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A POSSIBLE ROLE FOR 2-FURALDEHYDE IN THE BIOLOGICAL CONTROL OF WHITE ROOT DISEASE OF RUBBER

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ABSTRACT

Different control methods were tested against *Rigidoporus lignosus* mycelial cord growth in soil in highly artificial conditions. 0.2% furfuraldehyde in malt extract agar caused 50% inhibition of the growth of *R. lignosus*. Effect of soil fumigation with furfuraldehyde, addition of sterilized oat bran to soil and addition of spores of *Trichoderma* strains to soil were evaluated. A 45% inhibition of mycelial cord growth was achieved by soil fumigation with furfuraldehyde, whereas a 100% control was achieved by the addition of spores of *Trichoderma harzianum* (strain TV12b) along with sterilized oat bran as 0.5% supplement to soil. Combination of treatments did not increase the inhibition than did either treatments alone. Different *Trichoderma* species behaved differently in fumigated or unfumigated soil. Soil fumigation with furfuraldehyde enhanced growth of other fungal inhabitants simultaneously reducing *R. lignosus* growth.

Key words: biocontrol, mycelial cords, *Rigidoporus lignosus*, *Hevea brasiliensis*, rubber, furfuraldehyde.

INTRODUCTION

Among the root diseases of *Hevea brasiliensis* (Willd. ex Adr. & Juss) Mull. Arg., (rubber tree), white root disease is the most destructive in most of the rubber growing countries. In some rubber growing areas such as West Africa, the white root rot of *H. brasiliensis* is responsible for yield loses up to 50% in the old plantations (Nandris *et al.*, 1987). During the early 1970s, the severity of the white

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root disease in Sri Lanka increased alarmingly (Liyanage *et al.*, 1977) causing unproductive bare patches in mature plantations and death of young plants in immature plantations. *Rigidoporus* survives in infected roots (inocula) and logs of diseased trees. New infections occur through root contact with a pathogen-affected root of a nearby tree or with a decaying *Rigidoporus* inoculum in soil. In some incidence, mycelial aggregates (cords) of *Rigidoporus* may also cause infection through soil (Bancroft, 1912).

Most of the chemicals in the triazole group significantly reduced the mycelial growth of *R. lignosus* in culture (Gohet *et al.*, 1991). However, the activity *in vitro* was not correlated to their activities in soil probably due to many factors such as temperature, pH, texture or chemical nature of soil, rainfall, systematic properties and stability. From their results cyproconazole and triadimenole were recommended for application at every 6 months to manage white root disease in *Hevea*. In Sri Lanka, 2% Pentachlorophenol in bituminous base appeared to have been successful in effective and economical control of white root disease (Jayasinghe *et al.*, 1995).

In field conditions, *R. lignosus* also grows through soil as mycelial aggregates (cords) from diseased roots of standing trees or major woody crop residues in soil and infects nearby trees. Therefore, one way to control the disesase is to prevent or slowdown the spread of mycelial cords in soil. This might be achieved by making the soil inhibitory to the fungus by physical, chemical or biological manipulation.

Inocula of pathogens surviving in woody roots of their hosts are difficult to eradicate by using antagonistic organisms alone. Instead it is often necessary to weaken the pathogen in its host residues so as to enable antagonists to invade these residues and replace the pathogen (Ohr *et al.*, 1973). Sulphur amendments to soils in rubber plantations is one of the principal traditional means of alleviating damage by *R. lignosus* (Peries, 1965). For other crops, sublethal dosages of fumigants (Strashnow *et al.*, 1985) or sublethal heating or drying (Munnecke *et al.*, 1976) bring the same effect in weakening pathogen inoculum and enabling antagonists such as *Trichoderma* spp. to invade the host residues. However, many of the recently used fumigants are highly toxic to workers and also have damaging environmental consequences. The use of furfuraldehyde as a possible fumigant on controlling *Sclerotium rolfsii*, was described recently (Cannullo *et al.*, 1992), renewing interest in this compound as a fumigant. As furfuraldehyde is cheap and relatively non-toxic (QO Chemicals Inc., 1989), most of the preliminary studies in this paper were focused on it's potential for use in control of *R. lignosus in vitro* conditions.

