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Effect of extracts of *Thevetia peruviana* (Pers.) K.Schum on development of *Phytophthora megakarya* causal agent of black pod disease of cocoa

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ABSTRACT

Objective: The *in vitro* and *in vivo* effects of seed extracts of *Thevetia peruviana* (Apocynaceae) on the growth and the germination of spores of *Phytophthora megakarya* causal agent of black pod of cocoa was evaluated .

Methodology and results: Five extracts obtained by soxhlet apparatus (extracts of hexane, ethyl acetate, acetone, methanol and aqueous extract) were used. One fungicide (*Ridomil ® gold* plus) served as control reference. Five strains of *P. megakarya* (Mbal 121, Ngoke 111, Nom121, Mbal 212, njo111) were tested. Three concentrations of extracts (6.25, 12.5 and 25 µl/ml) and one concentration of fungicide were used in V8 medium. Detached fruit bioassay was performed. Spores of five strains of *P. megakarya* were inhibited by methanolic and acetonic extracts of *T. peruviana* with an inhibition percentage ranging from 99-100 %. Thus, the smallest concentration of extract is capable of acting on the germination of spores of the fungi. Only hexane extract did not inhibit the growth of strains. Methanol extract reduced the growth from 60 to 100% at 25 µl/ml. The detached fruits bioassays demonstrated the effectiveness of extracts on plant tissue when inoculated with *P. megakarya* zoospores.

Conclusion and application of findings: Statistical analysis shows that methanol and acetone extracts are as effective as the tested fungicide on the growth and germination of zoospores, aqueous extract are effective as Ridomil ® on growth on the black pod disease of cocoa. These extracts could be used in the integrated pest management strategy of the plant pathogens tested.

Key words. *Phytophthora megakarya, Thevetia peruviana,* extract, biocontrol, black pod disease.

INTRODUCTION

The protection of plants against crop diseases and pests is a key factor in the increase of crop production (Nguyemban, 1996). Tropical crops are the subject of special attention, due to plant pathogens that attack them and cause huge economic losses to farmers, and because of their economic importance for these tropical countries. In Cameroon, black pod disease, caused by *Phytophthora megakarya,* is the most important field disease in cocoa beans production and causes up to 80% loss when no protection measure is undertaken (Nyasse, 1992; Cilas and Berry, 1994). Several control methods are used against this plant pathogen to reduce its effects on crops. There are cultural

practices that aim to reduce the inoculum potential by regular health harvests and modification of the microclimate thereby creating unfavourable conditions for the development of the disease (frequent weeding and pruning). Chemical control, based mainly on the use of synthetic pesticides is effective. Unfortunately, this method has negative consequences on the environment and is very expensive. The pesticides generally used are not always accessible to farmers (Kebe, 1994). Developing genetic resistance against this disease is considered the most cost effective and reliable method for control. The clones developed by this gene are tolerant but do not have the total resistance (Iwaro *et al*., 1998).This method is usually slow in developing varieties for farmer use. Until resistant varieties can be developed and distributed to the farmer, the use of biological control agents and natural products are more practical alternative for an integrated pest management strategy. However the use of *Trichoderma* species (Tondje *et al*. 2003; Holmes *et al*., 2004; Derberth *et al*., 2007; Mpika *et al*., 2009) provided interesting results, but a number of limitations with respect to the action spectrum of these organism and their high cost accrue. A number of plant species have been reported to possess natural substances that are toxic to a variety of plant pathogenic fungi (Bautista-Banos *et al*., 2000; Imtiaj *et al*., 2005). Seeds, leaves, fruits and roots of *Thevetia peruviana* are considered as potential sources of biologically active compounds, such as insecticides (Reed *et al*., 1982; Ambang *et al*., 2005), rodenticides (Oji and Okafor, 2000) fungicides (Gata-Goncalves, 2001; Ambang *et al*., 2010), virucides (Tewtrakul *et al*., 2002) and bactericides (Saxena and Jain, 1990). *Thevetia peruviana,* has already shown its effectiveness in reducing the inoculum pressure as well as the incidence of brown rot (Ambang *et al*., 2010). The objective of this work was to evaluate, *in vitro* and *in vivo* antifungal potential of extracts of seeds of yellow oleander (*Thevetia peruviana* (Pers.) K. Schum) on germination and growth of *Phytophthora megakarya* spores.

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MATERIALS AND METHODS

Plant material: The plant material consisted of *Thevetia peruviana* seeds collected in Yaoundé, the pods varieties SNK10 (sensitive) and BBK1016 (moderately tolerant) from the "Caobisco" station IRAD (Institute of Agricultural Research for Development) of Nkolbissong Yaoundé, Cameroon.

Fungal material: The fungal material consisted of *P. megakarya* strains obtained from cocoa pods showing symptoms of brown rot collected from the Centre and Littoral regions of Cameroon.

