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# *In vitro* Antifungal Potential of Aqueous Seeds Extracts of *Azadirachta indica* and *Thevetia peruviana* against *Phytophthora megakarya* in Cameroon

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author SBM designed the study, wrote the protocol and wrote the first draft of the manuscript. Author ZA designed the study, wrote the protocol, wrote the first draft of the manuscript and reviewed all drafts of the manuscript. Author AN reviewed the experimental design and performed the statistical analysis. Author JPND managed the experimental process and identified the fungal strains. Author FME performed the statistical analysis and reviewed all drafts of the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

This study aims to evaluate the antifungal effects of aqueous extracts of *Azadirachta indica* (AEAI) and *Thevetia peruviana* (AETP) seeds taking it from a biological perspective under laboratory conditions. A randomized sample block design containing four treatments (*T-:* absolute control, AEAI, EATP and Ridomil Gold Plus at the dose of 3.33 mg/ml) in three repetitions was used. Plant extracts were used in three concentrations: C1: 12.5 µl/ml; C2: 25 µl/ml and C3: 50 µl/ml. The study took place in University of Yaounde 1, Faculty of Sciences, Department of Plant Biology, laboratory

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of Phytopathology and Microbiology in Cameroon, during the years 2013-2014. The chemical screening and the chromatographic profiles of the extracts, the aggressiveness of the *P. megakarya* strains, the inhibition of the strains by the extracts, the minimum inhibiting concentration ($MIC_{50}$ and $MIC_{90}$) and the extract characteristic were determined by the usual phytochemical and phytopathological methods. Obtained results show that seed extracts of both plants contain many chemical compounds such as sterols, sugars, alkaloids, anthraquinons, saponins, coumarins, and fatty acids. The two extracts in high concentrations (50 µl/ml) induced an inhibition rate of 100% on the mycelium growth of all the strains tested. Using the extract in concentration C2, the inhibition rate varies from 42.5 to 100% and from 27.0 to 88.0% respectively for AETP and AEAI. The values of $MIC_{50}$ and $MIC_{90}$ registered were 0.48, 2.63 µl/ml for AETP, 4.76 and 19.11 µl/ml for AEAI, indicating that AETP is more efficient when compare with AEAI. At concentrations C3 and C2, these two extracts indicated a fungicide effect, while at a weaker concentration the effect was fongistatic. This study shows that at high concentrations, the efficiency of AETP and AEAI seems to be the same with the one of Ridomil Gold Plus in the repression of *P. megakarya* growth *in vitro*. These natural substances can be integrated in control program of this *P. megakarya* strains in Cameroon.

*Keywords: Biocontrol; Phytophthora megakarya; Thevetia peruviana; Azadirachta indica; aqueous extracts.*

## 1. INTRODUCTION

Cocoa (*Theobroma cacao* L.*)* is a little tree cultivated in many tropical zones of America, Africa, and south East Asia [1]. This plant is cultivated for its beans which are used as raw material in various industries for the manufacture of various chocolate, pharmaceutical, cosmetics and starch [2]. In Cameroon, cocoa bean is essentially destined for exportation and it serves as an important source of revenue, with a production of about 228 910 tons in 2012 [3]. Cameroon cocoa is subject to many parasite infections amongst them the pod rot caused by *P. megakarya*, a Stramenopile of the Oomycetes class is the most serious cocoa infection [4]. In natural conditions, its attack causes losses that can reach more than 80% when environmental conditions are favorable for its development and in the absence of phytosanitary measures [5].

The amount of energy applied by the farmers in order to control and reduce the incidence of *P. megakarya* are enormous and more or less efficient. The most widely used method is the chemical approach. Meanwhile, the cost and the non-availability of synthesized fungicides is evoked by the cocoa farmers as being the principal constrains in their utilization [6]. Moreover, the chemical method presents a high risk of environmental pollution and the intoxication of users [7,8,9].

The problems that it raises today in the cocoa chain due to this constrains related to the market and the environment, remains an important elaboration strategy not only for the environment, but also for human health. In the search for efficient alternatives that are non-pollutants, the control of phytopathogens with naturals gears towards substances of plant origin, presents a high interest in plant protection against diseases and other plant parasites [10,11,12,13]. The aqueous extracts of *Thevetia peruviana* (AETP) and of *Azadirachta indica* (AEAI) seeds seem to have a high inhibiting potential against parasitic attacks in the farm and during storage of the product. Due to this effect, so much work has been done to show some effects such as nematicides [14,15,16], insecticides [17,18,19, 20], bactericides [21,22], rodenticides [23] and fungicides [12,13,11,24] of this two plants. However the fungicides properties of the two plants remain very little to be proven. This research work is registered at the level of a biological control and the principal objective is to evaluate the fungicide effect of aqueous seed extracts of *T. peruviana* and *A. indica* by comparing it to that of Ridomil Gold Plus (fungicide synthesis) on the growth of certain strains of *P. megakarya in vitro.*

