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WEAKENING EFFECT OF 2-FURALDEHYDE ON *RIGIDOPORUS LIGNOSUS* THE CAUSE OF WHITE ROOT DISEASE OF RUBBER

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ABSTRACT

Furfuraldehyde is recognized as a potential fumigant which has the ability to weaken *Rigidoporus lignosus* in artificially or naturally infected rubber root inocula. Addition of sulphur to soil at 100 g per 75 kg of soil, inactivated or prevented the formation of *R. lignosus* mycelial cords from artificially or naturally infected inocula. Drenching 2.4% aqueous solution of furfuraldehyde in to soil (1 liter/75 kg) where *R. lignosus* inocula were buried, caused weakening or inactivation of *R. lignosus* in artificially or naturally infected inocula. However, combination of two treatments had no synergistic effect on both types of *R. lignosus* inocula in soil.

Key words: anatagonism, furfuraldehyde, mycelial cords, *Rigidoporus lignosus*, rubber

INTRODUCTION

White root disease caused by *Rigidoporus lignosus* (Kl.) Imaz. is the most destructive root disease in rubber plantations. Presently around 5-10% of the cultivated lands in Sri Lanka are affected and under bare patches due to white root disease (Jayasinghe *et al.*, 1995). The pathogen spreads as mycelial strands on infected roots or directly as cords through soil. In Sri Lanka, the production of rubber is marginally economic (Pieris, 1966) so the focus of work in this investigation was to explore noval methods that might be as cheap as possible for control of the damaging white root disease. It was anticipated that such work might lead to exploitation of biocontrol agents either alone or in conjunction with soil chemical treatments to enhance the degree of control.

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Many attempts were made towards the control of *R. lignosus*, in which successful results were obtained using (a) some chemicals of the triazole group (Tran, 1985; Tan, 1990; Ng & Yap, 1990; Chan *et al.*, 1991; Gohet *et al.*, 1991; Lam & Chin, 1993), (b) 2% Pentachlorophenol in bituminous base (Jayasinghe *et al.*, 1995) (c) biological control agents (Tong-Kwee & Keng, 1990; Jayasuriya & Deacon, 1995) (d) fumigants such as furfuraldehyde (Jayasuriya & Deacon, 1996). In studies of Jayasuriya & Deacon (1996), furfuraldehyde completely stopped *R. lignosus* mycelial growth on Malt extract agar (MEA) supplemented with 0.3% furfuraldehyde. Furthermore, 3 hour exposure to 0.2 ml furfuraldehyde caused 80% inhibition of *R. lignosus* mycelial cord growth rate from established inocula. When applied to soil for fumigation, *R. lignosus* mycelial cord growth was suppressed and the activities of resident soil fungi were enhanced causing reduction of *R. lignosus* mycelial cord growth through soil.

In most studies the results were obtained *in vitro* conditions. However, furfuraldehyde appeared to be a potential chemical for effective control of *R. lignosus* as it reduced the growth of *R. lignosus* on culture media as well as in soil (Jayasuriya & Deacon, 1996). Therefore, experiments reported in this paper were focused on drenching furfuraldehyde to soil and exposing *R. lignosus* inocula to furfuraldehyde or its vapor to investigate its fumigative effect on *R. lignosus* inocula in soil. To achieve our targets three rubber growing sites in three agro-climatic regions were selected for the investigation: site 1- Rubber Research Institute premises experimental fields, site 2- Matara, site 3- Kegalle. The work reported in this paper was carried out both in Edinburgh, UK and Sri Lanka.

MATERIALS AND METHODS

Furfuraldehyde (Sigma Co. USA) was used as a 1% or 2.4% dilution to drench into soil. *R. lignosus* (isolate RT) isolated from infected roots collected from Ratnapura, a rubber growing area of the wet zone of Sri Lanka, was used throughout the study. The fungus was stored in 2 cm^3 sterilized elm wood blocks.

Preparation of *R. lignosus* inocula from rubber tree roots

Freshly cut 15 mm thick rubber tree roots were washed thoroughly under running tap water. Soil particles adhering to the surface were removed. Then 8 cm lengths were cut, soaked in distilled water for 1 hour, and autoclaved for 45 minutes at 121 °C in 500 ml glass beakers covered with tin foil. *R. lignosus* was grown on

MEA in 9 cm Petri dishes until the colony margin reached the edge. About 6 cm^2 blocks of MEA colonized by *R. lignosus* were cut from the margins of the plates and transferred aseptically to the autoclaved roots in the beakers. Then the foil on the beakers was sealed with masking tape and they were incubated for 8 weeks.

