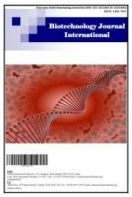

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In vitro Evaluation of the Fungicidal Potential of Aqueous and Methanolic Extracts of *Thevetia peruviana* on the Development of *Rigidoporus lignosus,* Causal Agent of White Root rot of *Hevea brasiliensis*

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Authors' contributions

This work was carried out in collaboration among all authors. Author ZA designed the study and identified the diseases. Authors SBM and MDA wrote the protocol and reviewed the first draft of the manuscript. Author SBM reviewed the experimental design, identified diseases, wrote first manuscript and performed the statistical analysis. Authors SBM, AH, GNN and JPND managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The objective of this work is to evaluate the effect of (aqueous (EAq) and methanolic (ME)) extracts of *Thevetia peruviana* on the *in vitro* development of *Rigidoporus lignosus*.
Study Design: A synthetic fungicide (Onazol 100), two extracts at differents concentrations: C1 = 3.5 mg / ml; C2 = 7 mg / ml; C3 = 15 mg / ml; C4 = 30 mg / ml; C5 = 50 mg / ml and C6 = 100 mg / ml for EAq; C1 = 3.5 ml/ml; C2 = 7 ml/ml; C3 = 15 ml/ml; C4 = 30 ml/ml; C5 = 50 ml/ml for ME and a negative control (T = 0 mg / ml) were used.
Place and Duration of Study: The study was conducted in pathology laboratory at University of Yaounde I and IRAD of Nkolbisson in 2018.
Methodology: Daily measurements of the pathogen's development was used to evaluate the average growth (D) of the mycelium. The inhibition percentages of the different doses of EAq, ME and ONAZOL 100 were calculated after 4 to 5 days of growth of the fungus.
Results: The major result obtained compared to the rate of rot showed that the treatments EAq, ME (C6) and ONAZOL 100 were closed. The disease rates at different concentrations of C1 to C6 were 13.91 to 100% for ME, 9.34 to 100% for EAq, and 100% for ONAZOL 100.
Conclusion: The two extracts are promising, and on small and medium scale, could be an effective and cheap formulation for the control of *Rigidoporus lignosus*.

Keywords: Biological control; Hevea brasiliensis; Rigidoporus lignosus; Thevetia peruviana; inhibition.

ABBREVIATION

IRAD : Institute of Agricultural Research for Development

1. INTRODUCTION

Rubber, a natural and synthetic compound, occupies a prominent place in the industry due to its remarkable properties. Although chemical advances combined with strong industrial demand have contributed to the growth of synthetic rubber, natural rubber remains irreplaceable due to its elasticity [1]. Of all rubber plants, only *Hevea brasiliensis* is industrially exploited for its better quality latex. The other plants produce a resin latex of low commercial value [2]. To this end, 99% of the world's natural rubber comes from *H. brasiliensis* plantations. For many countries in the humid tropics, rubber cultivation is one of the main economic resources. In addition, the very high rubber prices due to the development of the automotive industry have made rubber even more attractive. In Cameroon, however, the development of rubber cultivation remains the prerogative of large industrial companies, including the Cameroon Development Corporation (C.D.C.), the African Forest and Agricultural Society of Cameroon (SAFACAM) and Hevea-Cameroon (HEVECAM). This last company with a capital of 286,326 dollars, 10% of which is held by the Cameroonian State alone accounts for 45% of the national production, ranking it as Cameroon's third largest employer with 8,000 employees in 2016, including 5,000 direct and 3,000 indirect jobs [3]. Cameroon is the 15th largest producer of natural rubber in the world and the fourth largest in Africa, with 56,000 tonnes of production per year [3].

World production of natural rubber was 10.7 million tonnes in 2014 [3], for a market that is expected to reach 15.4 million tonnes in 2020 according to [4]. They also suggested that natural rubber will continue to play an important role in the industry because of its significant consumer preference over synthetic rubber, as manufacturers recognize that natural rubber has physical properties (elasticity and waterproofness) that are not possible with synthetic rubber. Despite the major advantages of this crop, it is threatened by several diseases and pests, including rubber root disease caused by soil fungi that attack the roots of many woody tree species. These fungi are: *Rigidoporus lignosus* (Kl.) Imaz. (formerly called Fomès lignosus) and *Phellinus noxius* (Corner) G.H. Cunn. (formerly called Fomès noxius). White root rot of rubber tree roots caused by *R. lignosus* is the most serious disease of rubber tree. This soil fungus, which grows on living stumps after felling forest trees, is transmitted from tree to tree through the lateral roots. It is present to varying degrees in all rubber-growing regions. It can cause more than 60% mortality of rubber plants in a plantation that is not or poorly treated for this disease [5]. *R. lignosus* is a root parasite of a large number of forest or cultivated woody species; it belongs to the basidiomycetes class

and to the polyporaceae family. To meet the higher future demand due to the estimated global rubber needs of 18 million tonnes in 2034, farmers need to use efficient technologies, ensuring the supply of rubber in a timely manner. Thus, to currently control the rubber fomès, several prophylactic and therapeutic methods are used, including: Integrated fomès control, which combines mechanical and chemical control.

