ANTIFUNGAL ACTIVITY OF PYROLYTIC OILS OF TAR FROM RUBBERWOOD (*HEVEA BRASILIENSIS*) PYROLYSIS

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HALIMAHTON MANSOR & RASADAH MAT ALI. 1992. Antifungal activity of pyrolytic oils of tar from rubberwood (*Hevea brasiliensis*) pyrolysis. The effect of three pyrolytic oil samples from rubberwood tar on three fungi species, *viz. Gloeophyllum trabeum* (brown rot), *Coriolus versicolor* (white rot) and *Bostryodiplodia theobromea* (blue stain) was studied with the agar dilution technique using malt extract agar media (MEA). With the three pyrolytic oil samples tested, the lowest concentrations causing a 50% reduction in fungal growth (ED_{50}) were 640.77 $\mu g\ ml^{-1}$ for *G.tabeum*, 736.88 $\mu g\ ml^{-1}$ for *C.versicolor* and 661.30 $\mu g\ ml^{-1}$ for *B.theobromea*. Although no correlation could be deduced between the antifungal activity and the phenolic components in the pyrolytic oils, the study showed that the tar oils from rubberwood pyrolysis have antifungal properties which made the tar possible to be used as a wood preservative or a fungicide.

Key words: Rubberwood - pyrolytic oils - antifungal - phenols - brown rot - white rot - blue stain

Introduction

Wood tar has wide applications such as wood preservatives, varnishes and adhesives (Halimahton 1990). The heavy oils of wood tars are generally used as preservatives, disinfectants and stain, while the light oils are good solvents. Creosote or oil prepared from coal tar distillation has long been used as wood preservative. Inoue *et al.* (1987) showed that sapwood stakes of *Cryptomeria japonica* and *Fagus crenata* treated with creosote oil with or without heavy oil, or with coal tar were generally sound after 28 years. Recent work by Doi *et al.* (1990) revealed the preservative effectiveness of distillates (b.p. 240-360°*C* and 280-320°*C*) from coal tar on wood rotting fungi, *Tyromyces palustris, Coriolus versicolor* and *Serpula lacrymans*. Extensive work has also been carried out on the preservative properties of pine tar, one of which involves the chemical modification of the tar by incorporation of toxic elements; results indicate that pine tar, incorporating 2.2% copper and 3.5% zinc, can be used for the development of wood preservatives (Rathor & Tewari 1983).

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As part of a study on the by-products of rubberwood pyrolysis, this preliminary investigation was conducted to test the activity of three pyrolytic oil fractions from rubberwood tar against three wood-rotting fungi, namely, *Coriolus versicolor* (white rot fungus), *Gloeophyllum trabeum* (brown rot fungus) and *Bostryodiplodia theobromea* (blue stain fungus). These three fungi were chosen since they are the most common wood-rotting fungi and their cultures were already available for testing.

Materials and methods

Rubberwood tar (100 *g*) collected from a pyrolysis retort was fractionally distilled at atmospheric pressure. Fractions distilled at 170 to 200°*C* and 200 to 235°*C* were analysed by gas chromatography (GC) and tested for their antifungal activity.

The individual phenolic components in the distilled fractions of rubberwood tar were identified by comparison with standard phenols. The analysis was conducted using a Shimadzu gas chromatograph model GC-9A equipped with a flame ionisation detector (FID) and a CR-3A Chromatopac data processor. A 250 × 0.24 *mm* PEG 20*M* glass capillary column was used. The analysis was run at 60°*C* for 30 *min*, then programmed from 60 to 175°*C* at 5°*C* min^{-1}, with N_2 as a carrier gas at a flow rate of 0.6 *ml* min^{-1}.

