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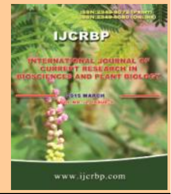

Original Research Article

Development of Cocoa Black Pod Disease (Caused by *Phytophthora megakarya*) in Cameroon when Treated with Extracts of *Thevetia peruviana* or Ridomil

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A b s t r a c t

The main objective of this work was to evaluate the impact of aqueous extract (AqE), methanolic extract (ME) of *Thevetia peruviana*, and Ridomil on cocoa black pod disease (*Phytophthora megakarya*) in Cameroon during two consecutive years in smallholder's plantation at Mbalmayo (Center Region of Cameroun). Disease distribution across various pod development stages showed that immature pods were the most susceptible to *P. megakarya*. Weekly observations of the pod distribution and disease progression at various developmental stages on cacao trees sprayed with Ridomil fungicide, methanol extract and aqueous extract of *Thevetia peruviana*, or untreated control trees indicated that the total pod production and incidence of black pod was significantly different between treatments. The disease rates were 53.65%, 67.6%, 36.92% and 73.95%, in the plots treated with AqE, ME, fungicide, and untreated respectively, in 2011, and 11.95%, 28.72%, 10.51% and 54.03% in the same plots in 2012. The major results obtained compared to the rate of rot showed that the aqueous extract and Ridomil treatments were close during these campaigns. Aqueous extract reduced the impact of this disease by 20.3% in 2011 and by 25.31 % in 2012. The rainfall records revealed 1532.6 mm and 889.9 mm of rain during 2011 and 2012 cocoa seasons respectively. This study showed that plant control of black pod disease with aqueous extract was promising, and on small and medium scale, could be an effective and cheap formulation for the control of black pod disease.

K e y w o r d s

Black pod disease
Cocoa seasons
Phytophthora megakarya
Plant extracts
Ridomil
Thevetia peruviana

Introduction

Cocoa (*Theobroma cacao* L.) is a plant native to tropical rainforests of Central and South America (Alverson et al., 1999; Whitlock et al., 2001). This plant is cultivated for its beans which are used as raw material in various industries for the manufacture of various chocolate, cosmetics, and starch. In Cameroon, cocoa is one of the major export crops among others. From the social perspective, the economy of more than 600,000 families is based on cocoa (Anonymous, 2009). Although the cocoa production has considerably increased in recent years (ICCO, 2007), global production during the 2012-2013 campaign was 3,931,000 tons, against a demand of about 4.091.000 tons. This application proposes the ICCO (International Cocoa Organisation) should reach 4.4 million tons by 2018. Seventy one percent of world production came from Africa (2.813.000 tons), of which 225 000 tons for Cameroon against 16 % for Latin America (618 000 tons) and 13% for Asia and Oceania (500 000 tons) (ICCO, 2013). However, production remains low due to the fact that in peasant milieu, cocoa farming is affected by parasitic hazards.

The pod rot of cocoa, is in Africa the most serious cocoa infection caused by Stramenopile (Martin and Tooley, 2003) of the genus *Phytophthora*. *Phytophthora megakarya* proves to be the most aggressive Stramenopile species that attack cocoa in Cameroon (Nyassé, 1997). Its attack causes losses that can reach more than 80% when environmental conditions are favorable for its development (Despréaux et al., 1988; Berry and Cilas, 1994). The imperatives of profitability that require high performance and quality products, in production systems, make plant protection a vital activity.