## 2. MATERIALS AND METHODS

### 2.1 Materials

Strains (08) of *P. megakarya* collected in different zones of cocoa production in Cameroon (Mbal1.2, Bia 1.3, Nko 1.1, Nko 4.1, Njo 1.1, Njo 2.1, Tik 1.1 and Tik 2.1) were used as fungal material. Seeds of yellow oleander (*Thevetia peruviana),* neem (*Azadirachta indica)* (Fig. 1),

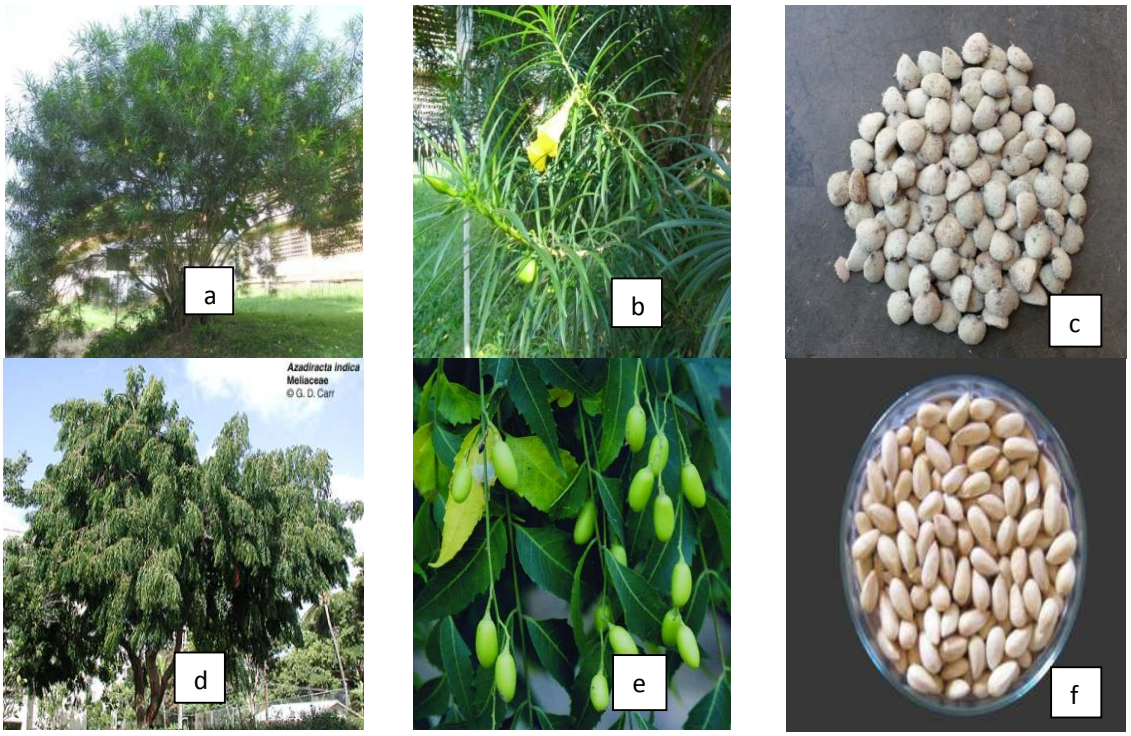


**Fig. 1. Plant materials used (a- *T. peruviana* plant, b- fertile branch of *T. peruviana* with yellow flowers, c- Seeds of *T. peruviana*, d- *A. indica* plant, e- fruits of *A. indica*, f- seeds of *A. indica*)**

pods of SNK 10 varieties of cocoa (sensitive) were considered as plant material and Ridomil Gold plus 66 WP (6% metalaxyl + 60% copper oxide) as chemical. In Petri dishes V8 medium was used for the growth of fungi [11].