Determination of antifungal activity

A 2% solution (2 *l*) of malt extract agar (MEA) was autoclaved and cooled to approximately 50°*C*. The pyrolytic oils in 95% ethanol were freshly prepared and aseptically added to the cooled agar. Preliminary trials indicated that ethanol concentration of more than 1% in the media retarded fungal growth. Levels below 1% of ethanol were therefore maintained in all the test media. The tubes were vigorously shaken to obtain a uniform mixture and aliquots (25 *ml*) were poured into a series of sterile glass petri dishes (9 *cm* diameter). Each dish was inoculated at the centre with a 4 *mm*-disc cut from the vegetative growing margin of 4 to 14-day-old cultures of the fungi *C. versicolor*, *G. trabeum* and *B. theobromea* maintained on MEA. The fungi, inoculated on 2% MEA amended with 95% ethanol, served as controls. Testing was carried out in triplicates for each concentration of the tested samples. The dishes were incubated at 27 ± 2°*C* for six days. The colony diameters taken as the mean of two diameters at right angles to each other were measured at 24-*h* intervals (Figures 1, 2 & 3).

Results and discussion

Three pyrolytic oil fractions used in the study were collected from the distillation of the rubberwood tar. The heavy oil A distilled at 200 to 235°*C* while the less viscous oil B distilled at 170 to 200°*C* and both were from the same pyrolysis run. The oil C was another heavy oil but from rubberwood tar obtained from a different pyrolysis run.

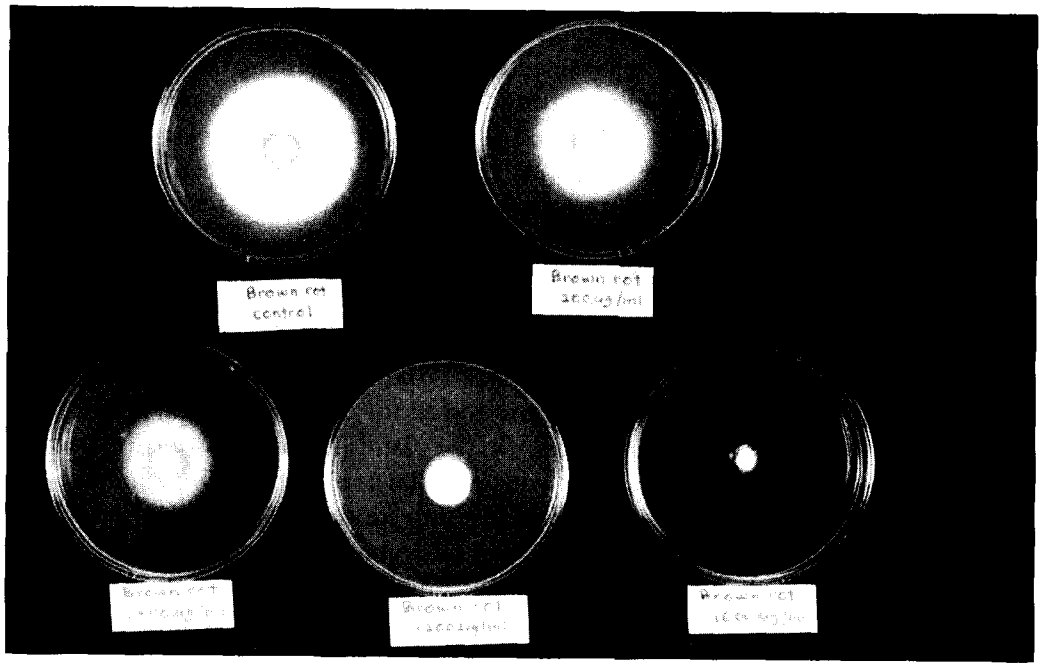


Figure 1. Effect of pyrolytic oil A from rubberwood tar on *G. trabeum* (Brown rot fungus) (Top left to right: Control, 200 *mg ml*$^{-1}$; Bottom left to right: 800, 1200, 1600 *mg ml*$^{-1}$)