Effects of sulphur and furfuraldehyde applied to soil on *R. lignosus* inocula.

Rubber root pieces (1-1.5 cm thick and 8 cm long) that were naturally or artificially infested with *R. lignosus*, were used as inocula. Naturally infected inocula were cut from large diseased trees on the day of the experiment or the previous day.

An experimental block in site 1 (site 1 - Rubber Research Institute premises experimental field) was prepared 3 days before the establishment of the experiment. A 2 m wide strip of land was cleared of ground cover and levelled. Ten bucket-shaped containers (made of PVC) which hold 75 kg of wet soil (30% w/w) were sunk into the soil up to ground level and filled with the same soil. The soil in these containers represented "disturbed soil". The containers had 2 holes drilled at the bottoms for drainage. The soil was allowed to settle for 4 days. Two types of inocula were then buried in 5 randomly selected containers about 15 cm below the surface. Each container received 9 pieces of inocula which served as 9 replicates. At other positions in the experimental site, equivalent inocula were buried at 25 cm depth by digging holes of 15 cm diameter. These sites represented "undisturbed" soil. The following treatments were used:

(1) natural inocula in undisturbed soil (NIUS), (2) artificial inocula in undisturbed soil (AIUS), (3) natural inocula in disturbed soil (NIDS), (4) artificial inocula in disturbed soil (AIDS), (5) natural inocula in disturbed soil with added sulphur, 100 g/75 kg soil (NIDS+S), (6) artificial inocula in disturbed soil with added sulphur, 100 g/75 kg soil (AIDS+S), (7) natural inocula in disturbed soil with added sulphur, 100 g/75 kg soil and 1 litre of 1% furfuraldehyde (NIDS + S + F1), (8) artificial inocula in disturbed soil with added sulphur, 100 g/75 kg soil + 1 litre of 1% furfuraldehyde (AIDS + S+ F1), (9) natural inocula in disturbed soil with 1 litre of 1% furfuraldehyde (NIDS+F1), (10) artificial inocula in disturbed soil with 1 litre of 1% furfuraldehyde (AIDS+F1), (11) natural inocula in disturbed soil with 1 litre of 2.4% furfuraldehyde (NIDS+F2.4), (12) artificial inocula in disturbed soil with 1 litre of 2.4% furfuraldehyde (AIDS+F2.4).

In treatments where sulphur was used, it was sprinkled on the soil at 100 g/75 kg soil in each container, and thoroughly mixed with the soil before the inocula were added. In treatments where furfuraldehyde was used, it was drenched into soil as 1 litre of 1% or 2.4% aqueous solutions per container, after burial of inocula. Theoretically, these two concentrations may provide furfuraldehyde concentration in the soil water solution of approximately 0.08% or 0.2% respectively (soil moisture content was 30%, w/w).

After 5 weeks, root pieces were retrieved for examination and for isolation of fungi that had invaded the inocula. By visual observations, records were made on the presence of mycelial cords on the inocula, number of mycelial cords formed and their length, status of epiphytic mycelia on the inocula and degree of decay of the root pieces. Soon after root pieces were brought to the laboratory, and cleaned carefully without damaging the existing mycelia on them. Then, three lengths of mycelial cords (approximately 5 mm long) were cut from the surface of each inoculum, surface-sterilized with 70% ethanol in sterilized distilled water and transferred to MEA supplemented with streptomycin and chlortetracycline (30 μg ml^{-1} of each), to see if *R. lignosus* could grow from them. Using a cork borer, (9 mm in diameter) plugs were removed from bark and wood of each inoculum piece and transferred on to PDA supplemented with antibiotics at the above rate. Plates were then incubated for 2-3 days. Fungal colonies growing out were transferred onto fresh PDA for purification. Each fungus isolated was later opposed to *R. lignosus* on dual membered plates as described by Dennis & Webster (1971) to assess its antagonistic properties. All fungi that suppressed *R. lignosus* were stored and maintained on PDA.