## 2.2 Methods

### 2.2.1 Obtaining aqueous seed extracts of *T. peruviana* and *A. Indica*

Ripe fruits of *T. peruviana* and *A. indica* were collected in urban and peri-urban zones of Yaounde (Centre Region) and Maroua (Far-North Region) of Cameroon respectively. The collection consists of directly harvesting ripe fruits on the plant and the picking up of those whose felt on the ground. These fruits are manually removed from the pod with the help of a stone. The grains obtained are dried at a room temperature (24-25℃) for 2-3 weeks. A mother solution of 500 µl/ml concentration is made by introducing 50 g of the ground grains of neem or yellow oleander in a sterile distilled water (100 ml) for 12 hours, then filtration with the help of a mousseline material [25]. Three doses or concentrations of extracts (12.5, 25 and 50 µl/ml) were obtained by using the formula: $C_i\ V_i = C_f V_f$ [26], where $C_i$ and $V_i$ are initial concentration and initial volume, $C_f$ and $V_f$ are final concentration and final volume. These doses of extract are made by measuring from the mother solution 1.5, 3 and 6 ml then adding to this 58.5; 57 and 54 ml of a cultural medium to have a final volume of 60 ml.

### 2.2.2 Phytochemical screening of extracts

The class of secondary metabolites present in aqueous extracts of *A. indica* and *T. peruviana* seeds was determined by an adaptation of the standard procedures [27]. This technics are based on the turbidity, precipitation, and the foaming of extracts in the presence of different reagents characterizing each class of the secondary metabolite. A small volume of the aqueous seed extract of neem powder and the yellow oleander was used to determine the qualitative presence of alkaloids, phenols, triterpene, sterols, flavonoids, saponins, anthocyanin, glycosides and tannins. Specially, 0.5 ml of extract was added to 1ml of sulfuric acid ($H_2SO_4$) found in test tubes, then homogenized manually before carrying it for boiling during 2 min. After boiling, 5 drops of Meyers reagent was added into each tube and the development of turbidity was considered as

an indication of the presence of alkaloids. About 0.5 ml of each extract was mixed in 1ml methanol contained in a tube carrying the testing sample, and then heated in a Bain Marie at 55℃ for 15 min. To this mixture was added 3 drops of ferric cyanide which were freshly prepared. The apparition of a green precipitate indicated the presence of phenol. Than 0,5 ml of each extract was introduced into the test tubes containing HCl, previously neutralized by 5% sodium hydroxide (NaOH) followed by Fehling (A+B) added drop by drop. The apparition of a brick red precipitate determined the presence of glycosides.

### 2.2.3 High Performance Liquid Chromatography of utilized extracts (HPLC)

High performance liquid chromatography was realized to determine the rich profile of the different extracts. The eluent system (Table 1) used was that of water/methanol at two levels of separation.

A few µl of the extract was diluted in methanol found in the Eppendorf tube. Water was removed from the pipe before the application of the products in the machine with mark Gynkotech. The speed was regulated at 0.8 ml/min. Observations were made at a wave length of 225 nm.

### 2.2.4 Isolation and purification of the pathogenic strains

The pods that presented symptoms of diseases were collected and brought to the laboratory where they were washed several times with tap water and disinfected with 95% alcohol, then sterilized with a flame. The pericarp which covers the zone of pathogenic growth is removed by the help of a scalpel; ten little fragments of the mesocarp are collected and incubated on Petri dishes containing cultural medium (V8) previously prepared for the growth of the pathogen. Six days afterwards fragments of mycelium are collected and transferred into Petri dishes containing the V8 medium. This process is done several times before obtaining pure strains *P. megakarya* [28].

### 2.2.5 Characterizing the different pathogenic strains used

*2.2.5.1 Mycelium growth*

The average growth (D) of *P. megakarya* mycelium is determined by the daily measurements of the mycelial growth 48 hours after incubation using a graduated ruler. Each diameter is respectively measured on one of the two straight lines forming a right angle passing through the center of the explant (Fig. 2).

The average radius growth is calculated by using the following formula:

$$D = \frac{d1+d2}{2} - d_0$$ [29].

*2.2.5.2 Pathogenicity*

For the entire test on the virulence of pathogens, eight good pods are utilized. Each of the pods is washed with tap water and allowed to dry. At 30 min later, cotton that was previously soaked with a spore solution containing 3-4 105 spores/ ml. of different mushroom strains is placed respectively on the middle part of the pods (Fig. 3). The pods are then introduced into a humidified dark enclosed tank. They are stored in these conditions for 2 days till the apparition of the rotten brown. The diameter of the necrosis was evaluated on two perpendicular diameters with the help of a meter ribbon. The level of infection was evaluated by measuring the surface of the necrosis according to the formula of [30]. The strain in which the necrosis started to appear first on the pod after inoculation is considered as more virulent than the others. This test was equally carried out in Petri dishes for 8 days by using purified pathogenic strains.