Mechanical control consists in detecting sick or dead trees in a plantation. These trees will be stumped, removed from the plots and all lateral roots, diseased trees are isolated by digging trenches with a radius of 0.80 to 1 m around the plant, lateral roots cut to a width of 30 cm to 60 cm deep; isolated trees are treated in the same way as healthy trees. Chemical control consists of spreading chemical products at the collar and on the roots. In the rainy season, the granulated formulation is the most recommended to limit leaching. The liquid formulation should be used in dry weather and avoided in rainy weather. The operation will be repeated every six months for three years, alternating products. The latter method is certainly effective, but has both financial and environmental disadvantages.

In the search for more effective and, above all, non-polluting alternatives, biological control with the use of natural substances of plant origin would be of interest in protecting plants against diseases, thus ensuring that soil integrity is maintained with respect to agricultural pollution and human health. Thus, like most products with a biodegradable pesticide effect, yellow laurel seed extracts (*Thevetia peruviana*) seem to have a strong inhibitory potential against attacks by plant parasitic fungi. *Thevetia peruviana* seeds contain a variety of compounds including terpenes, glycosides and sterols, which are known to have antifungal property. As a result, previous work has shown the effectiveness of extracts from certain plants, in particular *T. peruviana*, against certain fungal diseases of cultivated plants [6,7,8,9]. It is in this context that this work was realized with the objective of evaluating the effectiveness of *T. peruviana* on the development of *R. lignosus*.

2. MATERIALS AND METHODS

2.1 Presentation of the Study Site

This work was carried out in the Biotechnology and Environment Laboratory, Phytopathology and Plant Protection Research Unit of the University of Yaounde I and in the Plant Pathology Laboratory of the institute of agricultural research for development (IRAD) at Nkolbisson.

2.2 Obtaining Aqueous and Methanol Extracts from *Thevetia peruviana*

The dried, shell-free yellow laurel seeds were crushed with a Victoria brand hand mill to produce a powder. For the aqueous extract, a 500 mg/ml concentration stock solution was then prepared by introducing 50 g of seed powder into 100 ml of distilled water for about 12 hours, then filtered using the muslin cloth; the extract was used directly because it could not be preserved. For methanol extract, 500 g of seed powder was weighed and macerated in 2 liters of methanol and incubated for 72 hours [10]. The solvent-solute mixture was then transferred to the sonicator to maximize extractionAfter filtration with filter paper, the solution was transferred to the rota-vapor, for the separation of the solvent from the extractable compounds. The latter were then stored in a refrigerator (4°C) for later use.

2.3 Extraction Yield of Extracts

The extraction yields of the yellow laurel extracts were calculated on the basis of the weights of the plant material according to the formula below:

$$Yield\ (\%) = \frac{Mass\ of\ extract\ (g)}{Mass\ of\ plant\ material\ (g)} x100$$

Cit. [6]

The mass of the extract corresponds to the mass of the liquid obtained after extraction; the mass of powder corresponds to the mass of the crushed seeds.

2.4 Chemical Screening of the Various Extracts of Yellow Laurel seeds

The classes of secondary metabolites present in aqueous extracts of *Thevetia peruviana* seeds were determined by an adaptation of standard procedures [11]. These techniques are based on turbidity, precipitation, and foaming of extracts in the presence of the different reagents characterizing each class of secondary metabolites. A small volume of aqueous extract of yellow laurel seed powder was used to qualitatively determine the presence of alkaloids, phenols, triterpenes, sterols, flavonoids,

saponins, anthocyanins, glycosides and tannins. As a specific example, ½ ml of extract was added to 1 ml of sulphuric acid (H_2SO_4) contained in test tubes, then homogenised manually before being boiled for 2 minutes. After boiling, five drops of Meyer reagent were added to each tube and the development of turbidity was considered an indication of the presence of alkaloids. Approximately 0.5 ml of each extract was mixed with 1 ml of methanol contained in the test tubes and heated in a water bath maintained at 55 °C for 15 minutesTo this mixture, three drops of a freshly prepared ferric cyanide solution were added. The appearance of a green precipitate indicated the presence of phenols.