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, elimination of the diseased pods (Ndoumbe-Nkeng and Sache, 2003; Ndoumbe-Nkeng et al., 2004) and modification of the microclimate thereby creating unfavorable 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 for the majority of smallholders. The pesticides generally used are not always accessible to farmers (Kébé, 1999). Developing genetic resistance against this disease is considered the most cost effective and reliable method for control. The clones developed are tolerance but don't have the total resistance (Iwaro et al., 2000). This method is usually slow in developing varieties for farmers' use. Until resistant varieties can be developed and distributed to the farmers, the use of biological control agents and natural products are more practical alternatives for an integrated pest management strategy. However the use of *Trichoderma* species (Tondje et al., 2005; Holmes et al., 2004; Deberdt et al., 2008; Mpika et al., 2009) provided interesting results, but a number of limitations with respect to the action spectrum of these organisms and the problem of viability and formulation of this product accrue. Fight against *Phytophthora* using extracts is not developed, somewhat in South of Cameroun extract of *Cannabis sativa* are used by smallholders against *P. megakarya* (Coulibaly et al., 2002).

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* (Pers.) K. Schum are considered as potential sources of biologically active compounds, such as insecticides (Reed et al., 1982), rodenticides (Oji and Okafor, 2000), fungicides (Gata-Goncalves et al., 2003; Ambang et al., 2010; Ambang et al., 2011), virucides (Tewtrakul et al., 2002) and bactericides (Saxena and Jain 1990; Reddy 2010). *Thevetia peruviana*, has already shown its effectiveness in reducing the inoculum pressure as well as the incidence of black pod rot (Ambang et al., 2010). The objective of this work was to evaluate, the impact of aqueous and methanolic extracts of seeds of yellow oleander (*Thevetia peruviana*) and Ridomil in black pod disease and the dynamics of pod production.

Materials and methods

Obtention of plant extracts

The mature fruits were collected from different locations of the city of Yaounde. The seeds obtained from mature fruits of *T. peruviana* were dried in the laboratory at room temperature (22-25 °C) during one month and then almond obtained was weighed using balance 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 h each. The product obtained was concentrated with Rota vapor and then kept at 4°C (refrigerator) throughout the experiment (Gata-Goncalves et al., 2003). The product obtained was then concentrated with Rota vapor and then kept at 4°C (refrigerator) throughout the experiment (Ngoh Dooh, 2006).

To obtain the aqueous extract (AqE), 250 g pulp, was wrapped in muslin fabric and soaked directly in sterile distilled water (10 l) during at least 12 h. The next day the tissue containing the dough was gently squeezed to extract the maximum amount of product (Stoll, 1994; Ngoh Dooh et al., 2014a), which was applied directly in the field.

Meteorological measurements and experimental design

Total rainfalls (mm), were recorded daily by the Mbalmayo meteorological station and transmitted to the meteorological station of Yaoundé. The minimum and maximum temperatures and humidity were also taken using hygro thermometer apparatus in the four experimental sites, under the shadow and the means were recorded. The study was conducted in a smallholder's plantation located in Mbalmayo town (Ekombitie), in the Central Region of Cameroon during two consecutive years (2011 and 2012). This area is located in the agro-ecological zone with humid bimodal rainfall pattern. This plantation had not been treated over the three previous years before experimentation.

The experimental trial was at least 1 ha in size with cacao germplasm consisting of a mixture of hybrids and "German Cocoa" (according to the survey conducted among farmers). The spacing between cacao trees within a plot varied from 2.5 × 2.5 m to 3.0 × 3.0 m. Trees were on an average 50 years old and intercropped with other wild and domesticated fruit trees (coconut palms, mangoes, avocado pear and plum/safou trees). The tree height is about 4-8 m and shading was generally dense.

The experimental design used was randomized block design. It included three blocks. Each block contained four treatments (control, AqE, ME and Ridomil) in the elementary plots. Each elementary plot contained 25 cacao trees randomly chosen.

Each cocoa tree had a label that indicates the number of the tree, the block and the type of treatment to receive. The experimental plots were separated from each other by one or two border rows, made up of cocoa trees. Each border was "sanitary cordon" sprayed with fungicide Ridomil Plus Gold 66WP (metalaxyl + copper oxide, 3.33g/l) every 4 weeks (once a month). On each tree, two heights (H) were defined as follows: H_1 from 0 to 1.5 m above the ground and H_2 from 1.5 to 3 m in height on the stem (Silatsa, 2006).