Extraction Method: The mature fruits were collected from different locations of the city of Yaoundé. The seeds obtained from fruits were dried in the laboratory at room temperature and then crushed using a hand mill (brand: "Victoria"). The resulting powder was loaded into cartridges and placed in a soxhlet apparatus. Extraction solvents with high polarity, hexane, ethyl acetate, methanol, and acetone were used in the process. The different extracts were obtained after 48 to 72 hours each. The product obtained was then concentrated with Rota vapour and then kept at 4°C (refrigerator) throughout the experiment (Gata-Goncalves, 2003). The aqueous extract was obtained by maceration of the seed powder in sterile distilled water for at least 12 hours (Stoll, 1994).

Isolation and purification of pathogen strains: Isolation was performed from infected pods showing symptoms of brown rot. The pods were washed several times with tap water, disinfected with an alcohol swab (95%) and then sterilized with a flame. Using a scalpel, the epicarp covering the area of infection was removed. Mesocarp fragments containing the fungus were then collected and incubated in petri dishes containing water-agar culture medium. Four to five days after, the mycelium obtained was transplanted in boxes containing V8 medium. This last step was repeated several times in order to obtain pure strains of the fungus (Nyasse, 1992; Ondo, 2006).

Obtaining of spores: Two methods were used to obtain spores. The first was to scrape the mycelium of a pure culture and homogenize in a few ml of sterile distilled water before putting ice and allowing in the dark for 5 minutes. This heat shock allowed the release of zoospores of *P. megakarya*. The second was to scrape the white powder from a diseased pod and put it in ice-cold distilled water. The resulting solution was then placed in the dark for at least 5 minutes (Ondo, 2006; Deberdt 2008).

Evaluation of the effect of the extracts on spore germination: Different concentrations (6.25 µl /ml, 12.5

µl /ml, 25 µl /ml) were prepared from a stock solution of 500 µl /ml (Gata- Gonçalves, 2001). The dose equivalent to the lowest concentration of the extract was prepared with Ridomil®. For the aqueous extract, the concentrations were 6.25, 12.5 and 25mg/ml obtained in the same manner. The different media supplemented with extracts, fungicide and solvent dilution (Tween) were cast on the viewing strip, and after solidification, 20 µl /ml of a spore suspension calibrated at 3-410^5 spores / ml using a Mallasez's cell (hematimeter) was spread by means of a micropipette on each preparation. The whole was incubated in the dark for at least 12 hours. Each dose was repeated three times. The experiment was repeated once. Counting of spores germinated or not was made on 100 spores on three different areas of each blade. 300 spores per repetition and 900 spores for each treatment were counted under an optical microscope (magnification × 20). A total of 1,800 spores per treatment were counted (Wildmer and Laurent, 2006) for both tests.

Evaluation of the effect of extracts on the growth of strains. Mycelia disks obtained using a cookie cutter 7 mm in diameter and taken through a loop in cultures from 6 to 7 days and *P. megakarya* were deposited in the center of each Petri dish containing V8 medium enriched with different extracts or fungicide. A negative control non -supplemented in extract and a solvent control dilution were prepared. Each treatment was repeated 3 times in each of two tests. The plates were incubated at 25 °C and measurements were taken every day from the second day. However, the experiment was stopped when the Petri dishes were completely covered by the fungus. The radial growth of the strains used was evaluated by measuring daily (48 h after inoculation) and at the same time, the two perpendicular diameters of the tracks on the back of the Petri dish. The average of two perpendicular measurements of the diameter minus diameter of explants represents the measurement of the radial growth of the fungus.

Fungistatic and fungicidal activity of each extract and their effectiveness: At the end of each test, the explants from mycelium boxes where growth was completely inhibited, were aseptically collected and deposited on the culture medium containing no extract. After 7 days on this new culture, if the fungus grows, then the extract is said to be fungistatic. However, if on the contrary, the fungus does not grow, the extract is considered fungicidal (Pandey *et al.*, 1982; Kishore *et al.*, 1993).

Determination of minimum inhibitory concentrations: The values obtained after counting the spores and growth were used to determine the percentages of inhibition using the following formula:

IP = [(A-B)/A] x 100 (Leroux *et al.*, 1978).

Where IP = inhibition percentage; A = number of spores or average culture diameter found in control medium ; B = number of spores or average culture diameter with plant extract or fungicide.

From the linear regression equation between the natural logarithms of concentrations along the abscissa and the percentage inhibition of germination in ordinate, the concentrations reducing growth and germination of spores of 50% (MIC50) and 90% (MIC90) were determined (Dohou *et al.*, 2004). The minimum lethal concentrations (lethal MI) were obtained for extracts that showed complete inhibition (100%).