2.5 Evaluation of the Effects of *Thevetia peruviana* Seed Extracts on *Rigidoporus lignosus* in the Laboratory

2.5.1 Isolation and purification of the pathogen strain

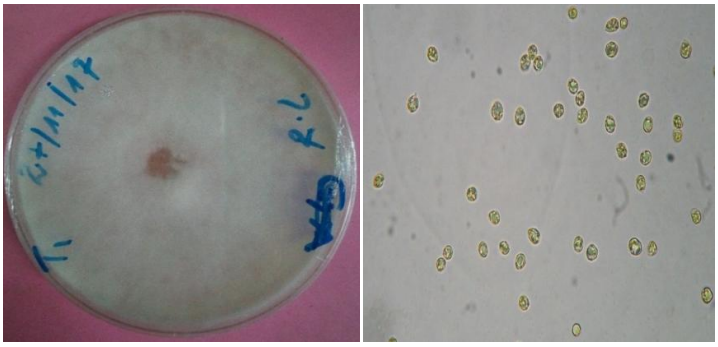
After identification in a rubber plantation where the disease is severe (HEVECAM), tree roots that showed symptoms of the disease, as well as carpophores present at the final stage of development of the disease, were harvested most preferably in areas where the disease is most advanced. These were brought back to the laboratory where they were washed several times with tap water. They were then disinfected with alcohol and rinsed with distilled water, and finally passed over a flame to be sterilized. Small fragments were collected and incubated in Petri dishes containing previously prepared culture media where the pathogen was to grow [12]. A few days after sowing (4 to 5 days), the fungus was fully grown; discs 0.6 cm in diameter were taken from the Petri dish and transplanted into new culture media for fungus growth. This process was performed several times in order to obtain pure strains. Microscopic observations of spores were carried out to confirm the identity of the isolated microorganism (Fig. 1).

2.5.2 Obtaining the different doses of aqueous extracts

Starting from the 500 mg/ml concentration stock solution previously developed and according to the formula (CiVi = CfVf) [13], six doses were prepared from the stock solution: 3.5; 7; 15; 30; 50 and 100 mg/ml by taking 0.28 ml; 0.56 ml; 1.2 ml; 2.4 ml; 4 ml and 6 ml respectively from the stock solution, adding 39.72; 39.44; 38.8; 37.6. 36 and 34 ml of culture medium for a final volume of 40 ml. This final volume was poured into 90 mm Petri dish, each containing 10 ml. For negative controls, a 10 ml solution of medium was dispensed directly into each Petri dish.

2.5.3 Obtaining the different doses of aqueous extracts

From the previously prepared stock solution, six doses "3.5; 7; 15; 15; 30; 50 and 100 ml/ml" of methanol extracts are prepared in the same way as the aqueous extract (EAq). To obtain the doses of the synthetic fungicide, the manufacturer's dosage (1L of product for 200L of water) was used. Indeed, a stock solution of ONAZOL 100 was previously prepared by mixing 1 ml of ONAZOL 100 with 200 ml of sterile distilled water. Using the formula CiVi = Cf.Vf., a volume of 0.2 ml was taken from the stock solution and mixed with 39.8 ml of culture medium for a final volume of 40 ml. This mixture was then poured into Petri dishes at a rate of 10 ml per dish.



A B

Fig. 1. Pure strain (A) and spores (B) of *Rigidoporus lignosus*

2.5.4 Determination of radial growth

The average growth (D) of the mycelium of *R. lignosus* was determined by daily measurements of the pathogen's development using a graduated ruler.

Each diameter was measured on one of the two straight lines forming a right angle through the center of the seeded explant (Fig. 2). The average radial growth was calculated using the following formula:

$$D = \frac{d1+d2}{2} - do \quad [14]$$

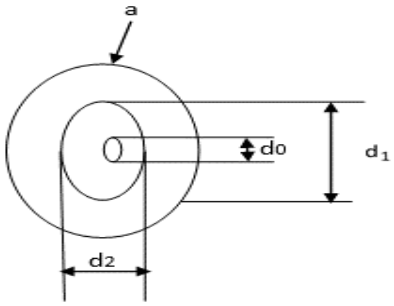


Fig. 2. Diagram showing the method of measuring the radial growth of the pathogen
d0 = explant diameter; d1 and d2 = perpendicular diameters of the pathogen; a = diameter of 90 mm Petri dish

2.5.5 Determination of effective doses and fungicidal status of the extracts tested

2.5.5.1 Percentage of inhibition of extracts

The inhibition percentages (I%) of the different doses of aqueous, methanolic extracts and ONAZOL 100 were calculated after 4 to 5 days of growth of the fungus This inhibition, expressed as a percentage (%), was determined using the following formula [14].

$$I(\%) = \frac{Dto\ (mm) - Dxi\ (mm)}{Dto\ (mm)} \times 100$$

Where:

I (%) = percentage inhibition;
Dt_0 = average diameter of the pathogen in the control;
Dxi = mean diameter of the pathogen in the extract or ONAZOL 100

2.5.5.2 Evaluation of the fungicidal or fungistatic activities of the extracts of yellow laurel leaf and ONAZOL 100 2.5.4.2.

The fungicidal or fungistatic activity of ONAZOL 100 was demonstrated by sowing the fungus in Petri dishes containing a mixture of culture medium and ONAZOL 100. After incubation, treatments in which the growth of the fungus was completely inhibited were identified and the explants were collected and transferred to a new culture medium without an extract. When growth resumed in this new medium, the extract was designated as fungistatic; otherwise it was designated as fungicidal [15,16].

2.5.5.3 Correlations between concentration and inhibition

The correlation between concentration and percentage inhibition was determined from the equation y = ax + b with x = concentration, and y = percentage inhibition. In this case, if a < 0, the slope is negative; if a > 0, the slope is positive; if r is between 0.8 and 1 then the correlation is perfect and positive; if r is between -0.8 and -1 then the correlation is perfect and negative; if r < 0.8, the correlation is positive but imperfect; if r > -0.8, the correlation is negative but imperfect; r is the correlation coefficient [17].