These blocks were allocated based on the homogeneity of the experimental site (density of shade and trees, relief of the site). The control plots received no treatment. The maintenance of the plots was done every two weeks by weeding and pruning of trees when necessary. The diseased pods were removed each week. Cultural control methods which consisted of weekly removal of diseased pods and regular harvesting and pruning, were practiced in all experimental plots, as recommended by Ndoumbè-Nkeng et al. (2004). No fertilizer was applied.

Application of extracts and Ridomil

The mixtures were prepared by mixing the extracts obtained in 15 liters of water. Ten g of soap used as wetting agent was added to the obtained liquid (Stoll, 1994). For ME and AqE, dose was that obtained in previously *in vitro* and *in vivo* tests. For ME dose was 6.25 ml/l and for the AqE, the dose was 8.33 g/l (Ngoh Dooh et al., 2014b). The dose of Ridomil used was that recommended; a sachet of 50 g in 15 l (3.33 g/l). Treatments were applied with a knapsack sprayer to a height of about 4 to 5 meters at a rate of 15 L/ha.

Volume applied per plot was 4.8L in mean. The aqueous and methanolic extracts were applied to the soil around the tree and on the entire tree, while Ridomil Gold Plus WP 66 was applied only on the fruit (Silatsa, 2006). The frequency of application of treatments was once every two weeks for extracts and once every four weeks (once per month) for Ridomil Gold Plus 66WP (RD) in each block. Treatment of plants with extracts and Ridomil began before the onset of symptoms of black pod rot.

Epidemiological observations

The observations were recorded from the first week of treatments (April 2011). Healthy fruits at different heights and at various developmental stages including cherelles (less than 10 weeks old), immature pods (10 to 22 weeks old) and ripe pods were counted on all 25 replicate trees each week. The ripe fruit was counted, harvested or marked to avoid counting it twice. The number of cherelles with symptoms or wilted pods, rotten pods, pods damaged by rodents was recorded during each week before being discarded.

Statistical analyses

The calculation method for the weekly pod rot rate (TPt) was adapted from the formula used by Berry and Cilas (1994) and De Jésus (1992) as follows:

$$\text{TPt} = \frac{Pt}{Pt + MSt + CInt} x100$$

Where,
t is treatment; Pt is number of rotten pods in treatment t; CInt is the number of immature pods in treatment t in the last week of observation; MSt is the number of ripe pod in the last week of observation.

The black pod disease dynamic was also studied by calculating the cumulative weekly pod rot rates ($TPCH_n$) and disease temporal progression.

$$TPCH_n(\%) = \frac{\sum_{i=1}^{i=n} P_i}{CI_N + \sum_{i=1}^{i=n}(P_i + MS_i)} \times 100$$

Where,
i is the week of observation ; N is the last week of observation ; CI_N is the number of pods in the last week of observation ; P_i is the number of rotten pods in the week i ; MS_i is the number of ripe pods harvested in the week i.

The modified formulae of TPt permits to calculated the incidence at different developmental stages and different tree height in order to analyze the effect of the pod age and height on disease incidence and to identify a particularly susceptibility stage or height.

An analysis of variance (ANOVA) was done per year and per variable using SPSS 16.0 (the generalized linear model) and GraphPad InStat Softwares. Duncan test at 5 % were used to compare the averages. The interaction between pod age × treatment and tree height × treatment were also equally studied.

Results

Dynamics of the pod production

The weekly potential production per treatment or plot was estimated over the two consecutive years corresponding to 2011 and 2012 seasons. In 2011, the pod production was observed during the whole campaign. The production was higher in control and methanol plots with a production peak reaching 441 pods compared to the other plots. Production began at the same time in all the plots (Fig.1A). In 2012, production was very high with the plots reaching 1000 pods. Plot treated with AqE, presented low pod production, with at the week 17, about 800 pods compared to the other plots (Fig.1B). The potential production in the first season was lower compared to the second season.