Detached pods bioassay: The lethal dose and the lethal multiplied by 10 were applied to the pod. The ability of the extracts to reduce the frequency and size of lesions caused by *Phytophthora megakarya* on pods was evaluated according to a modification of the method of assessing resistance to *P. megakarya* by pod parts in the laboratory developed by Iwaro *et al.* (1997b, 2000). These changes involve two processing steps : first after spending 24 hours on a distilled water-soaked foam to make the most receptive cortex, pods clones (SNK10 and BBK 1016) were sprayed on their face side with an extract solution at different concentrations (Mpika *et al.* 2009). Then in the second stage, a second application was made, 12h at least after, by spraying with a zoospore suspension of *P. megakarya* calibrated at 3-410^5 zoospores / ml. For control tests, the pods were sprayed or not with a solution or soapy water. Pod sprayed were located approximately 30 cm from the sprinkler. An average of 20µl/ml inoculum was deposited per pod. Thus, inoculated pods were placed in bins on the foam soaked with distilled water. Incubation was carried out on pods inoculated at room temperature (28-30 °C) for 7 days. Pods were arranged in randomized complete block design in the tray. The severity of the infection was assessed by measuring the area of necrosis according to the formula of Blaha and Lotode (1976):

$$S = \frac{D \times d \times \Pi}{4}$$

Where S= area of necrosis (mm^2); D=large diameter; d= small diameter; Π=3.14

Statistical analyses: Data was analyzed by SPSS16.0 software and the Microsoft Excel software that performs the analysis of variance (ANOVA). Student tests and Duncan at 5% were used to compare the averages.

RESULTS

Effects of extracts on the growth of strains

Effect of hexane extract (HE) on the growth of *Phytophthora* strains: The HE had no effect on the growth of the strains tested. A growth stimulating effect was revealed with the studied strains. Strain 111 njo presented zero percent inhibition at all doses. The *Ridomil ® completely* inhibited all strains with the percentages of inhibition of the order of 100 %.(Fig.1).

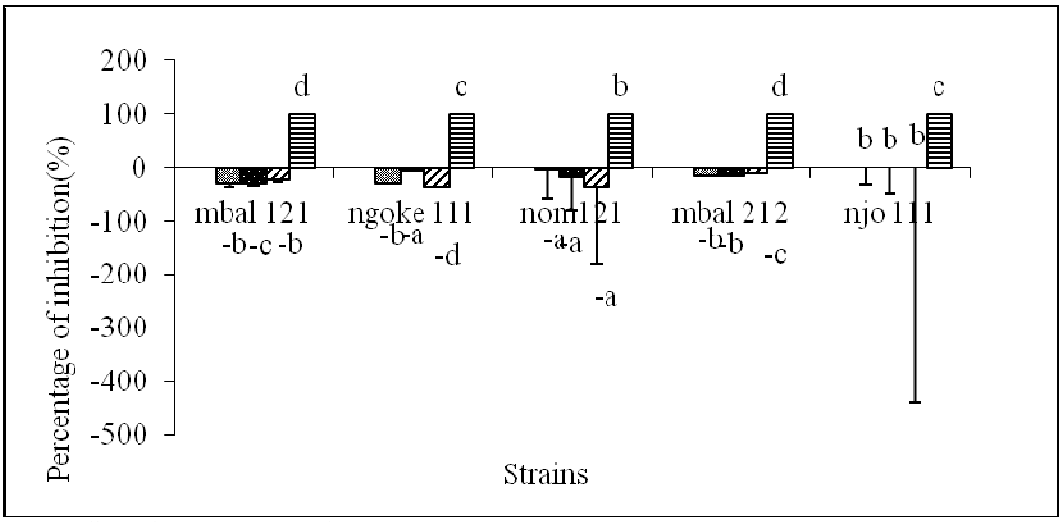

Fig.1: Effect of HE on growth of *P. megakarya* strains.
Means within the same strain followed by the same letter are not significantly different (P= 0.05) according to least significant differences

Effect of ethyl acetate extract (EAE) on the growth of *Phytophthora* strains: EAE showed significant inhibition on the growth of *Phytophthora* strains at the highest concentration (25µl/ml). At this concentration, the strain njo 111a had the lowest percentage of inhibition 37.66% against 100% inhibition for Mbal ngoke111 and 121 (Fig. 2).