2.5.5.4 Determination of minimum inhibitory concentrations

The values of the different inhibition percentages obtained from the formula of [14], made it possible to determine the minimum inhibitory concentrations. From the linear regression equation between the neperian logarithms of abscissa concentrations and the percentage inhibition of ordinate growth (Ci) = f (lnCi), concentrations reducing pathogen growth by 50, 90 and 95% were determined [18].

2.6 Statistical Analyses

At the end of the experimental studies, the data collected in the laboratory were subjected to an analysis of variance (ANOVA), using software R version 3.4.3. The averages were separated using multiple Turkey tests at the 5% threshold. The results in the form of a histogram were produced using the Excel application of the Microsoft office 2016 software.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Extraction yield

The yield, color and appearance of the different extracts obtained depend on the extraction solvent used. Aqueous extraction had the highest yield of 23% and methanol had the lowest yield of 7.44%. The aqueous extract is liquid (low oil content) and whitish in color, while the methanol extract has a viscous and brownish appearance (Table 1).

3.1.2 Phytochemical screening

The screening revealed the presence of many chemical compounds belonging to different families such as essential oils, sterols, coumarins, phenols, sugars, tannins, alkaloids and saponins. However, extracts of *T. peruviana* were found to be richer in sterols and sugars in EAq, while tannins, terpenoid glycosides, terpenoids and phenols were absent or in trace form. Alkaloids, anthraquinones, saponins, coumarins and oils were present but in small quantities. Methanol extract was the poorest in the chemical family because it contains little compared to aqueous extract, which has several families and is therefore richer (Table 2).

3.1.3 Effect of the different extracts on the radial growth of *R. lignosus* strain

In general, the various extracts tested significantly inhibited the development of the pathogen. This inhibition was more pronounced with EM because a total reduction in mycelial growth was obtained with the C5 dose (50 ml/ml).

However, a total reduction was also achieved with aqueous extracts at the highest dose C6 (100 mg/ml).

3.1.3.1 Effect of methanol (EM) extract from seeds of T. peruviana on radial growth of the strain in vitro

The evolution of mycelial growth of *R. lignosus* strain under the control of EM was significantly reduced. The increase in concentration significantly inhibited mycelial growth until total inhibition at C5 concentration (50ml/ml) (Fig. 3).

Radial growth of the pathogen was significantly reduced by EM throughout the experiment (from 2 DAT to 5 DAT). Significant differences at the 5% threshold according to the Turkey test between the different concentrations and controls tested were observed each time between all treatments except for the positive control (T+),

Table 1. Extraction yield (%) and characteristics of the extract obtained

Extract	Solvent	Yield (%)	Color	Aspect
Aqueous (EAq)	Water	23	Whitish	Very viscous and not very oily
Methanol (EM)	Methanol	7,44	Brown	Low viscosity and very oily

Table 2. Appearance of natural products in extracts of *Thevetia Peruviana*

Family of compositae	Methanol	Aqueous
Essential oils	+	+
Saponified oils	-	+
Coumarins	+	+
Phenols	T	-
Flavonoids	-	-
Saponins	+	+
Gallic tannins	-	-
Catechic tannins	+	-
Anthraquinones	-	+
Terpenoids	T	-
Sterols	+	+++
Alkaloids	+	+
Sugars	T	+++
Triterpenoic glycosides	T	-

'-' absence, '+' presence, '+++' abundant presence.

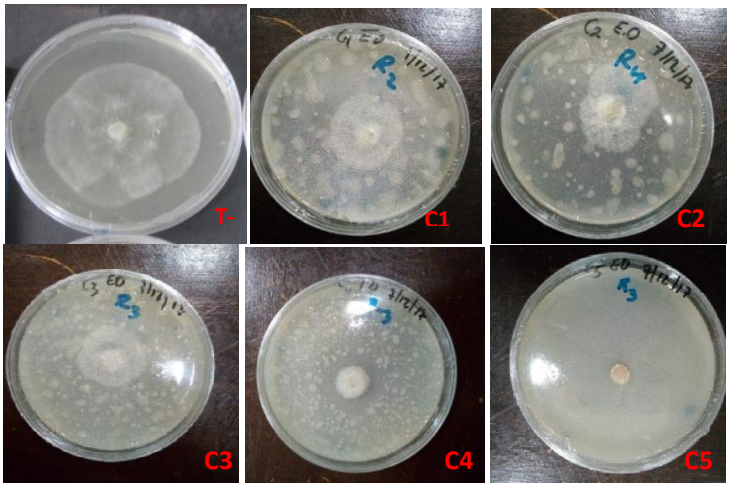


Fig. 3. Radial growth of the fungus under the effect of EM on the second day after treatment (2 DAT) with C1 = 3.5 ml/ml; C2 = 7 ml/ml; C3 = 15 ml/ml; C4 = 30 ml/ml; C5 = 50 ml/ml.