*2.2.5.3 Spores production*

To determine the number of spores produced by each strain used, a solution with fungi spores was prepared by removing the white powder containing the sporocyst with the help of a spatula and introducing it into a beaker containing 10 ml of sterilized distilled water. The solution is then transferred into a refrigerator (4℃) for 30 min then into a dark enclosed container for one hour. This operation went further to provoke a thermal choc favorable for the liberation of spores from sporocysts (Fig. 4). After liberation of spores, the Hematimater was used to count number of zoospores produced.

### 2.2.6 Evaluation of the effect of AETP and AEAI on the growth of different strains

Explant containing the mycelium was collected and placed at the center of the Petri dish containing the culture medium (V8) either added with the extracts or with the fungicides. Five treatments were used: T- or control (0 µl/ml);

**Table 1. The HPLC eluent system**

| Time | 0 | 6 | 8 | 34 | 37 | 40 |
|---|---|---|---|---|---|---|
| MeOH | 75 | 75 | 75 | 75 | 75 | 75 |
| H2O | 25 | 25 | 25 | 25 | 25 | 25 |

three concentrations of each plant extracts, C1 (12.5 µl/ml); C2 (25 µl/ml) C3 (50 µl/ml) and one dose of Ridomil (25 µg/ml). The dose equivalent to the lowest concentration of the extract was used to prepare Ridomil® dose. Each treatment is repeated three times. The bottles are incubated in an ambient temperature (25℃) and the mycelium growth is taken two days after the start of the experiment and this is done on a daily bases, till the total colonization of the mycelium was observed. The radial growth was evaluated through daily measurements at the same time with the two perpendicular diameters on the back of the Petri dishes. The average radial growth is calculated according to the formula [29]. After that, the inhibition percentage was evaluated following the formula:

$$I\,(\%) = \frac{\mathbf{Dt0\ (mm) - Dxi\ (mm)}}{\mathbf{Dto\ (mm)}} \mathbf{X\ 100}$$

Where: I (%) = inhibition percentage; $Dt_0$ = average diameter of a necrosis in the test sample; $Dx_i$ = the average diameter of a necrosis in the extract or the Ridomil.

### 2.2.7 Fungicidal and fungistatic test of different aqueous extracts

After incubation, the treatment in which the growth of mycelium was completely inhibited is counted and explants are transferred into a new culture with no extract. Seven days after, if there is a sign of regrowth in the medium, the extract is qualified as fungistatic; in the contrary case it is qualified as fungicide [31,32].

### 2.2.8 Determination of minimal inhibiting concentrations

From the linear regression equation between the neperian logarithms of the concentrations in abscisse and the inhibition percentage of ordered growth, the reducing concentrations of 50% (MIC 50) and 90% (MIC 90) of the growth is determined [33].

## 2.3 Statistical Analysis

An analysis of variance (ANOVA) was done using SPSS 19.0 (the generalized linear model). Duncan test at 5 % were used to compare the averages.

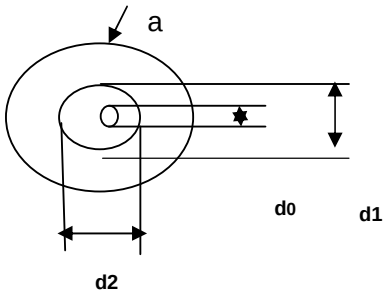


**Fig. 2. Diagram indicating the method of measuring the radius growth of pathogens**
*d0= diameter of the explant (0.7cm); d1and d2=diameter perpendicular to pathogens; a=petri dishes of 90mm in diameter*


**Fig. 3. Infected cocoa pods by three strains of *P. megakarya* 5 days after inoculation**

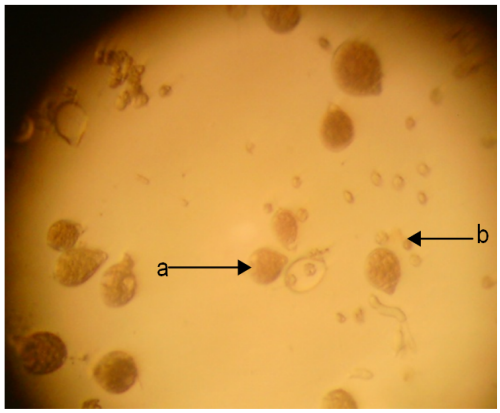


**Fig. 4. Liberation of spores after a thermal choc. a: Sporocyst, b: Liberated spore**

## 3. RESULTS AND DISCUSSION

### 3.1 Results

#### 3.1.1 Phytochemical screening of extracts

The screening revealed the presence of many families of chemical compounds such as essential oils, sterols, coumarines, phenols, sugars, tannins, alkaloids and saponins. However, the *T. peruviana* and *A. indica* aqueous extracts contained more sterols and sugars. Alkaloids, anthraquinons, saponins, coumarins and oil were present but in small quantity (Table 2).