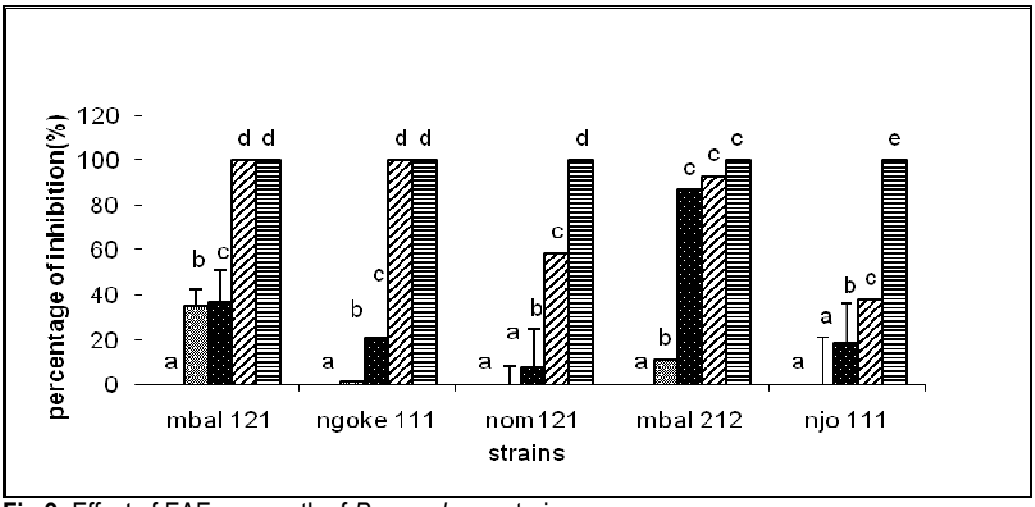

Fig 2: Effect of EAE on growth of *P. megakarya* strains

Effect of acetone extract (AE) on the growth of *Phytophthora* strains: The AE inhibited all strains except ngoke111, a significant difference was observed in 5% between control and different doses. Ngoke strain proved less sensitive to the extract with the inhibition percentages of 0%, 1.30% and 2.60% at the respective doses of 6.25µl/ml, 12.5µl/ml and 25µl/ml. Nom 121 and mbal 121 strains were more sensitive to the extract with percentage inhibition of 100% at the concentrations of 12.5 µl/ml and 25µl/ml (Fig.3)

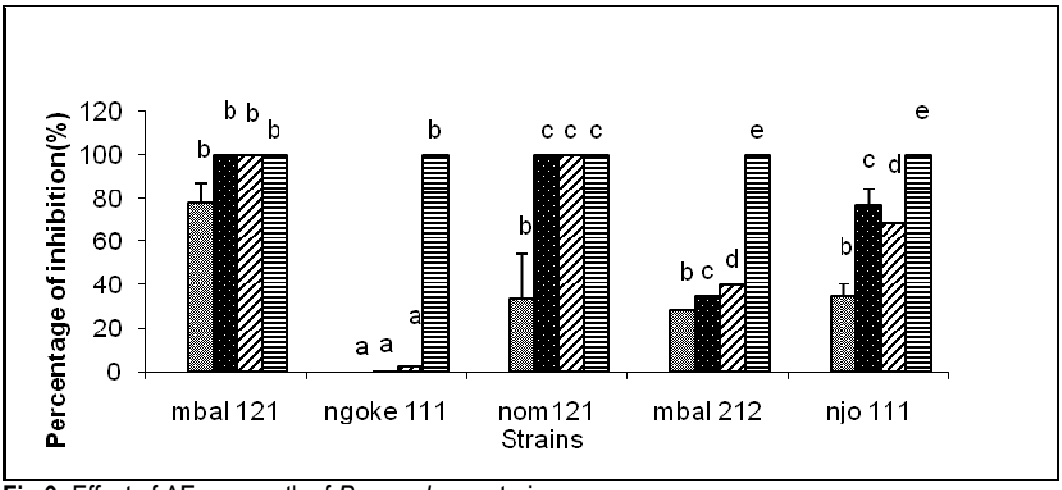

Fig.3: Effect of AE on growth of *P. megakarya* strains

Effect of methanol extract (ME) on the growth of *Phytophthora* strains: The ME proved very effective against the growth of *P. megakarya*. With the smallest dose, the percentage of inhibition ranged from 72.73% for njo111 to 100% for Mbal 212 and Nom 121. Mbal 212 and Nom 121were the most sensitive strains with total inhibition (100%) at all concentrations. The effectiveness of ME was substantially equal to that of *Ridomil ® (*fig 4).

