (*Table 5*). The application of *Trichoderma* with sulphur would be useful as the population of *Trichoderma* is enhanced by sulphur[7].

The application of *Trichoderma*, two months after the nursery plants were drenched with fungicides produced inconsistent results (*Table 4*). In the case of tridemorph and triadimefon, control was higher when these fungicides were combined with *Trichoderma* in only some trials. The reason for the differences in the effectiveness of the combinations is unknown but it may be related to the influence of weather and different preparation of *Trichoderma* cultures on the population of *Trichoderma* in the soil. Similar results were obtained in the field trials whereby the addition of *Trichoderma* did not improve tree survival. On the contrary, the presence of *Trichoderma* lowered the survival of the trees treated with propiconazole (*Tables 4* and *6*). The reduced effectiveness was insignificant in the case of triadimefon but significant for propiconazole. The reduced effectiveness could be due to the interaction of the fungicides with the organic matter used as carriers for *Trichoderma*. Proper choice of carriers of the biocontrol organism is necessary if fungicide is to be integrated with biocontrol so as to avoid deactivation of the fungicides by the carriers of the biocontrol organism.

In the field trial, originally only one application of fungicide was planned. However, after one year, as an important proportion of the trees treated with fungicides and/or *Trichoderma* were still not cured and tree death cannot be tolerated, a second drenching was performed. As the result more than 80% of the treated trees still survived three years after treatment. With the good effects of two drenching of fungicides on disease control, the contributory effects of *Trichoderma* was not seen

The fungicide drenching was more effective in the trial on field plants where the soil is loamier as compared to the nursery trial where the soil is very sandy. Soil structure may influence the effectiveness of fungicides when applied by soil drenching. To date, there are no available reports on the influence of soil structure on the control of white root disease by drenching of fungicide.

Trichoderma has been used in Indonesia for the control of white root disease[9]. In the current study, *Trichoderma* delayed death of infected trees. Further work is necessary to explore its potential especially on means to maintain high population for a prolong period The results presented here indicated that there was no benefit of integrating *Trichoderma* with fungicide application. In the literature,

integrated control using *Trichoderma* and fungicides was more successful with pathogens such as *Rhizoctonia Fusarium* and *Sclerotium*[11 13] Nevertheless *Trichoderma* successfully colonised *A mellea*, another rhizomorph producing fungus only after *A mellea* was weakened by fumigants[10] In this study, the fungicides were applied only to the narrow regions around the bases of trees. Rhizomorphs of *R lignosus* on lateral roots and progressing to the base of the trees may not be sufficiently exposed to the fungicide to allow parasitisation by *Trichoderma* The ability of *Trichoderma* to parasitise rhizomorphs of *R lignosus* has yet to be shown as to date only *in vitro* antagonism and lysis of mycelia were indicated In addition to *Trichoderma* other microorganisms should be investigated. Several of these organisms have also been identified[8]

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