C5 (50 ml/ml) and C6 (100 ml/ml) doses where no significant differences were observed for all growing days (Fig. 4). For example, two days after incubation, a radial growth of 5.49 cm was observed for the C1 dose (3.5 ml/ml) and 1.71 cm for the C4 dose (30 ml/ml). Four days after incubation, this radial growth was 7.71 cm for the C1 dose (3.5 mg/ml) and 2.35 cm for the C4 dose (30 mg/ml).

3.1.3.2 Effect of aqueous extract (EAq) from seeds of T. peruviana on radial growth of the strain in vitro 3.1.3.2.

The aqueous extract also exhibited an increasing level of inhibition with increasing concentration until total inhibition of radial growth at the highest (C6) concentration (100 mg/ml) (Fig. 5).

As with EMs, the radial growth of the pathogen was significantly reduced by EAq throughout the experiment (from 2 DAT to 5 DAT). Significant differences at the 5% threshold according to the Turkey test between the different concentrations and controls tested were observed each time between all treatments except for the positive control (T+) and C6 doses (100 mg/ml) where no significant difference was observed for all growing days (Fig. 6). Two days after incubation, radial growth was observed at 5.83 cm for the C1 dose (3.5 mg/ml) and 2.29 cm for the C4 dose (30 mg/ml). Four days after incubation, this radial growth was 7.9 cm for the C1 dose (3.5 mg/ml) and 2.56 cm for the C4 dose (30 mg/ml).

3.1.4 Inhibition percentage of *T. peruviana* seed extracts on the radial growth of the strain *in vitro*

Overall, the results of this work show that the different extracts tested significantly reduced the radial growth of *R. lignosus* strain with a very high inhibition percentage (73.89 to 100%). Four days after incubation, the analyses revealed that for EAq, T+ and C6 concentration (100 mg/ml) are not significantly different; they all showed 100% inhibition. However, these two treatments are significantly different from the other treatments which showed an inhibition percentage of 9.34; 17.54; 35.49; 68.65 and 79.1% respectively for C1 (3.5 mg/ml); C2 (7 mg/ml); C3 (15 mg/ml); C4 (30 mg/ml) and C5 (50 mg/ml) concentrations. For EM, analyses show that there is no significant difference between T+ and C5 (50 ml/ml) and C6 (100 ml/ml) concentrations; their inhibition percentages were in the order of 100%; 94.36% and 100% respectively. However, there are significant differences between T+ and all other treatments that have shown inhibition percentages in the order of 13.91; 23.36; 42.75; and 73.89% for C1 (3.5 ml/ml); C2 (7 ml/ml); C3 (15 ml/ml); and C4 (30 ml/ml) doses, respectively. With regard to the analysis comparing the two extracts, it appears that the behavior of the two extracts is not significantly different for doses C1; C3; C4 and C6, which showed inhibition percentages in the order of : 9.34% and 13.91% respectively for EAq and EM at C1 dose: 35.89% and 42.75% respectively for EAq and EM at C3 dose; 68.65% and 73.89%

respectively for EAq and EM at C4 dose and 100% for both extracts at C6 dose (Fig.7).

- histograms with the same lowercase letters show that there is no significant difference between the doses of aqueous extracts at the 5% threshold according to the Turkey test; - histogram dengan huruf kecil yang sama menunjukkan bahwa tidak ada perbedaan yang signifikan antara dosis ekstrak air pada ambang batas 5% menurut uji Turki;

- histograms with the same capital letters show that there is no significant difference between the doses of methanol extracts at the 5% threshold according to the Turkey test; - histogram dengan huruf kapital yang sama menunjukkan bahwa tidak ada perbedaan yang signifikan antara dosis ekstrak metanol pada ambang batas 5% menurut uji Turki;

- the histograms with the same numbered letters show that there is no significant difference between the doses of aqueous and methanolic extracts at the 5% threshold according to the Turkey test. - histogram dengan huruf bernomor yang sama menunjukkan bahwa tidak ada perbedaan yang signifikan antara dosis ekstrak air dan metanol pada ambang batas 5% menurut uji Turki.

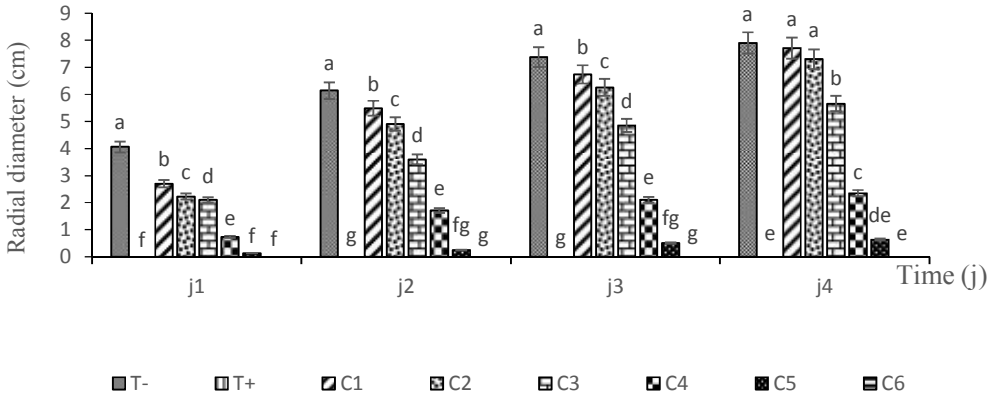


Fig. 4. Effects of EM on radial growth of the fungus as a function of time
With T- = 0 ml/ml C1= 3.5 ml / ml; C2= 7 ml /ml; C3= 15ml / ml; C4= 30 ml / ml; C5= 50 ml / ml; C6 = 100 ml / ml