Survival of weakened *R. lignosus* inocula in different soils in different agro-climatic zones of Sri Lanka

Artificially and naturally infested root inocula were prepared as described earlier. Two experimental plots (1.5 m x 5 m) were selected at each of the sites, Kegalle (site 3) and Matara (site 2), on land where rubber had grown for a considerable period. White root disease incidence is negligible in site 2 compared to site 3. Holes, 20 cm deep and 15 cm diameter, were dug at a distance of 50 cm from each other within and between rows. Soil from each hole was bulked and pH was measured. Both types of inocula were fumigated in sealed glass desiccators by placing a cotton wool plug soaked with 0.2 ml furfuraldehyde on the base of the desiccator without touching the inocula. After two hours of exposure, the inocula were placed in the holes. Two pieces of inocula were placed in each hole on a randomized design. One inoculum piece was considered as one replicate and six replicates were employed for each treatment. Inocula were buried for 5 weeks about

20 cm below the ground surface. This level was selected for burial, as Pearce & Malajczuk (1990) indicated that significantly ($P < 0.001$) more rhizomorphs (mycelial cords) were produced by *Armillaria luteobubalina* at 12 cm depth than at 28 cm depth in soil. Then the holes were filled with same soil and the area was demarcated with wooden pegs. The experiment consisted of the following treatments:

(1) Healthy freshly cut root piece.(control), (2) Naturally infested inoculum (NI), (3) Artificially infested inoculum (AI), (4) Naturally infested inoculum fumigated with 0.2 ml furfuraldehyde for 2 hours (NI-FUM), (5) Artificially infested inoculum fumigated with 0.2 ml furfuraldehyde for 2 hours (AI-FUM).

After 5 weeks, the inocula were retrieved. Then the inocula were cleaned of adhered soil particles. Visual observations were made on the degree of decay, number of mycelial cords formed and their lengths, and the presence or absence of epiphytic mycelia of *R. lignosus* on the inocula. Inocula were then brought to the laboratory in sealed polyethylene bags. Then fungi were isolated from bark and wood as described earlier. All the cultures of potential antagonists were preserved and maintained on PDA.

Isolation of fungi antagonistic against *R. lignosus* from buried inocula in soil

The isolation of antagonistic fungi that could possibly colonize *R. lignosus* inocula was carried out as a part of the experiments. Isolation was carried out from all types of inocula buried under soil treated differently (*i.e.* soil acidified with sulphur, from inocula weakened and buried in different soils in different agro-climatic zones of Sri Lanka). Bark and woody plugs (0.5 cm in diameter) were punched from buried inocula and transferred onto PDA supplemented with antibiotics (streptomycin 30 $\mu g\ ml^{-1}$ and chlortetracycline 30 $\mu g\ ml^{-1}$). The mycelia growing out from the plugs were transferred onto fresh PDA plates and opposed against *R. lignosus* on dual membered plates as described by Dennis & Webster (1971) to assess the antagonistic properties.

RESULTS

The separate and combined effects of addition of furfuraldehyde and sulphur to soil on *R. lignosus* inocula

The experiment was designed to study the effects of furfuraldehyde drenched into soil and the addition of sulphur to soil, on the survival of *R. lignosus* in its food base. Sulphur was added to soil at 100 g per pot containing 75 kg of soil (30% water

capacity) and furfuraldehyde was drenched as 1% and 2.4% solutions to soil after burial of inocula. Inocula buried in the pots after soil treatment were excavated and examined after 5 weeks.

In all the treatments where sulphur was used, no mycelial cords were formed from the inocula and the pre-existing cords on the inoculum surfaces seemed to be non-viable (Table 1). In all the treatments involving artificial inocula, the pre-existing mycelial cords on the inocula were weakened or inactivated during burial, whereas at least in some treatments involving naturally infested inocula the pre-existing cords on the inocula were able to produce viable mycelia on agar after burial. Apart from the sulphur treatment, the only other treatment that seemed to kill *R. lignosus* in the inocula (and prevent its growth into soil as mycelial cords) was the treatment (11) involving naturally infested inocula under soil drenched with 2.4% furfuraldehyde. Even though *Trichoderma* was not added as an experimental treatment, *Trichoderma* spp. most commonly were isolated from buried inocula.

