

## Ecofriendly heartwood extracts from different tree species as a safe natural fungicide against *Phytophthora*.

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### ABSTRACT

Attempts were made to evolve natural fungicides for the control of diseases of rubber by using water extracts of the heartwood of five tree species which are reported to contain phenolic compounds. The extracts were screened in the laboratory for inhibition of *Phytophthora* spp., which causes a variety of diseases in rubber.

Heartwood extracts of *Enterolobium saman* showed the highest antifungal activity. Rosewood extract caused fungicidal activity against the sporangia. Anjily wood extracts showed antifungal activity against airborne fungi. The importance of using ecofriendly fungicides is discussed.

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### Introduction

*Phytophthora* species is the single major pathogen which causes a large number diseases of the rubber tree *Hevea brasiliensis* (Willd. ex A. de Juss.) Muell. Arg. No part of the rubber tree is unaffected by the fungus. *Phytophthora* leaf fall is the most serious leaf disease causing the loss of crop in susceptible clones. Pod-rot and fruit-rot infection can do much havoc in seed collection areas tremendously reducing the number of healthy seeds. At present, leaf disease in susceptible clones, shoot-rot in nurseries and immature plantations and pod-rot in seed collection areas are prevented by routine prophylactic spraying of copper fungicides<sup>1</sup>. Bark-rot or black stripe also result in crop loss on clones in which tapping is continued during the rainy season. Stem patch canker can be very damaging in nurseries, budwood nurseries and immature plantations. At present, these diseases are all treated using Dithane M-45<sup>2</sup>.

Of late, it has become universally accepted that development cannot subsist upon a deteriorating environmental resource base and hence alternative and safe pest management strategies, ideally based on renewable resources that eliminate the use of toxic chemicals, is the need of the day. Attempts at developing suitable methods for prophylaxis of *Phytophthora* diseases based on renewable resources by exploitation of the biochemical defence mechanisms of certain tree species have been initiated. These attempts have been made with a view to the evolution of suitable disease management methods which are most important for sustainable agricultural development and a cleaner environment. This paper describes work done to tap the potential of the extractives of wood waste of a few heart-wood species for their fungicidal properties against *Phytophthora*, the major fungal pathogen of the rubber tree.

It is a common observation that the heartwood of tree species is generally immune to biodeterioration. The dark colour of the heartwood is due to the presence of phenolic compounds. Polyphenols are the most common extractives and are present in all heartwoods. The nonstructural components of the wood tissues of plants are commonly called extractives or "extraneous components". The largest amounts are found in the cell lumens but they may also be present in the cell walls.

It was decided to test the antifungal property of the heart-wood extractives of locally available common tree species against *Phytophthora* in order that they be tried for prophylaxis or therapy of the different *Phytophthora* diseases of the rubber tree. These extractives would be expected to be fundamentally safe biofungicides especially suitable in the present age of a desire for a cleaner environment.

## Materials and methods

### Experiment 1

Water extracts of waste from the heartwood of five tree species, (Teak wood, Jack wood, Rosewood, Anjily wood, Enterolobium wood and Mahogany wood) were prepared by cooking 200g in 1 litre water after which solid media with 2% agar were prepared. The media were poured into petridishes (10 replicates), inoculated with culture discs of 7- day-old PDA culture of *Phytophthora meadii* McRae. and incubated at  $20 \pm 2^\circ\text{C}$ . Rubberwood extract medium prepared as above and a rubber leaf extract medium similarly inoculated served as controls. Growth measurements were recorded daily. Measurements recorded on the 3rd day were compared for difference.

### Experiment 2

Extracts of the five species along with Rubberwood as control were kept at the rate of 20ml per plate as broth and 5ml each of sporangial suspension of *Phytophthora* was added. The dishes were incubated at  $20 \pm 2^\circ\text{C}$ . After 24 hours, the suspensions were mixed with 3 drops of cotton blue lactophenol and 6 slides each were prepared and examined under the microscope for a sporangial count and sporangia germinated or dead. The percentage of dead sporangia was then calculated and the differences in the percentage of dead sporangia was analysed.

### Experiment 3

Solid agar media, as in the first experiment, were each plated into petridishes and exposed uniformly to the air for 1 hour before being incubated at  $20 \pm 2^\circ\text{C}$  along with Rubberwood and leaf extract media as controls. After one week, the developed fungal colonies were counted and the colony diameter measured. The mean number of colonies per plate and the mean diameter of colonies in the case each media were calculated and analysed.

## Results and discussion

The growth rate of *Phytophthora meadii* in the different wood waste extract agar media showed highly significant differences compared to the control thereby proving the antifungal property of the wood extracts of the heartwood species tested. Enterolobium showed the highest antifungal property closely followed by Anjily wood and Rose wood, Jack wood and Teak wood (Table 1).