**The values of the same day with different letters are significantly different at p < 0.05 (Turkey test)*

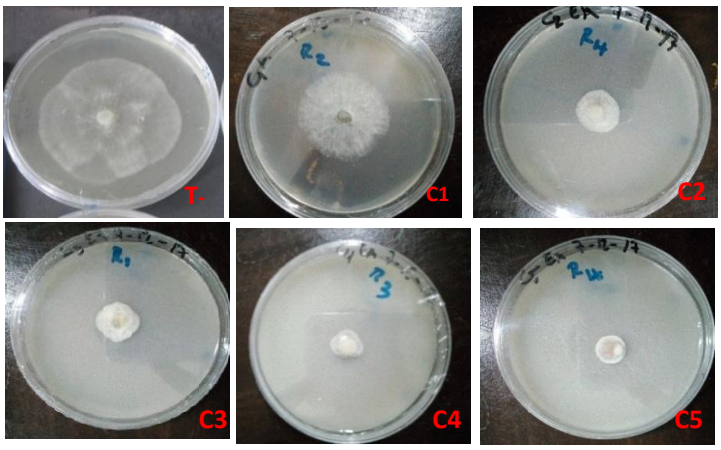


Fig. 5. Radial growth of the fungus under the effect of EAq on the second day after treatment (2 DAT)

3.1.5 Antifungal or fungistatic activity of extracts and synthetic fungicide (ONAZOL 100)

Following the evaluation of the extracts tested on the mycelial growth of the pathogen, the treatments that showed total inhibition were listed, which enabled us to evaluate the fungicidal and fungistatic activities of the different extracts tested. The lethal extracts tested were all found to be fungistatic. Only the synthetic fungicide showed fungicidal activity (Table 3).

3.1.6 Correlation between concentrations and inhibition percentages obtained with extracts

The purpose of this test was to see if there is a linear relationship between the decrease and increase in inhibition with the different concentrations of organic and aqueous extracts The regression lines obtained after analysis revealed similar behaviors of the strain with respect to extracts (organic and aqueous). It appears that all the lines obtained have positive slopes and linear correlation coefficients r > 0.8 (r = 0.96 for the EM and r = 0.98 for the EAq) This shows the existence of perfect and positive correlations between concentrations and different percentages of inhibitions (Fig. 8).

3.1.7 Minimum inhibitory concentration CMI50, CMI90 and CMI95

From the regression lines obtained after the correlation tests, the concentrations of the different extracts inhibiting by 50% (CMI50); 90% (CMI90) and 95% (CMI95) the growth of the strain were determined. Overall, the results show that the lowest inhibitory concentrations were obtained with methanol extracts. The values of CMI90 for example were about 4.08 ml/ml with EM and 4.30 mg/ml with EAq (Table 5).

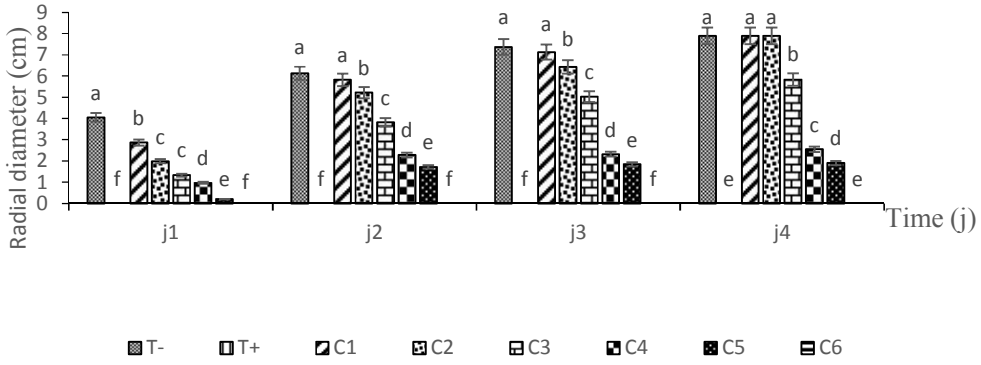


Fig. 6. Effects of EAq on radial growth of the fungus as a function of time With T- = 0 mg/ml; C1= 3.5 mg / ml ; C2= 7 mg /ml ; C3= 15mg / ml ; C4= 30 mg / ml ; C5= 50 mg / ml