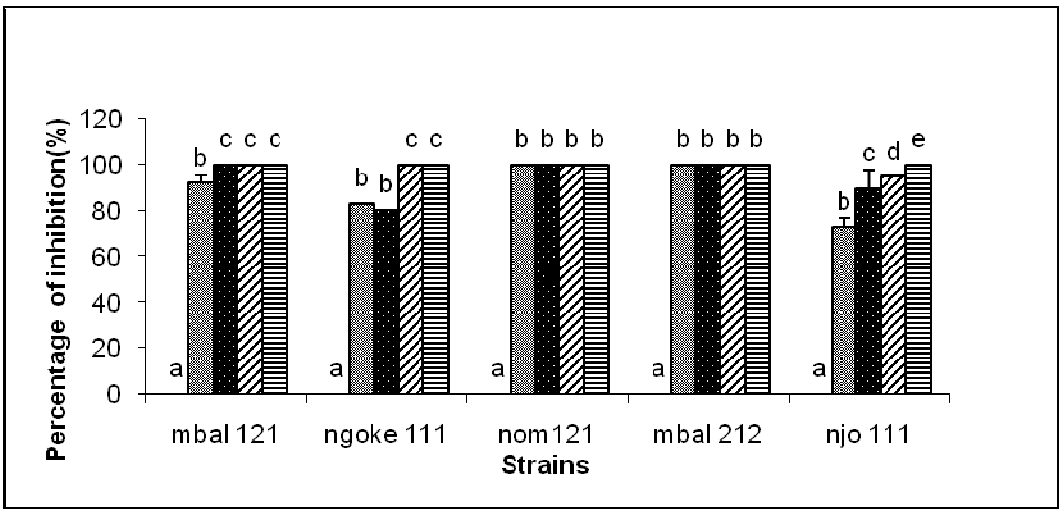

Fig. 4: Effect of ME on growth of *P. megakarya* strains

Effect of aqueous extract (AqE) on the growth of *Phytophthora* strains: AqE was very effective against the growth of *P. megakarya* strains. A significant difference was revealed to 5%. Mbalmayo strains were inhibited completely and at all concentrations. The lowest percentage of inhibition was obtained with nom 121, 46.58% at the concentration of 12.5µl/ml (Fig. 5).

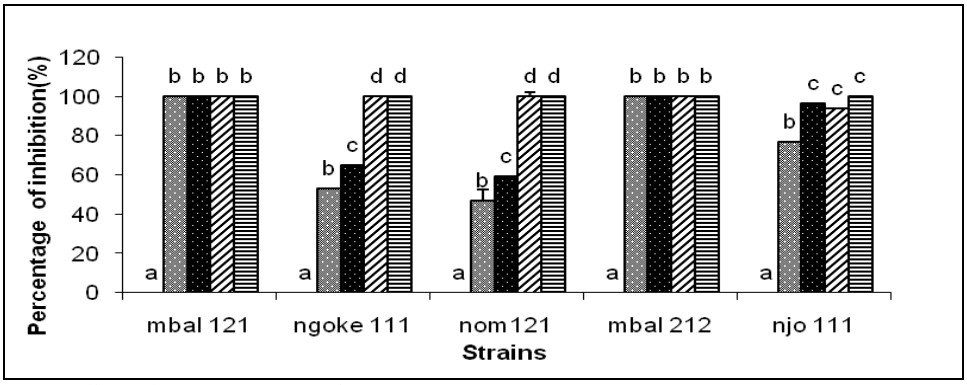

Fig.5: effect of AqE on growth of *P. megakarya* strains

Effect of extracts on germination of *P. megakarya* spores

Effect of hexane extract (HE) on spores' germination: The HE had no significant inhibitory effect on the germination of spores of the fungus. Ngokè strain had the highest percentage (11%). No significant difference was revealed at *P*≥5% between the control and the different concentrations of extracts. Only Ridomil ® totally inhibited spore germination (table1).

Table1: Percentage of non-germinated *P. megakarya* zoospores on V8 agar amended with different concentrations of plant extracts and fungicide after 12h

	***P. megakarya* isolate**				
Treatment[1]	**Mbal 121**	**nom 121**	**ngoke 111**	**mbal 212**	**njo 111**
T-	0 a[2]	0 a	0 a	0 a	0 a
C1	2,5a	4,1b	11,4c	4,4b	4,1b
C2	1,2a	5,1b	6,2b	6,2b	5,1b
C3	7,1b	4,7b	5,8b	4,4b	7,1b
T+	100c	100c	100d	100c	99c

[1]T- control; C1-6,25µl/ml ; C2- 12,5µl/ml ; C3- 25µl/ml ; T+ -*Ridomil*®

[2]Means within the same column followed by the same letter are not significantly different (P= 0.05) according to least significant differences.

Effect of ethyl acetate extract (EAE) on *Phytophthora* spores germination: EAE does not significantly affect the germination of spores despite the 29.9% obtained with the mbal 121, *P*≥ 5% (table 2).

Table 2: Percentage of non-germinated *P. megakarya* zoospores on V8 agar amended with different concentrations of plant extracts and fungicide after 12h

	***P. megakarya* isolate**				
Treatment[1]	**Mbal 121**	**nom 121**	**ngoke 111**	**mbal 212**	**njo 111**
T-	0 a[2]	0 a	0 a	0 a	0 a
C1	2,2a	29,9c	1,6a	0,9a	3,2b
C2	2,4a	20,4b	4,8b	0,7a	2,0b
C3	0,6a	12,3b	3,3b	4,7b	3,3b
T+	100b	100d	100c	100c	99c

[1]T- control; C1-6,25µl/ml ; C2- 12,5µl/ml ; C3- 25µl/ml ; T+ -*Ridomil*®.