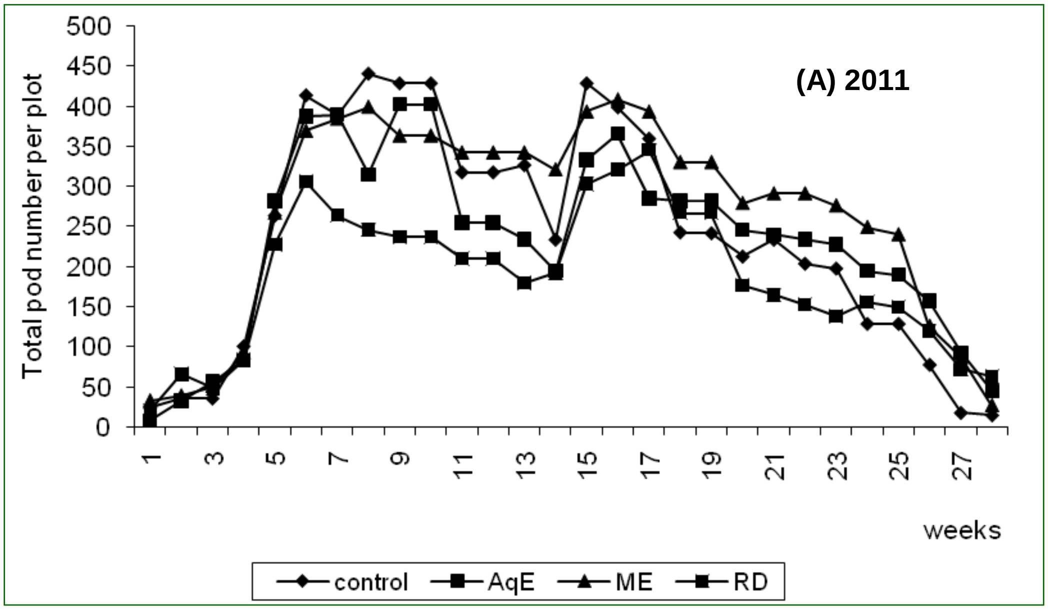
Dynamics of the pod rot progression

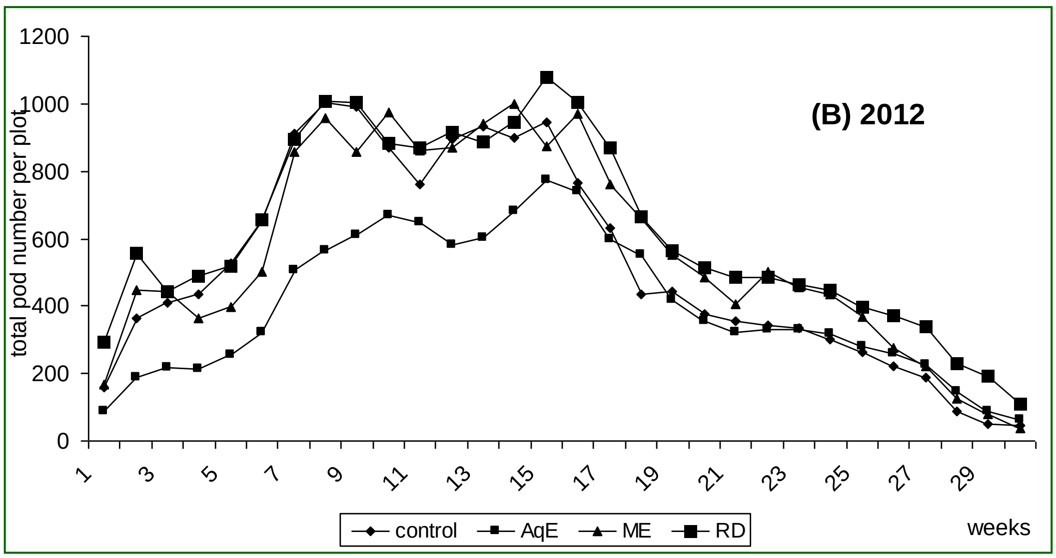
A descriptive study of the progression of black pod disease depending on weather conditions during the 2011 campaign

