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ANTIFUNGAL ACTIVITIES OF ESSENTIAL OILS AGAINST *RIGIDOPORUS LIGNOSUS*, CAUSATIVE AGENT OF WHITE ROOT-ROT DISEASE OF THE RUBBER TREE IN CAMEROON

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Abstract:-

Investigations were conducted to evaluate the antifungal activities of the essential oils of Eucalyptus saligna, Cymbopogon citratus and Cupressus lusitanica collected from the locality of Dschang, West region of Cameroon against Rigidoporus lignosus (clone GT1) causative agent of root rubber trees rot. The agar well diffusion method was used to evaluate fungal growth inhibition at various concentrations. The obtained results showed that essential oils of of Eucalyptus saligna, Cymbopogon citratus and Cupressus lusitanica found to be inhibitory to Rigidoporus lignosus. The antifungal activity of these essential oils were varied significantly depending on plant species. Eucalyptus saligna and Cupressus lusitanica essential oils completely suppressed de growth of Rigidoporus lignosus at 5000 ppm with fungicide effect for Eucalyptus saligna. However Cymbopogon citratus essential oil has been more active than the other two and also showed a fungicide effect at 500 ppm. From these results, it can be concluded that the essential oil from the leaves of C. citratus may be rich in antifungal compounds which possess considerable antifungal properties. It is a good alternative to harmful chemical pesticides and can be effectively used to control growth of Rigidoporus lignosus.

Keywords: - *Rigidoporus lignosus, Antifungal activity, Essential oils, White root-rot disease*

INTRODUCTION

Hevea brasiliensis (Willd. ex Adr. De Juss) Muell. Arg.) is principally valued for its latex content, latex or or Natural Rubber which is very significant in world's industrialization. This importance has been expressly emphasized in the production of elastomers, the use of which is indispensable in space, water, and ship technologies (Jacob, 2006).

The dependence of world industrialization on natural rubber production is further underscored especially now considering the diminishing reserves of petroleum with increasing environmental hazards (Irogue, 2012). Currently, rubber is the main provider of natural rubber in the world. The tyre industry consumes 65 percent of global production of natural rubber (Deon, 2012). On average, 10 million tonnes of natural rubber are produced each year worldwide. Cameroon with 55,000 tons of natural rubber production contributed 0.5% in the share of world production in 2012 (IRSG, 2012). The rubber trees are subject to a plethora of economically important pathological problems, mainly of fungal origin (Igeleke, 1998). Fungi belonging to the group of basidiomycetes cause rotting root and collar on trees of tropical forests including rubber (Mohammed *et al*., 2014). In field plantations, root diseases pose a serious problem especially in the first few years after planting.

From planting to exploitation period of latex (5-6 years) and during wet weather, the young rubber trees are exposed to a risk of invasion by telluric fungal pathogens (Kaewchai *et al*.. 2009). Fungal infections especially those which attack the root system of rubber can cause a high reduction of latex productivity and lead to the death of trees (Wilhelm, 1973; Nandris *et al*., 1987a; Evueh and Ogbebor, 2008). The infective fungal organism of the white root rot disease is *Rigidoporus lignosus* (klotzsch) Imazeki.

In the wet forests of Cameroon *R. lignosus* is responsible for the loss of mature *Hevea* tree (over 40%) (Rapport IRAD, 2007). Similarly, in Cote d´lvoire, *R. lignosus* is the main cause of tree losses with 40-60% of the trees destroyed over a period of up to 21 years (Nandris *et al*. 1987). In Nigeria, the most serious disease of rubber tree is the white root rot. It accounts for about 94% of incidences of all root diseases and kills up to five *Hevea* trees/ha (Otoide, 1978). Over a period of time, half of the rubber trees in a plantation are lost to the disease.

To fight this disease, several control methods are recommended, and the main concern, is to protect the rubber against pathogens invasions (preventive) by farming and agricultural methods and to reduce the disease incidence (curative struggle) by using chemical method. Among these methods, systemic fungicides belonging of "Triazole" group like propiconazole, hexaconazole (Lam and Chiu, 1993) triadimenol, pentachloronitrobenzene (PCNB), triadimefon (Ng and Yap, 1990), pentachlorophenol (PCP) (Jayasinghe *et al*., 1995) and phenol are effective against *Rigidoporus microporus*. However these fungicides are the most expensive available on the market (Jayasinghe, 2010), and inaccessible to the village plantation owners and not providing satisfactory economic returns. In this regard one of such alternatives is the use of natural plant preservatives such as essential oils, to control *Rigidoporus microporus*, since plant products have for many generations been used by small scale farmers in parts of Africa for their antifungal activity. The present study was undertaken to evaluate the antifungal effect of essential oils of *Eucalyptus saligna*, *Cymbopogon citratus* and *Cupressus lusitanica* against *Rigidoporus lignosus*.