MATERIALS AND METHODS

Fungal cultures

The *R. lignosus* (isolate RT1) used throughout the study was obtained from infested rubber roots collected from Ratnapura, a rubber growing area in the wet zone of Sri Lanka. The fungus was stored in 1 cm^3 elm wood blocks that had been autoclaved. *Trichoderma harzianum* (strain TV12b) was obtained from the collection of ICMB, University of Edinburgh, UK. *Trichoderma longibrachiatum* (isolate DZE10) and *Trichoderma* sp. (isolate KGRAO) were isolated from soils collected in Sri Lanka by plating on a modification of *Trichoderma* selective medium (TSM) described by Papavizas (1982). *Trichoderma* isolates were identified using the morphological and reproductive characters (Rifai, 1969). Furfuraldehyde (Sigma Co., USA) was used at a known dilution for soil fumigation, and in pure form where applied elswhere.

Effect of furfuraldehyde on the growth of *R. lignosus* in culture media

Furfuraldehyde was mixed with molten malt extract agar (MEA) at 40 °C at 0.1%, 0.2%, 0.3%, (v/v) and 15 ml aliquots were transferred into 9 cm petriplates. After setting, plates with furfuraldehyde supplemented MEA were centrally inoculated with *R. lignosus* inocula (5 mm plugs) obtained from growing cultures and incubated at 30 °C [optimum growth temperature for *R. lignosus* -(Liyanage *et al.*, 1977)] in darkness. The linear growth of *R. lignosus* was recorded periodically for up to 9 days.

Vapor effect of furfuraldehyde on *R. lignosus*

The soil tube method used to assess the mycelial cord growth was similar to that used by Fox (1963) to demonstrate the effect of food base on the growth of *Rigidoporus* mycelial strands (cords) through sterile and non-sterile soil. In present study, *R. lignosus* was grown on basal plugs of 10ml of MEA in large boiling tubes (28 x 2 cm) for 7 days. The tubes were then placed horizontally and 0, 0.2, or 0.4 ml furfuraldehyde in non-absorbent cotton-wool plugs were placed at the center of horizontal tubes. Then the tubes were closed air-tight with rubber bungs and kept at 23 ± 1.5 °C for 1, 2 or 3 hours. Treatments were replicated 6 times, including a

control which was not exposed to the chemical. After the exposure, the cotton plugs were removed along with excessive furfuraldehyde on the glass surface. Soil (clay loam, pH 6.4) collected from a wheat field at Edinburgh, UK, passed through a 2 mm mesh and maintained at 20% (w/w) was inserted to the tubes by firmly pressing to get the soils equally compacted throughout the tube. Then the tubes were sealed with tin foil and masking tape, and incubated horizontally at 23 ± 15 °C. The growth of mycelial cords that developed from the basal fungal colony through soils were recorded periodically.

Effect of fumigation and adding oat bran to soil on *R. lignosus* mycelial cords

R. lignosus (isolate RT1) was grown for 7 days on basal plugs of 10ml MEA in large boiling tubes as described earlier. Soil, (pH 6.4) was collected from a British wheat farm, sieved through a 2 mm mesh and treated as follows:

(1) Control - no treatment. (2) Supplemented with 0.5% sterilized oat bran thoroughly mixed into the soil. (3) Mixed with furfuraldehyde (0.46 g kg^{-1} soil) and kept for 7 days in air-tight glass containers at 23 ± 1.5 °C. (4) As in (3) but then supplemented with 0.5% sterilized oat bran before use.