#### 3.1.2 HPLC of different extracts

Chromatographic analysis revealed the presence of about 30 chemical compounds (Fig. 5). Ultra - minority compounds were proven to be numerous in the AETP than in the AEAI and have different retention periods. One compound was found to be a majority in the AETP (Fig. 5a) while five of them were identified in AEAI (Fig. 5b).

#### 3.1.3 Characterization of pathogenic strains

Concerning the radial growth of the strains, the strain Njo 2.1 presented an average maximum growth of 7.4 cm against an average maximum growth of 4.1cm for those with the Mbal 1.2 strain. For the most aggressiness of the strains tested, the Tik 1.1 strain proved to be more aggressive on the pods used with a necrosis growth of 11.5 cm against 0.8 cm in diameter for the Njo 2.1 strain. For those which had to be involved in the production of zoospores, the Tik 1.1 strain produced more spores more than the other strains at a rate of 6.5 x $10^5$ spores/ml against 0, 05 x $10^5$ spores/ml for the Njo 2.1 (Table 3).

**Table 2. Presence of natural products in aqueous extracts '-': absent, '+': present, '+++': present abundantly**

| Family of compounds | | Abundance of the compounds | |
|---|---|---|---|
| | | ***Thevetia peruviana*** | ***Azadirachta indica*** |
| oils | essential (monoterpenes, parfum) | + | + |
| | saponifiables (production of savon) | + | + |
| Coumarins | | + | |
| Phenols | | - | - |
| Flavonoids | | - | - |
| Saponins | | + | + |
| Tannins (phenolic molecules) | Gallic (full of phenols) | - | - |
| | catéchiques | - | - |
| Anthraquinons | | + | + |
| Terpenoids | | - | + |
| Sterols | | +++ | +++ |
| Alkaloids | | + | + |
| Sugars | | +++ | +++ |
| cardio tonic glycosides | | - | - |

**Table 3. Characteristic of the different strains tested**

| Strains | Mycelium diameter (cm) | Spore production x $10^5$ zoospores/ml | Necrosis diameter (cm) |
|---|---|---|---|
| Bia 1.3 | 6.2c | 1.5b | 7.2b |
| Njo 1.1 | 6.1c | 5.0d | 10.8d |
| Njo 2.1 | 7.4d | 0.05a | 0.8a |
| Mbal 1.2 | 4.1a | 2.6c | 9.3c |
| Nko 1.1 | 5.6b | 4.8d | 10.5d |
| Nko 4.1 | 5.5b | 2.9c | 9.5c |
| Tik 1.1 | 5.6b | 6.5e | 11.5e |
| Tik 2.1 | 5.4b | 2.7c | 8.7c |