Death of sporangia differed significantly among the treatments compared to the control Rubberwood extract whereby it is proved that the different wood extracts possess antifungal property against *Phytophthora meadii*. Among the five wood extracts tested, Rose wood extract showed maximum fungicidal property closely followed by Jack wood extract, Anjily wood extract and Teak wood extract. Mahogany extract showed the least antifungal property (Table 2).



Table 1 *Growth of Phytophthora culture on different wood extract media.*

Treatment	Mean diameter in cms
Rubber leaf extract agar	5.65
Rubberwood extract agar	7.00
Enterlobium ( <i>Enterlobium saman</i> ) wood extract agar	0.70
Teak ( <i>Tectona grandis</i> ) wood extract agar	1.69
Anjily ( <i>Artocarpus hirsuta</i> ) wood extract agar	0.78
Jackwood ( <i>Artocarpus integrifolia</i> ) extract agar	1.28
Rosewood ( <i>Dalbergia latifolia</i> ) extract agar	0.88
C.D. (P = 0.05)	0.17

Table 2 *Sporangial death in different wood extract broths.*

Treatment	Percentage
Rubberwood extract broth	5.91 (14.10)
Mahogany extract broth	61.52 (51.79)
Enterlobium ( <i>Enterlobium saman</i> ) wood extract broth	19.35 (23.51)
Teak ( <i>Tectona grandis</i> ) wood extract broth	74.94 (62.65)
Anjily ( <i>Artocarpus hirsuta</i> ) wood extract broth	85.33 (68.16)
Jackwood ( <i>Artocarpus integrifolia</i> ) extract broth	83.39 (68.39)
Rosewood ( <i>Dalbergia latifolia</i> ) extract broth	93.02 (77.59)
C.D. (P = 0.05)	12.37

The different wood extracts also exhibited antifungal properties against other common air-borne fungi. This was evidenced by a highly significant difference in the number of fungal colonies that developed in the wood extract agar media prepared from the heartwood species compared to the Rubberwood and leaf media controls. Maximum antifungal efficiency was exhibited by Anjily wood followed by Jack wood. Enterlobium wood showed the least antifungal property (Tables 3 and 4).

## Discussion

This is the first instance of work on the antifungal efficacy of the extractives of the heartwood of tree species against *Phytophthora*, the cause of a number of major diseases of *Hevea brasiliensis*. Although this is an *in vitro* study, it has a high potential which will be a boon in the field of disease management subject only to the success of *in vivo* studies. These products are low input, renewable, indigenous, natural and will totally eliminate the use of chemical fungicides thereby contributing to a cleaner environment. This may therefore pave the way for an economically viable and ecologically sound disease management technology against *Phytophthora* diseases. In the case of shoot-rot disease in nurseries and immature plantations, and even for mature trees of susceptible clones, the use of wood extracts with suitable spreader-sticker either in a water base or even an oil base can be considered. The most desirable use of such extracts is for the treatment of trunk infections such as bark-rot and patch canker where non-toxic natural pesticides could totally eliminate the use of toxic chemical fungicides.

**Table 3**      *Airborne fungal colonies developed in different media prepared using wood-waste extracts.*

Treatment	Number of colonies developed
Rubber leaf extract agar	4.55 (20.80)
Rubberwood extract agar	4.71 (22.60)
Enterlobium ( <i>Enterlobium saman</i> ) wood extract agar	4.17 (17.80)
Teak ( <i>Tectona grandis</i> ) wood extract agar	4.16 (17.40)
Anjily ( <i>Artocarpus hirsuta</i> ) wood extract agar	1.77 (3.20)
Jackwood ( <i>Artocarpus integrifolia</i> ) extract agar	3.67 (13.60)
Rosewood ( <i>Dalbergia latifolia</i> ) extract agar	3.97 (15.80)
C.D. (P = 0.05)	0.60

**Table 4**      *Growth of airborne fungal colonies*

Treatment	Colony diameter in cms
Rubber leaf extract agar	18.00
Rubberwood extract agar	19.57
Enterlobium ( <i>Enterlobium saman</i> ) wood extract agar	6.48
Teak ( <i>Tectona grandis</i> ) wood extract agar	7.95
Anjily ( <i>Artocarpus hirsuta</i> ) wood extract agar	2.22
Jackwood ( <i>Artocarpus integrifolia</i> ) extract agar	5.51
Rosewood ( <i>Dalbergia latifolia</i> ) extract agar	4.47
C.D. (P = 0.05)	2.23

### References

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