Effect of furfuraldehyde vapour on naturally and artificially infested *R. lignosus* inocula in soils of different sites of Sri Lanka

The incidence of white root disease of *Hevea brasiliensis* is comparatively higher in some regions of Sri Lanka, such as Kalutara, Ratnapura and Kegalle, than in others such as Galle and Matara. This variation may be due to soil or agro-climatic factors such as precipitation of particular regions or due to different levels of antagonist in the soil microflora. This experiment was designed to investigate the mycelial cord formation from weakened inocula buried in different soils in Sri Lanka. Inocula were exposed to 0.2 ml of furfuraldehyde for 2 hours in air-tight glass containers (12 pieces of inocula were exposed to 0.2 ml of furfuraldehyde). Then the inocula were examined and analyzed after 5 weeks. Subsequently, antagonistic fungi that had been invaded on to *R. lignosus* were also isolated and tested on dual culture plates as described by Dennis & Webster (1971) for their *in vitro* antagonism against *R. lignosus*.

As shown in Table 2, new mycelial cords had formed from all types of inoculum in the two soils tested, but in the soils from site 3 (Kegalle) there were generally fewer new cords formed from fumigated inocula than in the soils from site 2 (Matara). However, as shown in Table 3, only *Trichoderma* spp. were isolated from the inocula buried in site 2 whereas different fungi including *Aspergillus niger*, *Trichoderma* sp. and unknown fungi were isolated from inocula buried in site 3.-Pre-existing cords of inocula buried in site 2 were generally killed or weakened whereas those inocula buried in site 3 were viable.

Weakening effect of furfuraldehyde

Table 1. *Effect of sulphur and furfuraldehyde in soil on inocula of* R. lignosus

Treatments*	Degree of decay of inocula	Status of mycelial cords on the inocula**	Number of mycelial cords formed***
1. NIUS	fresh	active	1-3
2. AIUS	50%	weakened	1-3
3. NIDS	50%	active	4-6
4. AIDS	50%	weakened	1-3
5. NIDS + Sulphur	fresh	inactive	none
6. AIDS + Sulphur	50%	inactive	none
7. NIDS + Sulphur+1% Furfuraldehyde	fresh	inactive	none
8. AIDS + Sulphur+1% Furfuraldehyde	50%	inactive	none
9. NIDS + 1% Furfuraldehyde	fresh	active	1-3
10. AIDS + 2.4% Furfuraldehyde	fresh	weakened	1-3
11. NIDS + 2.4% Furfuraldehyde	50%	inactive	none
12. AIDS + 2.4% Furfuraldehyde	50%	inactive	1-3

* NI= natural (root) inoculum; AI= artificially colonized inoculum; DS= disturbed soil; S= sulphur addition; furfuraldehyde= drenched to soil; US= undisturbed soil; DS= disturbed soil,**active, inactive or weakened: when mycelial cord fragments were surface sterilized and transferred to MEA, active cords produced active mycelia on agar, weakened cords produced active mycelia from less than 50% of the fragments, inactive cords did not produced active mycelia at all, ****number of cords formed from the edges of inocula.

Table 2. *Effect of fumigation of rubber tree root inocula of* R. lignosus *with furfuraldehyde before burial in soil in two agro-climatic regions of Sri Lanka.*

Inoculum type*	Degree of decay**	Status of mycelial cords on surfaces of inoculum***	No. of mycelial cords formed from each inoculum+
Site 2 - Matara			
control (fresh root)	50%	-	
NI- unfumigated	50%	Pre-existing cords not viable	1-3
AI- unfumigated	>75%	Pre-existing cords not viable	2-4
NI- fumigated	50%	Pre-existing cords not viable	2-3
AI- fumigated	50%	Pre-existing cords not viable	2-3
Site 3 - Kegalle			
control (fresh root)	fresh	-	
NI- unfumigated	fresh	Pre-existing cords viable, new cords formed	1-3
AI- unfumigated	50%	Pre-existing cords viable	4-6
NI- fumigated	50%	Pre-existing cords viable, new cords formed	1-3
AI- fumigated	50%	Pre-existing cords not viable	1-2 or none

* NI= naturally infested rubber tree root inocula; AI= artificially infested rubber tree root inocula, **Degree of decay was observed visually by examining the status of bark and the wood, *** pre-existing cords were visually identified as they were discoloured and the newly formed once were clearly distinguishable as they were white coloured, + number of mycelial cords formed from edges of each inoculum was counted.