[2]Means within the same column followed by the same letter are not significantly different (P=0.05) according to least significant differences.

Effect of acetone extract (AE) on spore germination: The acetone extract proved to be very effective in inhibiting the germination of spores of the fungus *P*≤ 5%. At all concentrations tested and with all the strains, the

percentage of inhibition was 97% to 100%. No significant difference was obtained between this extract and the synthetic fungicide commonly used, the *Ridomil ® (*table 3).

Table 3: Percentage of non-germinated *P. megakarya* zoospores on V8 agar amended with different concentrations of plant extracts and fungicide after 12h

	***P. megakarya* isolate**				
Treatment[1]	**Mbal 121**	**nom 121**	**ngoke 111**	**mbal 212**	**njo 111**
T-	0 a[2]	0 a	0 a	0 a	0 a
C1	100b	100b	100b	100b	97b
C2	100b	100b	100b	100b	98b
C3	100b	100b	100b	100b	99b
T+	100b	100b	100b	100b	99b

[1]T- control; C1-6,25µl/ml ; C2- 12,5µl/ml ; C3- 25µl/ml ; T+ -*Ridomil ®*

[2]Means within the same column followed by the same letter are not significantly different (P=0.05) according to least significant differences

Effect of methanol extract (ME) on spores' germination : The methanol extract showed efficacy in inhibiting spore germination with a percentage of 100% inhibition at three concentrations tested with strains Mbal 121, Nom 121, ngoke111 and mbal 212, *P*≤ 5%.. Only njo111 strain showed efficiency less than *Ridomil ® P*≥ 5%. (Table 4).

Table 4: Percentage of non-germinated *P. megakarya* zoospores on V8 agar amended with different concentrations of plant extracts and fungicide after 12h

	***P. megakarya* isolate**				
Treatment[1]	**Mbal 121**	**nom 121**	**ngoke 111**	**mbal 212**	**njo 111**
T-	0 a[2]	0 a	0 a	0 a	0 a
C1	100b	100b	100b	100b	89,8b
C2	100b	100b	100b	100b	85,7b
C3	100b	100b	100b	100b	84,7b
T+	100b	100b	100b	100b	99,0c

[1]T- control; C1-6,25µl/ml ; C2- 12,5µl/ml ; C3- 25µl/ml ; T+ -*Ridomil®*

[2]Means within the same column followed by the same letter are not significantly different (P=0.05) according to least significant differences.

Effect of aqueous extract (AqE) on spore germination: The aqueous extract showed no significant inhibitory effect on spore germination compared to the *Ridomil ® positive* control, *P*≥ 5%. The highest percentage of inhibition was 12%, and was obtained with mbal 121 (Table 5).

Table 5: Percentage of non-germinated *P. megakarya* zoospores on V8 agar amended with different concentrations of plant extracts and fungicide after 12h

	***P. megakarya* isolate**				
Treatment[1]	**Mbal 121**	**nom 121**	**ngoke 111**	**mbal 212**	**njo 111**
T-	0 a[2]	0 a	0 a	0 a	0 a
C1	7,6b	2,5b	1,5a	8,0b	1,4a
C2	10,8b	2,9b	4,7b	8,3b	4,2b
C3	13,3b	3,4b	3,9b	8,3b	4,1b
T+	100c	100c	100c	100c	99c

[1]T- control; C1-6,25µl/ml ; C2- 12,5µl/ml ; C3- 25µl/ml ; T+ -*Ridomil®*

[2]Means within the same column followed by the same letter are not significantly different (P= 0.05) according to least significant differences

Fungicidal activity of extracts and fungicide: All the extracts showed fungistatic effect on all fungi at the concentrations of 6.25 and 12.5µl/ml (6.25 and 12.5mg/ml for aqueous extract). *Ridomil ® and* C3 concentration of different extracts showed fungicidal effect.

MIC_{50} and MIC_{90} determination: MIC calculated from equations derived from regression was higher for germination compared to growth. This shows the sensitivity of these samples at the stage of growth. The HE showed the highest MIC. Lethal CM (6.25µl/ml) was obtained with the AE and ME, both extracts having inhibited completely germination of all tested strains and the growth of some strains.