In 2011 cocoa season, the disease was more severe. It began three weeks after the beginning of the observation on the line of the control and for all the other curves, it appeared late in approximately fifteen weeks. The curves have generally the same shape. Curves of Ridomil (RD), aqueous extract (AqE) and methanol extract (ME) showed the exponential phase from the fifteenth week of observation until the last week. The control curve shows a stable phase from week 6 to 11 and the resumption of the disease then increases until the last week (Fig. 2).

The results obtained on all climate data showed that the 2011 campaign was marked by heavy rains. Rainfall was about 1532.6 mm. The maximum and minimum temperatures obtained ranged respectively from 23.1°C to 30.05°C and 22.8°C to 28.6°C. Minimum temperatures coincided with the highest rainfall months (July, August) and maximum temperatures coincided with the month of October (Fig. 3).

Fig.1: Pods production per plots treated with AqE, ME, Ridomil and untreated (control) in year 2011 (A) and 2012 (B).





A descriptive study of the progression of black pod rot depending on weather conditions during the 2012 campaign

Apart from the curve of Ridomil Gold Plus 66 (RD) which had a late start to black pod rot, all other curves are almost similar, highlighting five stages of disease progression**:**

- The start of the rot that occurred within the first five weeks of observation for control (relative latent phase) and three weeks later for AqE and ME and after ten weeks of observation for Ridomil**.**

- The exponential phase of the rot that follows the starting stage and from the fifth week of observation. It occurred in the short rainy season with an important passage at cherelles (stage 1) and immature pods (stage 2). It is much more pronounced in the curve of control which is thus detached from all other curves (Fig. 4)

- The first stationary phase of the disease: it took place in the least rainy months and corresponds to cumulated rot rate stability since the beginning of brown rot. It usually began from the seventeenth week of observation and coincided with a strong fruit production.

Fig. 2: Cumulated pod rot values (%) for trees treated with aqueous extract (AqE),methanol extract (ME), Ridomil (RD) and untreated control plots in year 2011.