DISCUSSION

The results of the experiments have showed that furfuraldehyde has a lethal or sub-lethal effect on *R. lignosus* in soil. A concentration of 0.1% in media was adequate for 50% inhibition of *R. lignosus* (Jayasuriya & Deacon, 1996). Furthermore, *R. lignosus* on culture media was exposed to furfuraldehyde vapour, the lethal effect of this volatile chemical was confirmed: growth of *R. lignosus* was reduced 80% by 3 hour exposure to the chemical, at concentrations that would be achievable by drenching the chemical around the collar of an infested tree. However, the effect of furfuraldehyde in field conditions need further study. For example, it is not known whether the chemical could penetrate into the tissues of infected plants or into plant residues to weaken the pathogen and enhance its susceptibility to biocontrol [A similar study was carried out by Lim *et al.* (1990), using fungicides of the triazole group by adding them to soils and assessing their persistence in soil and their vapour phase effect on *R. lignosus* growth. Tridemorph was found to be the most persistent, maintaining a level of inhibition of 60-70% at 100 μg a.i. kg^{-1} soil and 77-90% at 200 μg a.i. kg^{-1} soil]. Even partial weakening of surviving inoculum of *R. lignosus* by furfuraldehyde could be beneficial if the chemical also enables antagonistic fungi such as *Trichoderma* to grow and antagonize the residual inoculum of *R. lignosus*. Such an alteration of the soil microflora by furfuraldehyde was reported by Canullo *et al.* (1992).

Despite the substantial degree of control of mycelial cord growth by some soil treatments with *Trichoderma*, bran or furfuraldehyde (Jayasuriya & Deacon, 1996), the results of one comparison of sulphur with furfuraldehyde in soil (Table 1) showed clearly that sulphur treatment was highly effective in suppressing mycelial cord growth by *R. lignosus*, presumably due to its marked effect in lowering the soil pH. This experiment was done in field conditions and using in some treatments, natural inocula (infected root materials) of the pathogen. Because of the completely suppressive effect of sulphur alone, it was impossible to see if sulphur combined with a furfuraldehyde treatment would enhance the degree of control, but this possibility might be further explored, especially with larger pieces of host residue as inoculum units.

In a separate experiment in which *R. lignosus* inocula were buried in different sites, it was noted that all the epiphytic mycelial cords were inactive after burial for 5 weeks in site 2 (Matara). It was also important to note that only *Trichoderma* spp. could be isolated from the bark surfaces of buried inocula. However, new mycelial strands were formed from buried inocula in both sites.

From the results obtained herein, it may be difficult to predict that drenching 2.4% furfuraldehyde may totally kill the pathogen on buried inocula as it formed mycelial cords through soil. However, according to Table 1, it seems that the pathogen on the surface was weakened or killed. Therefore, it may be benifitial to supplement an antagonist to prevent forming mycelial cords. Apart from this, sulphur seemed to be highly effective on reducing and killing the mycelial cords.

Results from the Table 2 showed that all pre-existing mycelial cords were killed on inocula buried in site 2 which can presumably be due to the fact that weakened mycelia were more sensitive for an attack by antagonistic organisms in soil microflora. This can be proved by the fact that, from all the inocula buried in site 2, only *Trichoderma* spp. such as *T. harzianum* and *T. koningii* were isolated whereas from inocula buried in site 3, different fungi were isolated. As Jayasuriya & Deacon (1996) reported in their results obtained in soil studies, proliferation of *Trichoderma* in soil may be suppressed by other competitive soil fungi. This was demonstrated well in treatments in which soil was fumigated with furfuraldehyde, where *T. harzianum* (strain TV12b) sporulated abundantly.

CONCLUSIONS

The results obtained from the experiments reported here indicate that furfuraldehyde has a potential to control *R. lignosus in vitro*. Sulphur amendments to soil can cause detrimental effects on formation of *R. lignosus* mycelial cords presumably by altering the soil microflora in favour of acidophylic fungi such as *Trichoderma* spp. As there is no additive effect of furfuraldehyde and sulphur in soil, sulphur may be effective on reducing the mycelial cord growth by *R. lignosus*. It may be possible to suggest that furfuraldehyde can be used as a fumigant to weaken the *R. lignosus* pathogen and consequently amend an antagonistic organism such as *Trichoderma* sp. for a complete control..

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