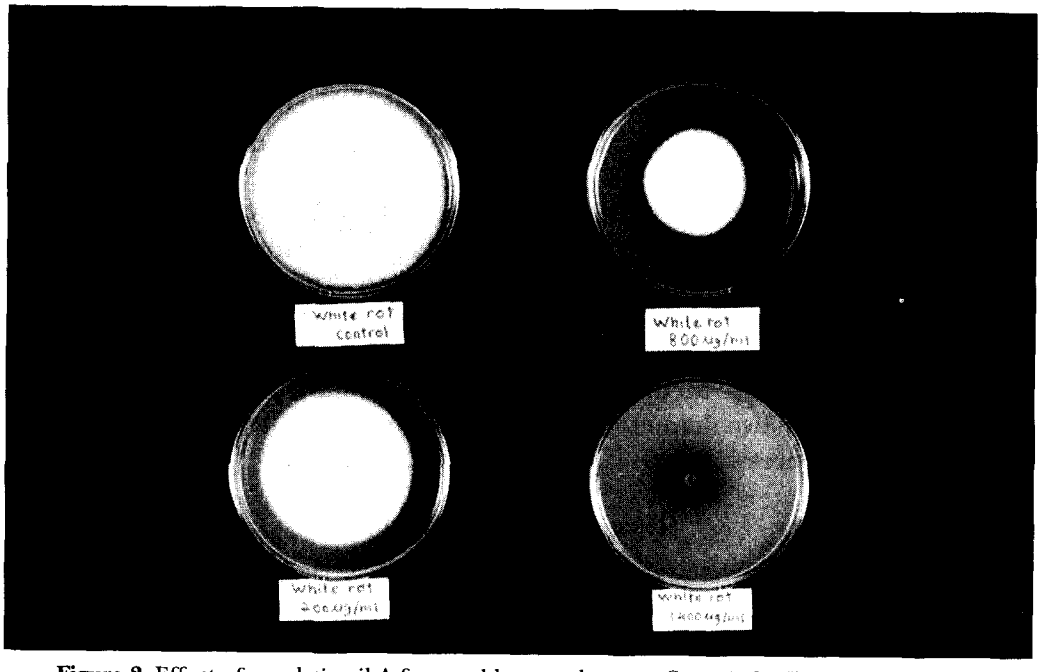


Figure 2. Effect of pyrolytic oil A from rubberwood tar on *C. versicolor* (White rot fungus) (Top left to right: Control, 800μg *ml*$^{-1}$; Bottom left to right: 200, 1400 μg *ml*$^{-1}$)

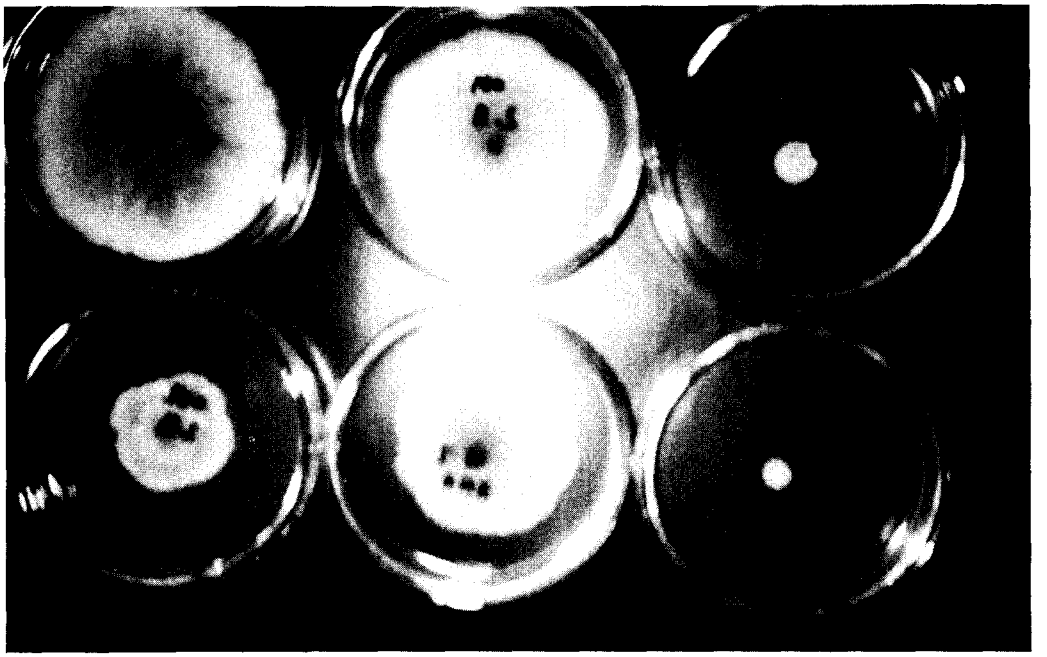


Figure 3. Effect of pyrolytic oil A from rubberwood tar on *B. theobromea* (Blue stain fungus) (Top left to right: control, 100 $\mu g\ ml^{-1}$, 700 $\mu g\ ml^{-1}$; Bottom left to right: 500 $\mu g\ ml^{-1}$, 300 $\mu g\ ml^{-1}$, 800 $\mu g\ ml^{-1}$)