**The values followed by the same letters are not significantly different when P< 0.05*

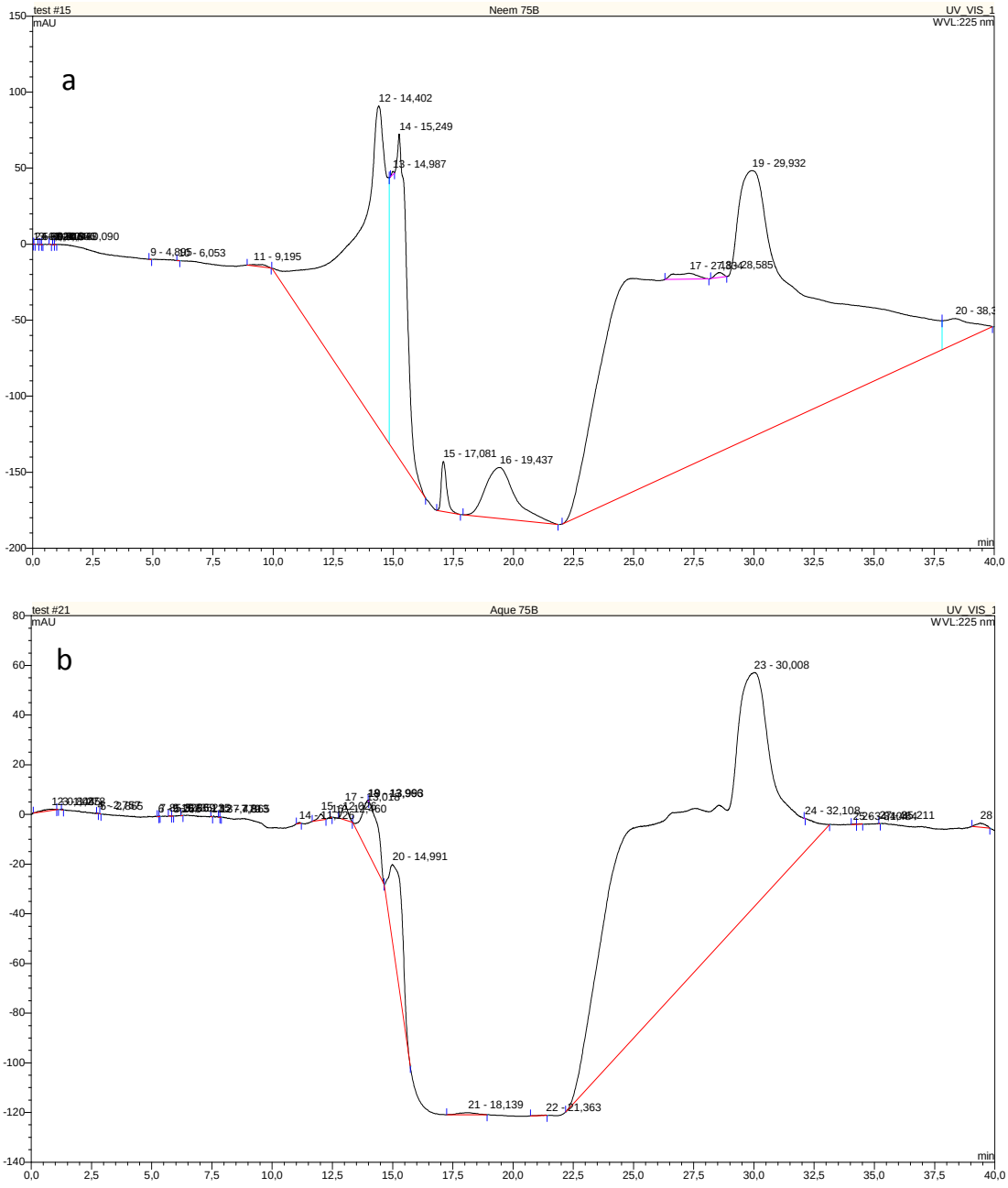


**Fig. 5. Chromatographic profiles of aqueous extracts with an eluent system of water/methanol, a- AEAI ; b- AETP**

### 3.1.4 Effect of grain extracts of *T. peruviana* and *A. indica* on the growth of different strains *in vitro*

AETP and AEAI considerably reduced the mycelium growth in a more pronounced way on the Nko 1.1 and Nko 4.1 strains on the different doses of the two plant extracts.

#### *3.1.4.1 Effect of AETP on the growth of different strains*

The aqueous extracts of *T. peruviana* proved to be very efficient against the growth of *P. megakarya*. An inhibition of 100% was obtained with the higher dose C3 (50 µl/ml) on all the strains tested. With the dose C2 (25 µl/ml) 100%

of inhibitions was equally obtained on the strains: Tik 1.1, Nko 4.1, Nko 1.1 and Mbal 1.2. At this dose (25 µl/ml), the weakest inhibition (42.5%) was observed on the Bia 1.3 strain. The smallest inhibition of 29.8% was obtained with dose C1 (12, 5 µl/ml) on the Bia 1.3 strain (Fig. 6).

*3.1.4.2 AEAI effect on the growth of different strains*

The AEAI equally proved to be efficient against the *in-vitro* growth of different *P. megakarya* strains. To this effect, 100% inhibition were obtained at doses of C3 (50 µl/ml) and C2 (25 µl/ml) on the Tik 2.1, Nko 4.1 and Mbal 1.2. The Tik 1.1, Njo 2.1, Njo 1.1 and Bia 1.3 strains, presented a very high resistance towards AEAI when use dose C3 with a rate inhibition of 70.86, 34.86, 37.04 and 37.64% respectively. A weak inhibition of 26.25% was obtained with dose C2 on the Njo 2.1 strain. On the other hand Mbal 1.2 and Nko 4.1 strains which proved to be very sensitive to extracts, the dose C1 (12,5µl/ml) proved to be ineffective against the group of pathogenic strains tested. The smallest growth inhibition obtained at this dose is 15.36% on the Njo 2.1 strain (Fig. 7).