Where furfuraldehyde was used, an aqueous solution in which the concentration was known was sprayed at the above rate using a hand sprayer to allow an equal distribution through soil and kept in-air-tight glass containers for 7 days at 23 ± 1.5 °C. Furfuraldehyde was then allowed to evaporate from soil for 2 hours before use. Moisture level of all the soils was maintained at 20% (w/w). After treatments 1-4 soil was inserted into the tubes and incubated in darkness at 27°C. The growth of mycelial cords was recorded in 4-14 days.

Proliferation of other fungi in soil was assessed after 7 days by examining the tubes with a binocular stereo-microscope. Other fungal hyphae that crossed a 5 mm length of the eye - piece graticule were recorded from ten random points on a spiral way along the tubes (*R. lignosus* mycelia were easily distinguished by their pattern of growth).

The individual and combined effect of fumigation and *Trichoderma* in soil on *R. lignosus*

Soils were fumigated with furfuraldehyde at 0.4 g kg^{-1} soil and were kept as described earlier. Then furfuraldehyde was allowed to evaporate from soils before use and soils were inserted to boiling tubes which contain *R. lignosus* (isolate RT1) on a basal plug of MEA. The following treatments were employed.

(1) Control soil (no supplement). (2) Soil with 0.5% sterile oat bran (3) Soil with 0.5% sterile oat bran and 10^6 conidia of *T. harzianum* (isolate ED2) g^{-1} soil. (4) Soil with furfuraldhyde at 0.4g kg^{-1} soil. (5) Soil with furfuraldehyde 0.4g kg^{-1} and supplemented with 0.5% sterile oat-bran. (6) As in (5) but with 10^6 conidia of *T. harzianum* (isolate ED2) g^{-1} soil..

Tubes were incubated and growth of *R. lignosus* mycelial cords was assessed as earlier. The density of *R. lignosus* mycelial cords along the tubes was also assessed after 30 days, by counting the number of cords that crossed a piece of cotton thread (0.8 mm thickness) wrapped around the tubes at 5 cm and 10 cm away from the agar surface (food base). Only mycelial cords thicker than the thread were counted.

Comparison of the effect of *Trichoderma* isolates on *R. lignosus* mycelial cord growth

Clay loam soil (pH 6.4) collected from a British Wheat farm was adjusted to 18% (w/w) saturation with sterilised distilled water. The *Trichoderma* isolates used in this experiment were *T. harzianum* (strain TV 12b), *T. longibrachiatum* (isolate DZE10) and *Trichoderma* sp. (isolate KGRAO). All were added to soil at 4 x 10^3 conidia g^{-1} soil with 0.5% sterile oat bran on the day of the establishment of the experiment. *R. lignosus* inoculum was supplied as 2 cm^3 elm wood inoculum blocks artificially colonised for 6 weeks. The following treatments were used.

(1) Control (no soil supplement). (2) Soil with 0.5% sterile oat bran. (3) Soil treated with furfuraldehyde (0.4 g kg^{-1} soil). (4) As in (3) but then supplemented with 0.5% oat bran. (5) Soil supplemented with o.5% sterile oat bran and 4 x 10^3 conidia of *T. harzianum* (strain TV 12b) g^{-1} soil. (6) As in (5) but *T. longibrachiatum* (isolate DZE10) was used. (7) As in (5) but *Trichoderma* sp. (isolate KGRAO) was used. (8) As in (4) but supplemented with 4 x 10^3 conidia of *T. harzianum* (strain TV 12b) g^{-1} soil. (9) As in (4) but supplemented with 4 x 10^3 of *T. longibrachiatum* (isolate DZE10) g^{-1} soil. (10) As in (4) but supplemented with 4x 10^3 conidia of *Trichoderma* sp. (isolate KGRAO).

R. lignosus (isolate RT1) inoculum was placed in the boiling tubes and the treated soils were then compacted and incubated as earlier. Mycelial cord growth measurements were recorded after 4 days.