The qualitative and quantitative compositions of phenols in the pyrolytic oils in this study are summarised in Table 1. Analysis of the overall chemical composition of the rubberwood tar oil has been reported (Halimahton *et al.* 1990). In this work, detailed analysis was restricted to phenolic contents which were expected to be primarily responsible for any antimicrobial activity. The results in Table 1 show that guaiacol and catechol were overall the main components in the pyrolytic oils. A significant difference in the distribution of these two phenols in the three pyrolytic oils was observed: catechol was present in a very much higher amount (43.59%) than guaiacol (5.99%) in C but *vice versa* in B in which guaiacol was present in higher amount (34.64%) than catechol (8.47%) whilst the difference in percentage of the two phenols was not as large in pyrolytic oil A.

The effects of increasing concentration of the pyrolytic oils on fungal growth are shown in Tables 2, 3 and 4. Overall, pyrolytic oil A exhibited a relatively significant inhibition of fungal growth and the smallest diameter of hyphal growth at the highest concentration, while B and C showed a more gradual change in their antifungal effect. Figures 1 to 3 show the effects of pyrolytic oil A on the three fungi.

The percentage inhibition of growth compared to maximum growth on control plates was calculated. A probit-log concentration analysis (Finney 1971) was carried out to determine the ED_{50} values, which is the concentration causing a 50% reduction in growth measured in $\mu g\ ml^{-1}$ (Luken 1971), and the slope of the probit-log concentration regression line of each fungus and compound (Figure 1). The

Table 1. Phenolic components of pyrolytic oils from rubberwood tar (% based on total phenol fraction)

Pyrolytic oils	guaicol	2,6- xylenol	cresol	o-cresol/ phenol	p-cresol	m-cresol	2,3-xylenol	3,5-xylenol	3,4-xylenol	syringol	catechol
	RT(27.40)	RT(28.60)	RT(29.60)	RT(30.80)	RT(32.40)	RT(32.50)	RT(33.80)	RT(34.40)	RT(35.50)	RT(36.70)	RT(37.90)
A	26.53	1.07	13.76	5.70	10.57	tr	9.12	5.11	1.34	13.24	13.57
B	34.64	4.00	12.09	5.97	9.73	tr	5.97	6.69	1.03	11.41	8.47
C	5.99	0.31	9.19	10.17	10.17	2.40	5.28	4.60	0.89	15.59	43.59

tr-trace, <0.31%
A - 200-235°C; distilled from tar collected at pyrolysis run with intrinsic temperature of 700°C
B - 170-200°C; distilled from tar collected at pyrolysis run with intrinsic temperature of 700°C
C - 200-235°C; distilled from tar collected at pyrolysis run with intrinsic temperature of 500°C

Table 2. The effect of pyrolytic oils on *Bostryodiplodia theobromea*

Concentration ($\mu g\ ml^{-1}$)	Diameter of hyphal growth (*cm*)*		
	A	B	C
200	6.1833 ± 0.08	6.1000 ± 0.00	5.9333 ± 0.06
400	5.8500 ± 0.05	5.5833 ± 0.06	5.6833 ± 0.08
600	4.5000 ± 0.00	5.0833 ± 0.07	5.3667 ± 0.03
800	3.3000 ± 0.00	4.2000 ± 0.05	4.9000 ± 0.00
1000	2.7500 ± 0.05	4.0833 ± 0.06	4.7000 ± 0.00
1200	1.8833 ± 0.02	4.0000 ± 0.00	4.1833 ± 0.03
1400	1.1000 ± 0.00	3.5333 ± 0.03	3.4500 ± 0.05
1600	0.9000 ± 0.00	2.9667 ± 0.03	3.3500 ± 0.08
Control	7.0000 ± 0.00	7.0000 ± 0.01	7.0000 ± 0.01