**The values of the same day with different letters are significantly different at p < 0.05 (Turkey test)*

Table 3. Antifungal or fungistatic activity of extracts from *T. peruviana* seeds and synthetic fungicide

Extracts	Lethal concentration	Effect
Methanol	100 ml/ ml	Fongistatic
Aqueous	100 mg /ml	Fongistatic
ONAZOL 100	0,005 ml /ml	Fongicidal

Table 4. Correlation between inhibition percentage and extract concentrations on the strain tested with EAq and EM

Extracts	Correlation coefficient ®	Observations
EM	0.96	Highly correlated
EAq	0.98	Highly correlated

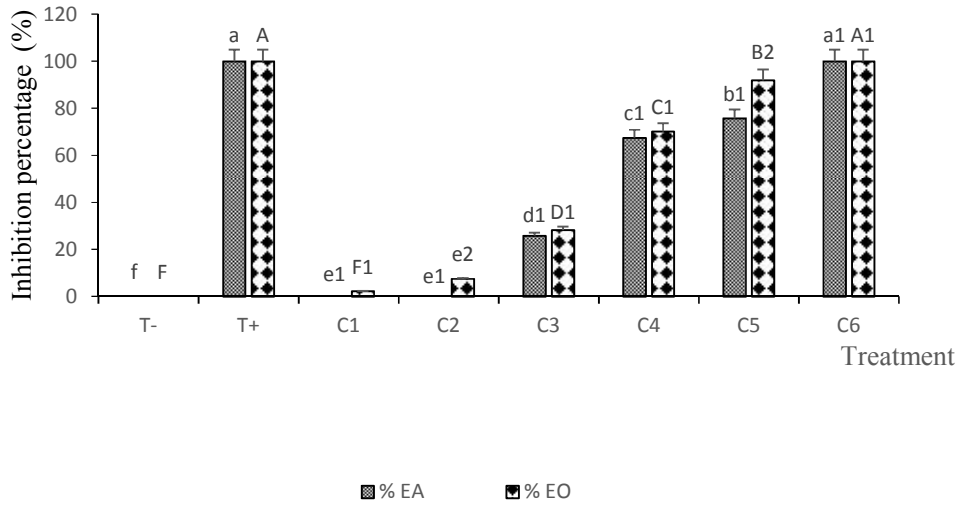


Fig. 7. Percentage of inhibition of extracts on day 4 after treatment
With T- = 0 mg/ml; C1= 3.5 mg / ml; C2= 7 mg /ml; C3= 15mg / ml; C4= 30 mg / ml; C5= 50 mg / ml; C6= 100 mg/ml

**The values of the same day with different letters are significantly different at p < 0.05 (Turkey test)*

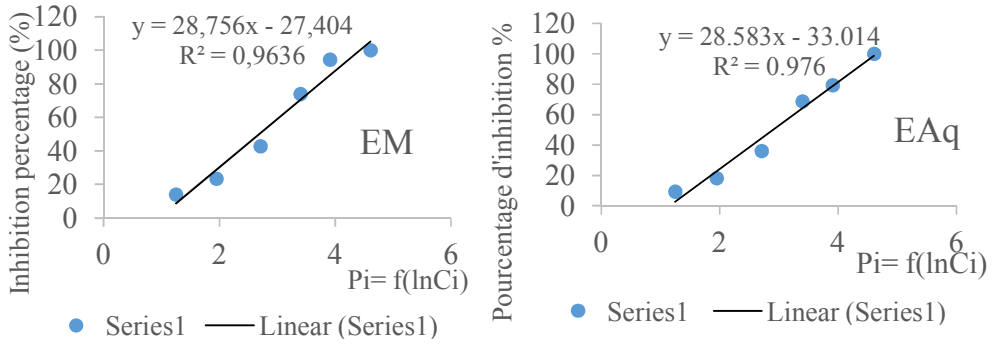


Fig. 8. Regression lines of mycelial growth at different treatments (EM= methanol extract, EAq= aqueous extract)

Table 5. CMI50; CMI90 and CMI95 of the mycelial growth of the strain with the different extracts tested (in ml/ml and mg/ml)

Extraits	CMI_{50}	CMI_{90}	CMI_{95}
EM	2.69	4.08	4.26
EAq	2.90	4.30	4.48

3.2 Discussion

The exploration of plant extracts as alternative or complementary sources to synthetic fungicides to reduce or eliminate parasitic pressure is nowadays a major topic in crop production. This work, based on the preparation of extracts (methanol and aqueous) from *Thevetia peruviana* seeds and the laboratory assessment of their fungicidal power on the fungus *Rigidoporus lignosus*, causal agent of white root rot in rubber roots, is part of this approach.