RESULTS

Effect of furfuraldehyde on *R. lignosus* in culture media

Results of the Table 1 show a relationship between the concentration of furfuraldehyde in MEA and mycelial growth rate of *R. lignosus in vitro*. An increase of 0.1% fold of furfuraldehyde in MEA caused significant ($\alpha = 0.05$) reduction of *R. lignosus* mycelial growth rate on MEA. Furfuraldehyde 0.1% in agar caused over 50% reduction of the growth of *R. lignosus* but more than 0.2% furfuraldehyde in agar was necessary to cause complete inhibition of the growth.

Table 1. *Linear growth rate of* R. lignosus *(means of 6 replicates) on malt extract agar supplemented with furfuraldehyde.*

Furfuraldehyde concentration in MEA (%)	0.0	0.1	0.2	0.3
Linear growth rate cmday^{-1}	0.7^a	0.28^b	0.12^c	0^d
% Inhibition	0	60	83	100

Values with same letters are not significantly different by Duncon's multiple range test.

Vapor effect of furfuraldehyde on *R. lignosus* on MEA

As shown in Table 2, *R. lignosus* was seemed to be affected in all treatments by the exposure to furfuraldehyde. Mycelial cord growth rate lowered when the exposure time increased. The highest inhibition was achieved in either treatments where the fungus was exposed to the chemical for 3 hours, irrespective of the volume of furfuraldehyde used for the exposure.

The individual and combined effect of soil fumigation and oat bran in soil

As shown in Table 3, the addition of bran alone had no marked effect on growth of mycelial cords. Soil fumigation or addition of bran with *Trichoderma* markedly reduced the mycelial cord growth through soil, though the degree of inhibition was only 13-17%. Counts on other unknown fungal hyphae (measured in 5 mm transects) showed that the addition of bran alone increased the number of unknown species of soil inhabitant fungi proliferated in soil. These fungi were

isolated from soil and found that they were slightly antagonistic to *R. lignosus* by producing difusible antibiotics. However, identification of thoes fungi was difficult as they did not produce reproductive organs on agar. Soil fumigation with or without bran addition caused marked increase in the growth of unknown fungal species. It thus seems that soil fumigation with furfuraldehyde enhances the growth of soil fungi simultaneously reducing the ability of *R. lignosus* to grow through soil. The density of *R. lignosus* mycelial cords was higher in most cases at 12 cm away from the agar surface.

Table 2. *Inhibitory effect of furfuraldehyde (furfural) vapor on* R. lignosus *assessed by subsequent growth of mycelial cords through soil.*

Volume ml/ exposed time to furfural vapor	Control	0.2ml/ 1h	0.4ml/ 1h	0.2ml/ 2h	0.4ml/ 2h	0.2ml/ 3h	0.4ml/ 3h
Mycelial cords growth rate cm/day*	0.77[a]	0.58[b]	0.64[b]	0.41[c]	0.42[c]	0.15[d]	0.15[d]
% Inhibition*	-	23.9	16.8	46.70	45.40	80.50	80.50

* Means of 6 replicate tubes. a-d: values with same letters are not significantly different by Duncan's Multiple Range Test.

Efficacy of *Trichoderma* isolates in soil on the suppression of *R. lignosus* mycelial cord growth

After 3 days, it was noted that *Trichoderma* proliferated abundently in soil. In the control soils, very thin mycelial cords of *R. lignosus* were formed, whereas in soils with 0.5% bran, relatively thick mycelial cords were formed. It was also noted that unknown species of soil inhabitant fungi have heavily colonised the bran particles (Table 4, treatment 2). In fumigated soils, no other soil fungi proliferated while *R. lignosus* mycelial cords were formed after 20 days, their growth was slightly less (Table 4, treatment 3). However, in fumigated and bran added soils, other soil fungi sparsely colonized on bran particles but less than where bran was added alone (Table 4, treatment 4). *T. harzianum* (strain TV12b) sporulated on bran particles but in fumigated soils, sporulation was comparatively abundant. This suggests that fumigation