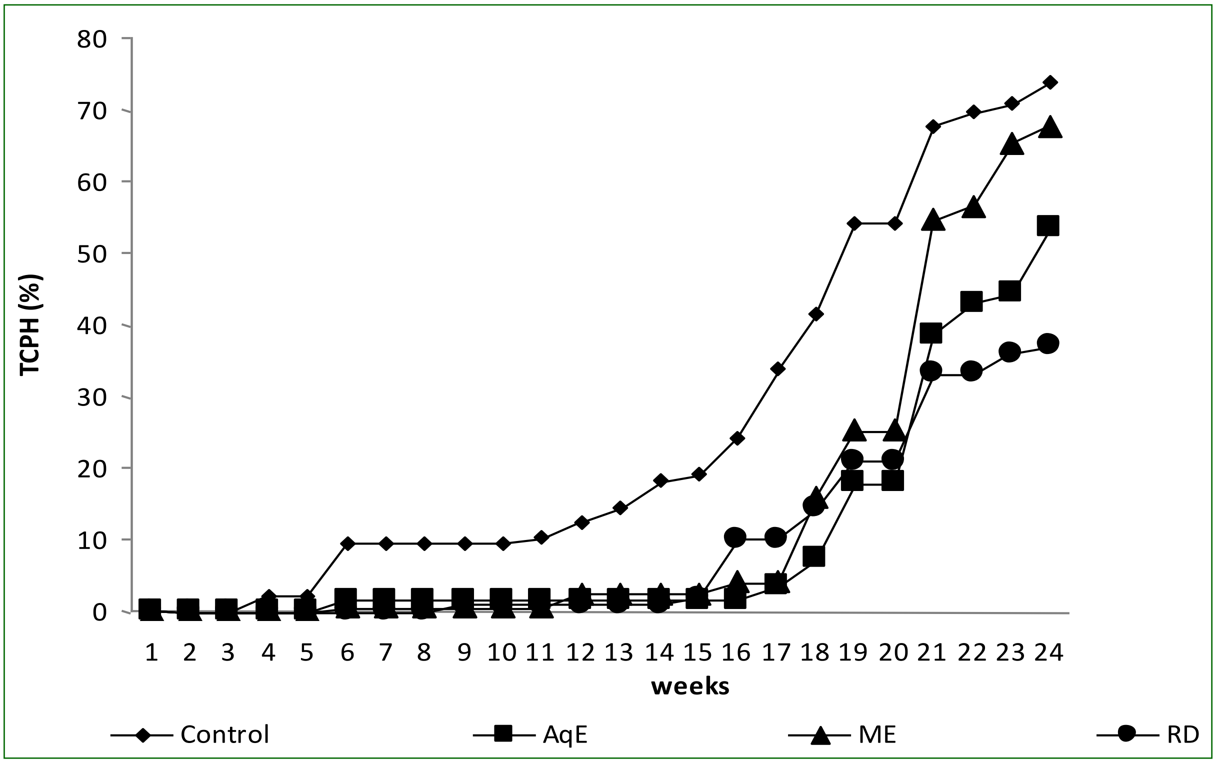
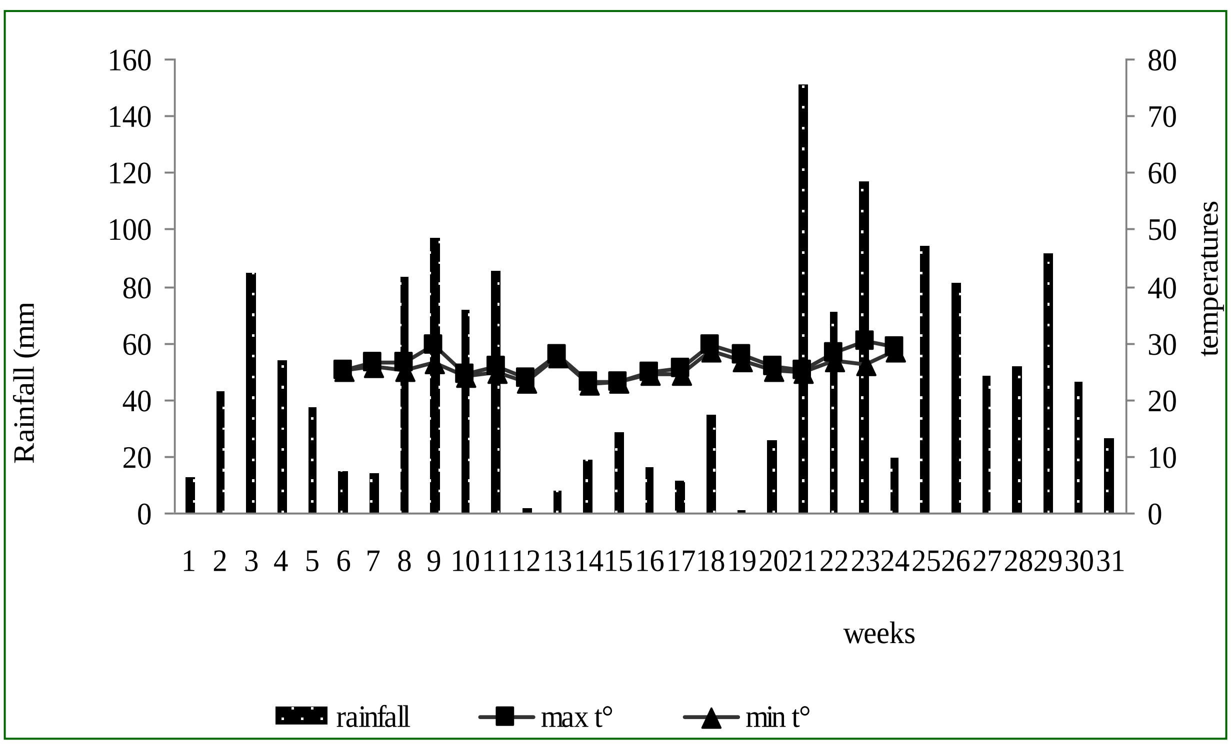