The extraction efficiency was around 23% and 7.44% for EAq and EM respectively. These

extraction yields therefore varied according to the solvents used. Indeed, [19] and [20] reported that environmental conditions, harvest period and age of the plant material can influence extraction yields. In addition, the polarity of solvents is believed to play a role in the extraction of many compounds [21]. Such results were obtained by [22] and [8] on seed extracts of *T. Peruviana* when they worked with *T. peruviana* aqueous extracts against *Phytoththora mergakarya.*

The results of the screening carried out showed the presence of several families of compounds that are natural bioactive substances such as essential oils, coumarins, sterols, saponins, sugars, terpenes and flavonoids. Several of these compounds have also been obtained by [23] and [24] on *T. peruviana* and *Azadirachta indica* respectively. Overall, the results of this work show that the various extracts tested (organic and aqueous) significantly reduced the radial growth of the *R. lignosus* strain with a 100% inhibition percentage obtained with the C6 (100 mg/ml) concentrations of EAq and C5 (50 mg/ml) concentrations of EM, thus demonstrating the strong antifungal properties of these natural substances. The effectiveness of these extracts can be explained by the presence of sterols, phenols, essential oils, monoterpenes, coumarins, tannins and sugars, which are secondary metabolites with very strong antifungal properties. With high performance liquid chromatography (HPLC), [23] obtained numerous secondary metabolites such as sterols, terpenes, lactones and showed the efficacy of these compounds on the development of *Cladosporium cucumerinum*. The work of [9]; [22] and [25] have already shown *in vitro* and *in vivo* the efficacy of *T. peruviana* extracts on the radial growth of *Arachis hypogea* and *P. megakarya* respectively. Similarly, [8] showed that aqueous extract of *T. Peruviana* (EATP) significantly inhibited the growth of different strains of *P. megakarya* with inhibition percentages in the order of 100% obtained at C3 = 50 mg/ml concentration on several strains.

However, this significant reduction in radial growth of the *R. lignosus* strain was more pronounced with methanol extract than with aqueous extract. These results are different from those of [6] who showed that aqueous extracts of *Jatropha curcas* were more effective than organic extracts of the same plant on the *in vitro* development of *Cercospora abelmoschus*, causal agent of okra cercosporiosis. Several suggestions emerge from these results: the use of solvents with a sufficiently high polarity makes it possible to obtain extracts of *T. peruviana* with more bioactive molecules. All these molecules, which are reputed to be bioactive, are believed to be responsible for the antifungal potential of *T. peruviana* seed extracts. The different concentrations of extracts tested significantly influenced the radial growth of the fungus, with the highest concentrations being the most inhibitory. Similar results had been reported by [7] with the use of aqueous neem seed extracts for the control of *Phakospora pachyrhizi*.

The various antifungal tests (fungicide or fungistatic) of the different extracts were all found to be fungistatic, while only the synthetic fungicide showed fungicide activity. This may be due to the activities of the active substances present in our extracts, the (relatively low) concentrations used, which possibly acted as a contact fungicide because they just inhibited the development of the strain without killing it entirely, and the variable resistance of the strain of the pathogen used. These results are on the one hand similar to those of [6] who obtained fungistatic activities with extracts from *J. curcas* seeds on the mycelial growth of *C. abelmoschus in vitro.* On the other hand, they are contradictory to the work of [9] who obtained a fungicidal effect with aqueous and methanol extracts of *T. peruviana* on the control of the *P. megakarya* strains. The percentages of inhibition of extracts on strain growth also varied with the increase in concentrations used. At high concentrations, organic and aqueous extracts showed total inhibition on the development of the fungus such as that obtained with the use of the synthetic fungicide made up of Cyproconazol 100. Cyproconazol 100. In other words, the higher the concentration, the more inhibition; this reveals the observed correlations between concentrations and percentage inhibition. Similar studies on the antifungal activity of some extracts have been reported by [7] which showed the efficacy of *A. indica* extracts on the *in vitro* development of strains of *P. pachyrhizi.*

The CMI50; CMI90 and CMI95 of the various extracts were determined. The low values obtained highlight the efficacy and antifungal properties of these different extracts on the growth of the strain. CMI50; CMI90 dan CMI95 dari berbagai ekstrak ditentukan. The EMs were therefore more effective than the EAq because the smallest CMI50 (2.69) was obtained with

these EMs. These results are in line with those of [26] who obtained a high inhibition of pathogenic fungal development with extracts of *Ocimum graticimum* with low MICs. Similarly, [6] and [7] obtained strong inhibition of *in vitro* growth of *P. pachyrhizi* and *C. abelmoschus* strains with *A. indica* and *J. curcas* extracts respectively with low MIC.

4. CONCLUSION

The objective of this work was to evaluate the fungicidal potential of extracts (aqueous and methanol) from *Thevetia peruviana* seeds on *Rigidoporus lignosus*. The tests carried out on *R. lignosus* have shown that, in general, the various extracts tested have considerably reduced the radial growth of the *R. lignosus* strain with very high inhibition percentages (73.89 to 100%). However, EMs were more effective than EAqs because they completely inhibited the mycelial growth of the pathogen with the C5 dose (50 ml/ml) compared to EAqs, which completely inhibited the radial growth of the pathogen at the C6 dose (100 mg/ml) However, these two extracts had the same effects as the synthetic fungicide made up of cyproconazole 100 and can therefore be integrated in the biological control of Rubber Fomès in plantations.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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