Table 3. *The individual and combined effect of soil fumigation, oat bran or* Trichoderma *in soil on* R. lignosus *mycelial cord growth in soil; soil saturation was 20% w/w (pH 6.4)*

Treatments	mycelial cord growth cm day^{-1}*	Growth as control %	Other fungal hyphae in 5mm transects**	Mycelial cords***	
				5 cm level	10 cm level
1. Control (no supplement)	0.81±0.02	100	0.38±0.07	6.0±1.0	9.1±0.83
2. Soil+0.5% Sterile oat bran	0.78±0.02	96.3	1.32±0.21	4.8±0.66	12.5±2.0
3. Soil+0.5% Sterile oat bran+ *T. harzianum* (isolate ED2) 10^6 conidia g^{-1} soil	0.67±0.03	82.7	4.05±0.56	8.8±0.79	9.8±1.35
4. Soil+Furfuraldehyde (0.4gkg^{-1})	0.64±0.02	79.0	6.98±0.73	7.2±1.1	7.7±0.9
5. Soil+ Furfuraldehyde (0.4gkg^{-1})+ sterile oat bran	0.68±0.02	83.9	6.7±0.73	7.0±0.64	5.7±0.89
6. As in (5) but with *T. harzianum* (isolate ED2) 10^6 conidia g^{-1} soil	0.66±0.02	81.4	6.45±0.68	6.2±0.66	10.8±2.2

* All data are means of 9 replicates ± SEM. **Measured microscopically through the glass of the tube. ***Assessed at 5 cm and 10 cm levels from the surface of the agar food base of *R. lignosus*.

Table 4. *Mean growth of* R. lignosus *mycelial cords through soil, when soil was fumigated and added with 0.5% oat bran and Trichoderma spp. *: soil saturation was maintained at 18% w/w (pH 6.4)*

Treatments	Mean growth cm day^{-1}	% Inhibition
1. Control (no supplement)	0.56 ± 0.04	-
2. Sterile oat bran	0.56 ± 0.09	-
3. Furfuraldehyde	0.31 ± 0.08	44.6
4. (3)+sterile oat bran	0.28 ± 0.08	50.0
5. Sterile oat bran+ *T. harzianum* (strain TV12b)	0	100.0
6. Sterile oat bran+ *T. longibrachiatum* (isolate DZE10)**	0.41 ± 0.1	26.7
7. Sterile oat bran+ *Trichoderma* sp. (isolate KGRAO)**	0.07 ± 0.05	87.5
8. Furfuraldehyde + *T. harzianum* (strain TV12b)	0.03 ± 0.02	94.6
9. Furfuraldehyde + sterile oat bran+ *T. longibrachiatum* (isolate DZE10)**.	0.16 ± 0.07	71.4
10. Furfuraldehyde + sterile oat bran+*Trichoderma* sp.(isolate KGRAO)**	0.27 ± 0.08	51.8

* All data are means of 9 replicate tubes ± SEM. **Isolated from Sri Lankan soils by placing on *Trichoderma* selective medium. Where applied *Trichoderma* were added at $4x10^3$ propagules g^{-1} soil. In all cases oat bran was added to soil as 0.5% supplement and furfuraldehyde was applied to soil at 0.4 g kg^{-1} soil.

of soil may have enhanced the activities of *Trichoderma* in soil possibly by inhibiting other competitive fungi. *T. longibrachiatum* (isolate DZE 10) and *Trichoderma* sp. (isolate KGRAO) proliferated less than did *T. harzianum* (strain TV12b) on bran particles. Their sporulation was not observed in fumigated soils (treatment 8). As shown in Table 4, in all the treatments involved *Trichoderma* or furfuraldehyde, *R. lignosus* mycelial cord growth was suppressed in some degree. The results on the mycelial cord growth in treated soil showed that fumigation caused reduction of mycelial cord growth. Addition of *Trichoderma* with bran also lowered the cord growth but *T. longibrachiatum* had only a slight effect compared with that caused by *T. harzianum* (strain TV12b) or *Trichoderma* sp. (isolate KGRAO).