* Values are Mean ± SEM (standard error of the mean) of inhibition, each analysed in triplicate; p>0.001 are statistically different from the respective control group

Table 3. The effect of pyrolytic oils on *Gloeophyllum trabeum.*

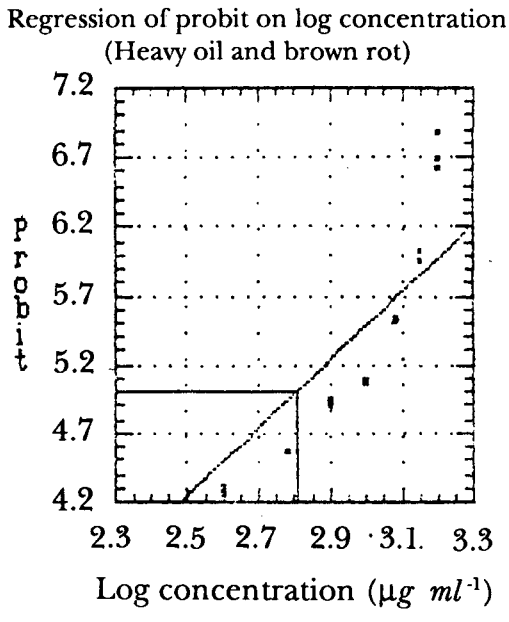
Concentration ($\mu g\ ml^{-1}$)	Diameter of hyphal growth (*cm*)*		
	A	B	C
200	5.5000 ± 0.00	7.0000 ± 0.00	6.0000 ± 0.00
400	5.4333 ± 0.03	6.4833 ± 0.03	5.9333 ± 0.03
600	4.8000 ± 0.00	6.0000 ± 0.00	5.7833 ± 0.03
800	3.9000 ± 0.05	6.0000 ± 0.00	5.7000 ± 0.00
1000	3.5000 ± 0.00	5.3333 ± 0.03	5.3333 ± 0.05
1200	2.3767 ± 0.03	5.0000 ± 0.00	5.0833 ± 0.03
1400	1.4333 ± 0.06	4.5500 ± 0.05	5.0000 ± 0.00
1600	0.6833 ± 0.07	4.1333 ± 0.07	4.2000 ± 0.00
Control	7.0000 ± 0.01	7.0000 ± 0.00	7.0000 ± 0.00

* Values are Mean ± SEM of inhibition, each analysed in triplicate; p>0.001 are statistically different from the respective control group

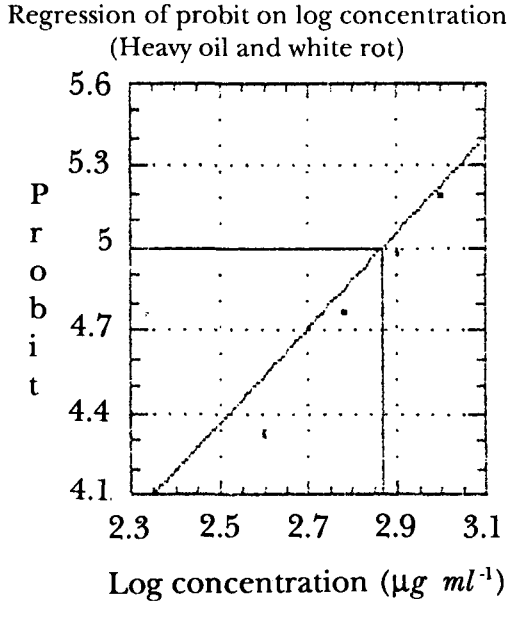
Table 4. Effect of pyrolytic oils on *Coriolus versicolor*