Table 6: MIC_{90} and MIC_{50} (in µl/ml) of the mycelia growth and germination of *P. megakarya* in the presence of *T. peruviana* extract

	Isolate	Mbal 121	Nom 121	Ngoke 111	Mbal 212	Njo 111
Minimum inhibitory concentrations (MICs) of the extracts on the growth						
HE	MIC_{50}	1,1 10^{7}	**	**	1 1,6 10^{7}	**
	MIC_{90}	5,4 10^{10}	**	**	29 10^{10}	**
EAE	MIC_{50}	8,8	13,9	23,9	9,7	39,5
	MIC_{90}	24,91	24,6	62,8	19,7	176,9
AE	MIC_{50}	6,25	15,21	6,7	78,15	7,98
	MIC_{90}	10,32	26,90	15,81	9754,37	42,68
ME	MIC_{50}	**	**	6,25	6,25	1,24
	MIC_{90}	3,2	14,58	6,25	6,25	16,10
AqE	MIC_{50}	6,25	6,2	7,5	6,25	**
	MIC_{90}	6,25	20,5	21,5	6,25	13,6
Minimum inhibitory concentrations (MICs) of the extracts on the germination of spores						
HE	MIC_{50}	1,5 10^{7}	8,3 10^{53}	**	233 10^{14}	1726 10^{14}
	MIC_{90}	2,8 10^{10}	**	**	68,9 10^{29}	42,4 10^{29}
EAE	MIC_{50}	**	**	281 10^{14}	106 10^{14}	17,6 10^{29}
	MIC_{90}	**	**	78,9 10^{29}	44 10^{14}	1,6 10^{54}
AE	MIC_{90}	6,25*	6,25	6,25	6,25	6,25
		6,25	6,25	6,25	6,25	6,25
ME	MIC_{90}	6,25	6,25	6,25	6,25	6,25
		6,25	6,25	6,25	6,25	6,25
AqE	MIC_{50}	208 10^{3}	2,8 10^{32}	556 10^{3}	1,06 10^{60}	52 10^{7}
	MIC_{90}	408 10^{6}	1,2 10^{59}	55 10^{3}	1,0 10^{98}	6,8 10^{20}

*Minimum concentration, which inhibited completely germination of spores

** Represents values that are undefined for being at zero statistically

The *in vivo* test: Test performed with AE, ME and AqE and *Ridomil* ® *on* detached pods, revealed a significant difference between the control and the different extracts at 5% by Duncan test. The rate of change of necrosis varies from one clone to another clone. SNK 10 was attacked in relation to clone BBK 1016 that had only a few small points of necrosis. It is found that increasing the concentration provides good protection to pods. Only pods treated with the aqueous extract showed an attack of fungus (Table 7).

Table7: Effect of AqE, ME and AE of *T. peruviana* on pods of different clones

	Clones pods	
Treatments	BBK 1016	SNK 10
control	3,14 a*	15,07 a
AqE	1,19 ab	5,93 b
ME	0,75 b	0,88 c
AE	0,00 b	0,00 c
AqE×10	0,00 b	0,15 c
ME×10	0,00 b	0,00 c
AE×10	0,00 b	0,00 c
Ridomil ®	0,00 b	0,00 c