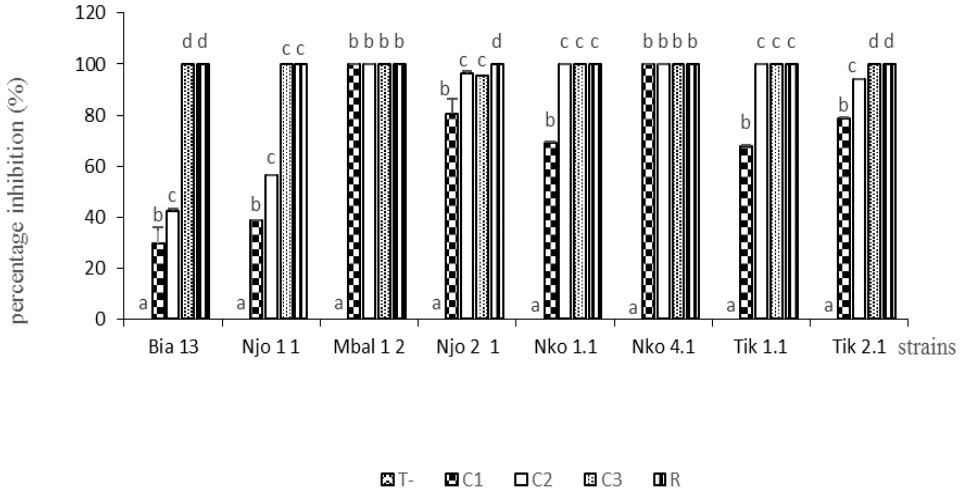


**Fig.6 . AETP effect on the growth of different *P. megakarya* strains**

**The values of the same strain carrying different letters are significantly different when P< 0.05*

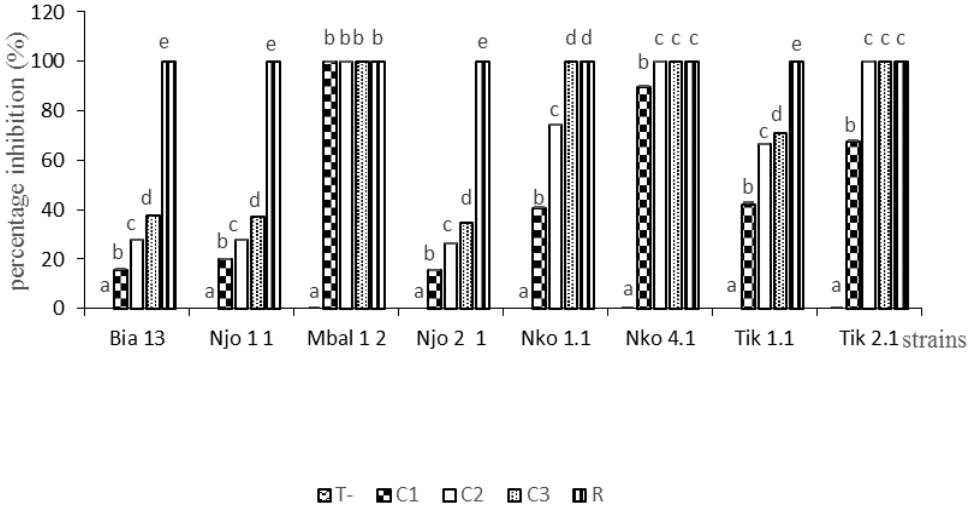


**Fig. 7. AEAI effect on the growth of different *P. megakarya* strains**

**The values of the same strain carrying different letters are significantly different when P< 0.05*

#### 3.1.5 Fungicidal/fungistatic properties of extracts

For fungicidal/fungistatic test carried on the different strains of *P. megakarya*, only the inhibiting doses of aqueous extracts are mentioned. As such, amongst all the strains studied, only Mbal 1.2 underwent a fungicide activity of AEAI at a concentration of $C_2$ = 25 µl/ml and $C_3$= 50 µl/ml while this same activity was observed on the quasi-totality of strains having the same concentrations with AETP (Table 4).

#### 3.1.6 Minimum inhibiting concentrations of $MIC_{50}$ and $MIC_{90}$ certain extracts used

The weakest inhibiting concentrations were obtained with AETP. The $MIC_{50}$ obtained on the totality of the eight strains tested varied from 0,48 to 7,46 µl/ml and for $MIC_{90}$ from 2,63 to 25,79 µl/ml while on this same strain tested with AEAI the $MIC_{50}$ and $MIC_{90}$ vary from 4,76 to 15,71 $10^6$ and from 19,11 to 3,47 $10^9$ µl/ml respectively (Table 5).

### 3.2 Discussion

This research was based on extracting natural substances from the seeds of *Thevetia peruviana* and *Azadirachta indica* and evaluating their antifungal rate on some strains of *Phytophthora megakarya.* The extraction, which is done in the cold, gave products with turnover ranging from 23 and 9.4% for AETP and AEAI respectively. This different level of yield registered was linked to atmospheric conditions, to the plant material at the time of harvest or even to the plant cycle [34].