Concentration (μg)	Diameter of hyphal growth (*cm*)*		
	A	B	C
200	6.4000 ± 0.05	7.1000 ± 0.08	6.0000 ± 0.00
400	6.1000 ± 0.00	7.0000 ± 0.00	5.6500 ± 0.02
600	4.9000 ± 0.00	6.1833 ± 0.08	5.3833 ± 0.03
800	4.2500 ± 0.00	5.1333 ± 0.03	4.8000 ± 0.00
1000	3.6300 ± 0.00	5.0000 ± 0.00	4.3000 ± 0.00
1200	2.5000 ± 0.00	4.1667 ± 0.03	4.1000 ± 0.00
1400	-	2.9500 ± 0.05	3.8000 ± 0.00
1600	-	2.7500 ± 0.05	3.3000 ± 0.00
Control	7.0000 ± 0.00	7.0000 ± 0.01	7.0000 ± 0.01

- No growth could be measured; *Values are Mean ± SEM of inhibition, each analysed in triplicate; p>0.0001 are statistically different from the respective control group



(1)



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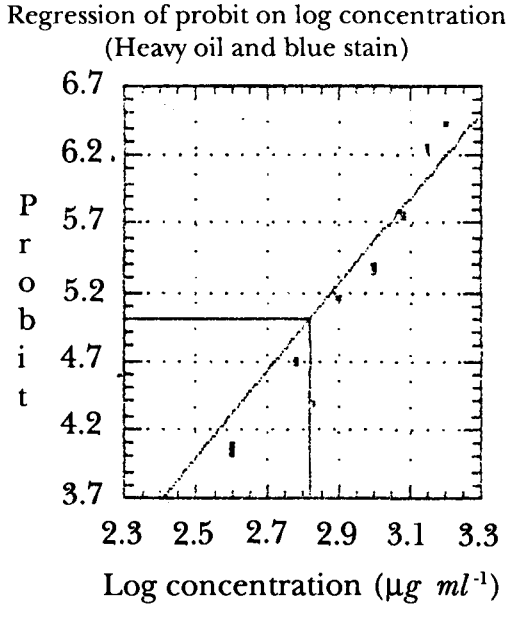

(3)

Figure 4. Probit-log concentration analysis for pyrolytic oil A; (1) - *G. trabeum*, (2) - *C. versicolor*, (3) - *B. theobromea*

slope is a characteristic of the toxicant as it measures potency with changing concentration of toxicant. Slope may describe, in part, the mechanism of action for the toxicant (Horsfall & Dimond 1957). Table 5 gives the values of ED_{50} obtained from the probit-log concentration analysis for each fungus with the corresponding oil sample tested. Results show that the three fungi were most affected by pyrolytic oil A as indicated by the ED_{50}values ranging from 641to 737 $\mu g\ ml^{-1}$. For B and C the ED_{50}values were higher for all the three fungi, varying from 1107 to 1944 $\mu g\ ml^{-1}$ and 1587 to 5620 $\mu g\ ml^{-1}$ respectively. From these ED_{50} values, it can be concluded that pyrolytic oil A was the most effective against the three fungi while pyrolytic oil C was the least effective. The relative antifungal activity of the three oils cannot be easily correlated with any single compound given in Table 1. However, it is a well observed phenomenon that antifungal activity may not only be due to individual constituents but often to the total oil composition. This is in line with the study by Walchli (1983) on the effectiveness of coal tar oils on several fungal species which showed that the content of acid phenols is no more important for the antifungal effect of creosote than the other fungicidal components present.

Table 5. Effective dose at 50% inhibition (ED_{50}) ($\mu g\ ml^{-1}$) for pyrolytic oils on different fungi

Pyrolytic oils	White rot	Brown rot	Blue stain
A	736.88	640.77	661.30
B	1107.39	1944.46	1237.94
C	1731.41	5619.53	1587.45

White rot fungus - *Coriolus versicolor* ; Brown rot fungus - *Gloeophyllum trabeum* ;
Blue stain fungus - *Bostryodiplodia theobromea*

Conclusion

The results obtained in this study show that the effect of pyrolytic oils from rubberwood tar against wood-rotting fungi depends on the chemical composition of the oils. It can be concluded that whatever the active compound(s), the tar oil from rubberwood pyrolysis offers a potential use as wood preservative or a fungicide. Studies on possible toxicity of the oils should not be overlooked.

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