MATERIELS AND METHODS

Pathogen

Isolates of *Rigidoporus lignosus* (clone GT1) were collected from infected rubber trees in village plantations of Nko'olong locality, South Region of Cameroon. Isolation and purification were done in Phytopathology Laboratory of IRAD Ekona.

Plant material and extraction procedure

Fresh leaves of *Eucalyptus saligna*, *Cymbopogon citratus* and *Cupressus lusitanica* were collected in june 2016 from the locality of Dschang, West region of Cameroon**.** Their identification was confirmed through consultation in the Herbarium of the Department of Plant Biology. The essential oils tested were extracted by water steam distillation using a Clevenger apparatus from the leaves of plant collected. The distilled essential oils were dried over a anhydrous sodium sulphate and stored in a refrigerator at 4°C (Tatsadjieu *et al*., 2007).

Effect of essential oils on fungal growth

Antifungal assay was performed using the agar disc diffusion method (De Billerbeck *et al*., 2001). Potato dextrose agar (PDA) medium with different concentrations of essential oils (50 ; 75 ; 100 ; 500 ; 1000 ; 1500 ; 2000 ; 3000 and 5000 ppm) based on the effectiveness of each oil, were prepared by adding the appropriate quantity of essential oil to the melted medium. About 20 ml of the medium was poured into glass Petri-dishes (9 cm × 1.5 cm). Each Petri-dish was inoculated at the center with a mycelia disc (6 mm diameter) taken at the periphery of a fungal strain colony grown on PDA for 48 h. The Penncozeb (53 ppm) has been used as a positive control while the Petri dishes without essential oil as a negative control. Plates were incubated at 30°C and the colony diameter was recorded each day. For each concentration, three tests were carried out. The growth inhibition percentage (IP) was calculated as follows (ShengYang *et al*., 2005):

$$IP = 1 - \frac{Go}{Gc} \times 100$$

Go = diameter of growth zone in the test plate

Gc = diameter of growth zone in the control plate.

Nature of mycelia growth inhibition
Fungicide effect of essential oils was determined while transferring disks coming from Petridish where the mycelia growth inhibition by the essential oil was total during incubation period, in a PDA medium without essential oil. The effect was fungistatic if there was resumption of growth and fungicide in the contrary case.

Statistical analysis
Data from three independent replicate trials for growth percentage inhibition were subjected to statistical analysis using Statistica .06, Statistical package (Statsoft, 1995). Differences between means were tested using Duncan Multiple Range Test.

RESULTS AND DISCUSSION
In the present investigations *R. lignosus* was isolated from infected rubber trees in village plantations of Nko'olong locality, South Region of Cameroon. Different concentrations of essential oil of *Eucalyptus saligna*, *Cymbopogon citratus* and *Cupressus lusitanica* were tested for their antifungal potential against *Rigidoporus lignosus*
Table 1 and 2 illustrates the effect of the different essential oils on growth percentage inhibition of *R. lignosus*. Overall, all essential oils have inhibited growth of *R. lignosus* at various degrees. It clear from the results that there were significant differences in the mycelia growth of oil supplemented samples compared to the control which was not supplemented with essential oil (ANOVA and Duncan Multiple Range Test, $P < 0.05$). Growth inhibition was significantly ($P < 0.05$) influenced by the incubation time and essential oil concentration. Mycelia growth was considerably reduced with increasing concentration of essential oil while their growth increased with incubation time.
Table 1 presents the antifungal effect of *Cymbopogon citratus* on growth of *R. lignosus*. According to this table, *Cymbopogon citratus* essential oil has completely inhibited the pathogen at 500 ppm as the positive control at 53 ppm. The concentraion 500 ppm exhibited marked inhibition as compared to 75 ppm and 100 ppm concentrations against *R. lignosus*. As far as concerning essential oils of *Eucalyptus saligna* and *Cupressus lusitanica* (presented in table 2), completely inhibition of *R. lignosus* was obtained at 5000 ppm. We also noted that the inhibition percentages obtained with doses 2 000, 1500 and 1000 ppm are not significantly different.
Table 3 shows the results of the nature of the inhibition of essential oils of *C. citratus, C. lusitanica* and *E. saligna*. The transfer of mycelial discs where growth inhibition was complete by *C. citratus, C. lusitanica* and *E. saligna* into PDA medium without essential oil, showed a variation on their effect. *Cymbopogon citratus* essential oil which completely inhibited the mycelial growth at 500 ppm, showed a fungicide effect against *R. lignosus* because it has no resumption of fungal growth. Similarly essential oil of *Eucalyptus saligna* which completely inhibited the mycelial growth at 5000 ppm, showed a fungicide effect against *R. lignosus*. However, essential oil of *Cupressus lusitanica* has completely inhibited the growth of *R. lignosus* at 5000 ppm with fungistatic effect.