***** For each clone, the values followed by the same letter in column are not significantly different at the 5% level.

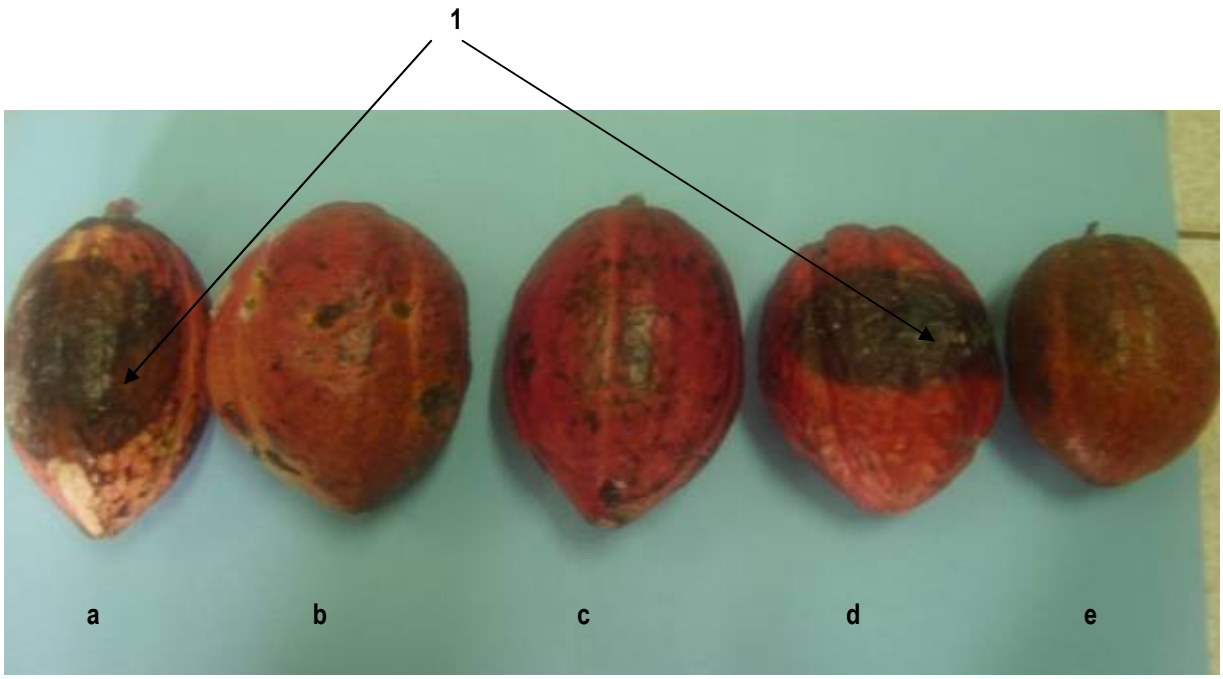

Fig 8 : Detached fruit bioassay seven days after inoculation with 3-4 10^5 zoospores of Mbal 212 using SNK10 variety treated with (**a**) water plus soap powder; (**b**) acetone extract plus soap powder; (**c**) methanol extract plus soap powder; (**d**) aqueous extract plus soap powder; (**e**) *Ridomil* ® plus water. 1= pods showing symptoms of black pod disease

DISCUSSION

The antifungal property of five extracts of *T. peruviana* was tested against five strains of *P. megakarya* at different concentrations (6.25µl/ml, 12.5 µl/ml, and 25µl/ml) and compared to that of *Ridomil* ® *(*25µg/ml). The HE stimulated the growth of all strains compared to the control. A similar phenomenon has been observed by Gata-Goncalves (2001) with the aqueous extract of the leaves of *Thevetia peruviana* obtained by infusion (80 °C) on the development of *Fusarium culmorum*. The growth stimulating effect of the fungus observed with the HE approximates the assumption of Roger (1951) which states that almost all fungicides act as stimulants in low

doses. Thus, Southan and Ehrlich cited by Roger (1951) gave the name of hormesis stimulus caused by harmful substance acting at very low doses. EAE, AqE, AE and ME were effective against the growth of strains with a strong inhibition for methanol extract. The antifungal activity of these extracts in this study could corroborate the work of Gata-Gonçalves *et al*. (2003) who explained it by the presence of the high molecular weight compounds (lactones and sterols). Other compounds in these extracts are known for their antifungal properties, triterpenes, phenols, sugars. Furthermore, these studies confirm those of Ambang *et al*. (2010) who obtained complete inhibition of the growth of the *P. megakarya* strains with methanol extract of seeds of *T. peruviana.* The results indicate that the extract with acetone and the methanol extract were found to be very active in the inhibition of spore germination for all strains with a percentage of inhibition between 98-100% in all concentrations tested. This could be due to possible concentration of compounds responsible in these two extracts. These results are in agreement with those obtained by Wildmer and Laurent (2006) who obtained complete inhibition of spore germination of *Phytophthora* spp. with plant extracts containing caffeic acid (lavender) and rosmarinic acid (rosemary) at doses of 3g / l and 6 g / l. Plant extracts have shown their effectiveness against the germination of fungal spores. This case with results of Achraf Khaldi et al. (2012) with the aqueous extract of Asphodelus tenuifolius and Zygophyllum album proved effective in inhibiting spore germination of *Penicillium expansum* with a percentage inhibition of 95.48% and 93.82%. *T. peruviana* includes bioactive substances such as saponins, tannins, sterols, terpenes, flavonoids in significant amounts, which possess antifungal and antimicrobial activity. Boulenouar *et al*. (2009) demonstrated the antifungal effect of flavonoids and the work of Gata-Goncalves (2003) showed the effect of extracts of *T. peruviana* against *Cladosporium cucumerinum.* The low MIC value obtained with AE and ME highlights the effectiveness of these extracts on the germination of fungal spores. These results are in agreement with those of Serghat *et al*. (2004) who argue that low MIC values inhibit conidial germination of *Pyricularya grisea* causative agent of rice blast. The fruits detached bioassay has demonstrated the effectiveness of extracts on plant tissues when they are treated by zoospores of *P. megakarya*. The same result was also obtained by Timothy and Lawrence (2006) on *Pyrus communis* (William variety) which treated with caffeic acid and rosmarinic acid and inoculated with zoospores of *Phytophthora spp* showed no symptoms. The heterogeneity observed in the percentages of inhibition and minimum inhibitory concentrations could be explained by the fact that fungi do not react in the same way with biopesticides.

CONCLUSION

Seed extracts of T. peruviana showed significantly effective inhibition rate against strains of *P. megakarya*. AE,ME and AqE are shown as antifungal for mycopathogen. Minimum inhibitory and minimum lethal concentrations were observed with, AE and ME. This study served as a basis for determining appropriate and effective concentration for the use of the plant for biological control against *P. megakarya*. AE,ME and AqE showed their inhibitory potency in causing brown rot on detached pods.

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