However, the ranking order of the effectiveness of *Trichoderma* isolates in fumigated soil was different from that of in non-fumigated soil. *T. longibrachiatum* (isolate DZE10) was more effective in fumigated soil whereas *Trichoderma* sp. (isolate KGRAO) was less effective. *T. longibrachiatum* (isolate DZE10) completely stoped *R. lignosus* on agar by volatile inhibition whereas *Trichoderma* sp. (isolate KGRAO) showed less but significant effect (Jayasuriya, 1994).

DISCUSSION

Furfuraldehyde was reported to alter the status of micro-organisms in soil consequently controling of *Sclerotium rolfsii* (Cannullo *et al.*, 1992). The results of the present study showed that furfuraldehyde has a lethal or sub-lethal effect on *R. lignosus* in culture media and in soil. A concentration of 0.1% in agar was adequate for 50% inhibition of *R. lignosus*. The lethal effect of furfuraldehyde was confirmed by exposing *R. lignosus* on culture media to furfuraldehyde vapor where the growth of *R. lignosus* was reduced by 80% in 3 hour exposure to the chemical. However, the effect of furfuraldehyde in field conditions needs 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 with fungicides of triazole group by adding them to soils and assessing their persistence in soil vapor phase and effect on *R. lignosus* growth (Lim *et al.*, 1990). Tridemorph was most persistent, maintaining 60-70% inhibition at 100μg 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 antagonise *R. lignosus* in the residual inoculum.

Fumigation of soil often enhanced the activities of resident soil fungi presumably by making organic matters available to them. This was evident especially in 20% w/w soil moisture level. Fumigation caused reduction of *R. lignosus* mycelial cord growth from agar or woody inoculum base. However, similar inhibition was also achieved by adding 0.5% bran with *Trichoderma* propagules and the combination of these treatments gave no further control than did either treatments alone.

Evidently there is a complex of factors that affect control of mycelial cord growth. But one crucial factor might be the strain of *Trichoderma* that is applied to soil or that becomes dominant in fumigated or bran-added soil. This was demonstrated in the results of Table 4 where some *Trichoderma* strains were compared. Some of them were more effective than others under different conditions in controlling *R. lignosus* mycelial cords. One of the most effective strains (*T. longibrachiatum* -isolate DZE10) had been shown to inhibit *R. lignosus* completely by producing volatile inhibitory metabolites (Jayasuriya, 1994). However, in present study, *T. longibrachiatum* (isolate DZE10) did not perform well in soil as did by *T. harzianum* (strain TV12b) in soil where *R. lignosus* cord growth was completely inhibited when supplemented to soil. But in studies of Jayasuriya (1994), *T. harzianum* (strain TV12b) stimulated the growth of *R. lignosus* by producing volatile metabolites. Scarselletti & Faull (1994) recently suggested a strong relationship between antifungal activity of *T. harzianum* strain and its production of 6-penlyl-α pyrone, an antibiotic that is a major component of the coconut aroma of *Trichoderma* (Collins & Halim, 1972). In further studies it would be desirable to test such a relationship for antagonistic activities of *Trihchoderma* strains to *R. lignosus*, both *in vitro* and in studies involved soil.

CONCLUSIONS

The focus of work in this investigation was to explore methods that might be as cheaper 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.

From the bank of the results obtained from the experiments indicated that furfuraldehyde has a potential to control *R. lignosus in vitro*. Results of the field tests also indicated that by drenching furfuraldehyde into soil, *R. lignosus* inocula burried in soil were either weakened or killed after one month time (unpublished data). Therefore, the performences of this chemical should be further tested in critical trials.

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