Discussion
The effectiveness of the essential oils varies according to the plant species. The results revealed that these essential oils caused significant inhibition in the mycelia growth of *R. lignosus*. Many researchs have been conducted for antifungal activities of these essential oils. They depend on their chemical composition and on the presence of certain compounds which are known for their antifungal activities (Periago *et al.,* 2002; Viollon & Chaumont, 1994).
The essential oil of *C. citratus* was found to be most effective against *R. lignosus*. The inhibitory effect of essential oil of *C. citratus* against the *R. lignosus* may be due to the presence of Citral and others phenolic compound. The GC analysis identified citral as major component with a percentage of 76%. Citral as main components of *C. citratus* an oxygenated terpenoid, which has been identified as a compound exhibiting antifungal properties (Paranagama *et al*., 2003). This monoterpene has proved effective in controlling mycelial growth and conidial germination of *C. gloeosporioides* (Palhano *et al*., 2004). Similarly it is reported that the anti-fungal activity of lemon grass oil may be due to the presence of its aldehyde containing the active constituent citral (Gupta *et al*.,2011). Paranagama *et al*, (2003) reported that *C. citratus is* of West Indian origin and yields an essential oil with high content of citral (>70%).
About the essential oil of *E. saligna*, its composition depends on the phenologic stage. In the vegetative phase, the major constituents were *p*-cymene (54.2%) and *γ*-terpinene (43.8%), while during the blossoming *α*-pinene became the major constituent followed by *p*-cymene (22.5%) (Patricia *et al*., 2007). Concerning *Cupressus lucitanica* essential oil, monoterpenes and sesquiterpenes were shown as major constituent (Florisvaldo *et al*., 2011). All of these components of *E. saligna* and *Cupressus lucitanica* essential oils presented antifungal activities less marked than *C. citratus*.
The activity of the oils would be expected to relate to the respective composition of the plant volatile oils, the structural configuration of the constituent components of the volatile oils and their functional groups and possible synergistic interactions between components (Hong Zeng *et al*., 2015; Li and Yu, 2015).

CONCLUSION
This study indicated that essential oils of *Eucalyptus saligna*, *Cymbopogon citratus* and *Cupressus lusitanica* may possess antifungal activity and can be exploited as an ideal treatment for eliminating *Rigidoporus lignosus* spread. However *C. citratus* essential oil has been more active than the other two and also showed a fungicide effect with respect to this fungus. It would be very interesting to test the *in vivo* effect of these oils on the development of *R. lignosus* on the rubber trees roots.

Table 1: Inhibition percentages of *Rigidoporus microporus* by essential oil of *Cymbopogon citratus*

Concentration	Mycelial growth Inhibition percentage
	Cymbopogon citratus
0 ppm (C-)	0.00 ± 0.00c
50 ppm	2.71 ± 8.64c
75 ppm	5.00 ± 3.71c
100 ppm	12.71 ± 9,44b
500 ppm	100.00 ± 0.00a
1 000 ppm	100.00 ± 0.00a
53 ppm (C+)	100.00 ± 0.00a

Means assigned with the same letter in the same column are not significantly different according to the Duncan test at P ≤ 0, 05. C- = Negative control; C+ =Penncozeb.

Table 2: Inhibition percentages of *Rigidoporus microporus* by essential oils of *Cupressus lusitanica* and *Eucalyptus saligna*

Concentration	Mycelial growth Inhibition percentage	
	Cupressus lusitanica	*Eucalyptus saligna*
0 ppm (C-)	0.0 ± 0.00f	0.00 ± 0.00e
100 ppm	15.42 ± 1.71e	23.33 ± 4.33b
500 ppm	39.17 ± 4.21d	28.33 ± 12.40d
1 000 ppm	50.21 ± 5.58c	53.33 ± 5.12bc
1 500 ppm	49.17 ± 6.09c	46.88 ± 11.81c
2 000 ppm	53.13 ± 3.29bc	51.88 ± 9.53bc
3 000 ppm	56.25 ± 8.56b	61.04 ± 4.21b
5 000 ppm	100.00 ± 0.00a	100.00 ± 0.00a
53 ppm (C+)	100.00 ± 0.00a	100.00 ± 0.00a

Means assigned with the same letter in the same column are not significantly different according to the Duncan test at P ≤ 0.05. C- = Negative control; C+ =Penncozeb.

Table 3: Nature of the toxicity of essential oils

Concentration	*Cymbopogon citratus*	*Cupressus lusitanica*	*Eucalyptus saligna*
500 ppm	F	/	/
1 000	F	/	/
1 500 ppm	F	/	/
2 000 ppm	F	/	/
5 000 ppm	F	f	F

F= fongicide; f= fungistatic; / = not evaluated

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