The results of screening done indicated the presence of many family compounds which are natural bioactive substances such as essential oils, coumarins, sterols, saponins, sugars, terpens, tannins, and flavonoids. Many of this compounds where equally obtained by [35] and [16] on *T. peruviana* and *A. indica* respectively.

**Table 4. Fungi and fungistatic activities of extracts**

| strain | Concentrations | AEAI | AETP |
|---|---|---|---|
| Mbal 1.2 | C1 | Fungistatic | Fungicidal |
| | C2 | Fungicidal | Fungicidal |
| | C3 | Fungicidal | Fungicidal |
| Bia 1.3 | C3 | Fungistatic | Fungicidal |
| Njo 1.1 | C3 | Fungistatic | Fungicidal |
| Nko 1.1 | C2 | - | Fungicidal |
| | C3 | Fungistatic | Fungicidal |
| Nko 4.1 | C1 | Fungistatic | Fungistatic |
| | C2 | Fungistatic | Fungicidal |
| | C3 | Fungicidal | Fungicidal |
| Tik 2.1 | C2 | Fungistatic | - |
| | C3 | Fungicidal | Fungicidal |
| Tik 1.1 | C2 | Fungistatic | Fungistatic |
| | C3 | Fungicidal | Fungicidal |

**Table 5. The $MIC_{50}$ and $MIC_{90}$ of the mycelium growth of different strains and tested extracts. (µl/ml)**

| Souches | EATP | | EAAI | |
|---|---|---|---|---|
| | $MIC_{50}$ | $MIC_{90}$ | $MIC_{50}$ | $MIC_{90}$ |
| Bia 1.3 | 7,46 | 21,54 | 105,64 | 1366,51 |
| Njo 1.1 | 4,95 | 13,87 | 145,47 | $3,91.10^9$ |
| Njo 2.1 | 0,48 | 13,6 | 142,59 | 2514,93 |
| Mbal 1.2 | * | * | * | * |
| Tik 1.1 | 4,62 | 25,79 | 15,64 | 106,7 |
| Tik 2.1 | 1,73 | 23,34 | 4,76 | 26,05 |
| Nko 1.1 | 4,22 | 25,28 | 15,18 | 19,11 |
| Nko 4.1 | * | * | 15,71 $10^6$ | 3,47 $10^9$ |

**Represents values that are not defined to be at zero statistically*

The chromatographic profile obtained with AETP and AEAI revealed the presence of minor and ultra-minor compounds than the major compounds; the efficiency of this two extracts was due to the presence of molecules which reacted at very tiny doses.

The strains tested revealed different behaviors within one another. The south east strains (Tik 1.1) indicated to be more aggressive (more virulent) with a high spore production rate of about 6,5 x $10^5$ spores/ml with a necrosis growth rate of 11,5 cm. such results were obtained by [13] on certain strains of fungi.

AETP and AEAI significantly inhibited the growth of tested mushroom strains. Percentage inhibitions in the order of 100% was obtained at a concentration of C3 (50 µl/ml) on many strains with the two extracts; However AETP proved to be more efficient with a rate of inhibition in the order of 42.5 to 100% at the concentration of C2 (25 µl/ml). A total growth inhibition was also observed on all the tested concentrations with the strains of Mbal 1.2 (with the two extracts) and that of Nko 4.1 (with AETP only). These results confirm those of [11,12,13] which indicated in vitro percentage inhibitions similar on other strains of *P. megakarya.* This efficiency of different extracts on the pathogenic growth was due to the presence of compounds like tannins, anthocyanins, saponins, sterols, and many others that are secondary metabolites contain a high antimicrobial and antifungal activity [36]. However, Azadirachtin can also explain the efficience of neem extracts. These plant extracts in higher doses may be considered as a potential biofungicide against *P. megakarya*.

## 4. CONCLUSION

In this study the comparison of antifungal effect of aqueous seed extracts of *T. Peruviana* and *A. indica* to that of Ridomil Gold Plus on the *in vitro* growth of *P. megakarya,* shows that the two plant extracts are efficient in the reduction of mycelium growth with the highest concentrations $C_3$ (50 µl/ml). However AETP proved to be more efficient than AEAI when using other concentrations $C_2$ (25 µl/ml) and $C_1$ (12,5 µl/ml). Therefore, these extracts in particularly AETP could be used as substitute to synthetic fungicides which are more pollutant in control of *P. megakarya*.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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