Fig. 3: Weekly rainfall, minimum and maximum temperatures in the locality of Mbalmayo during the 2011 campaign.



- The recovery of the disease: it began with the return of the rains and was mainly observed in all curves except RD curve (Fig. 4). It extended until the twenty-seventh week of observation in general on all curves except in RD curve which has not experienced this phase since the previous phase because it is almost horizontal. At the moment when this phase occurred fruits were at "immature pods" and "ripe pods" stage.

- The final stationary phase: it took place from twenty-seventh week of observation and corresponds to the end of the cocoa production (Fig. 4)

The 2012 campaign was less watered by rain. Rainfall was about 889.9 mm throughout the campaign. The maximum and minimum temperatures obtained ranged respectively from 27.33°C to 34.96°C and from 27.2°C to 32.0°C. These temperatures were higher than the previous season. Temperature fluctuations were low during this season (Fig. 5). Despite these high temperatures compared to 2011, humidity was higher in 2012 compared to 2011 (results not shown).

Fig. 4: Cumulated pod rot values (%) for trees treated with aqueous extract (AqE), methanol extract (ME), Ridomil (RD) and untreated control plots in year 2012.

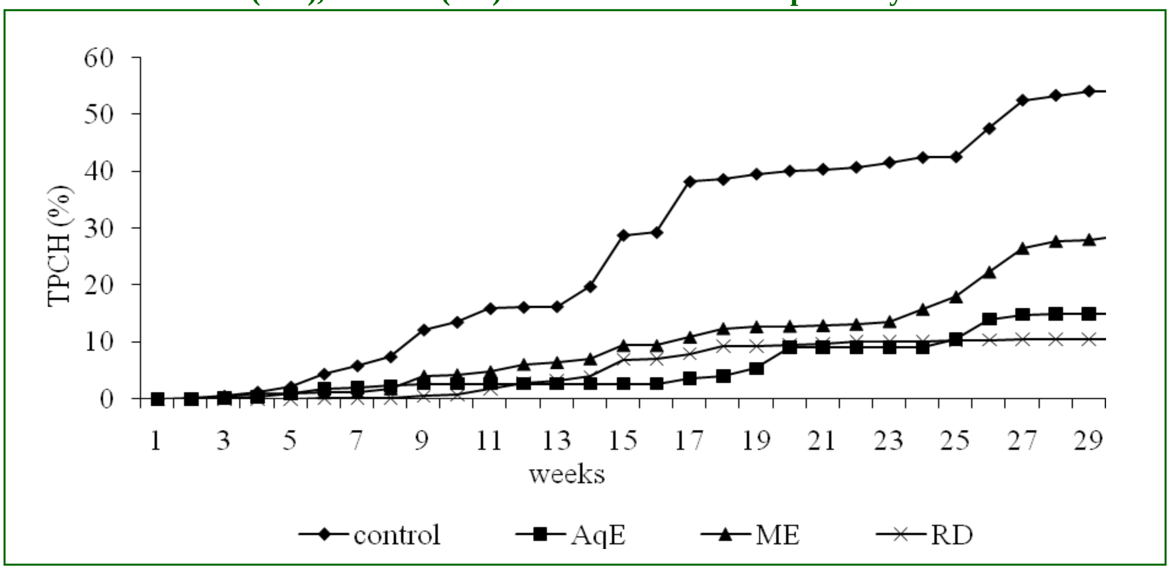
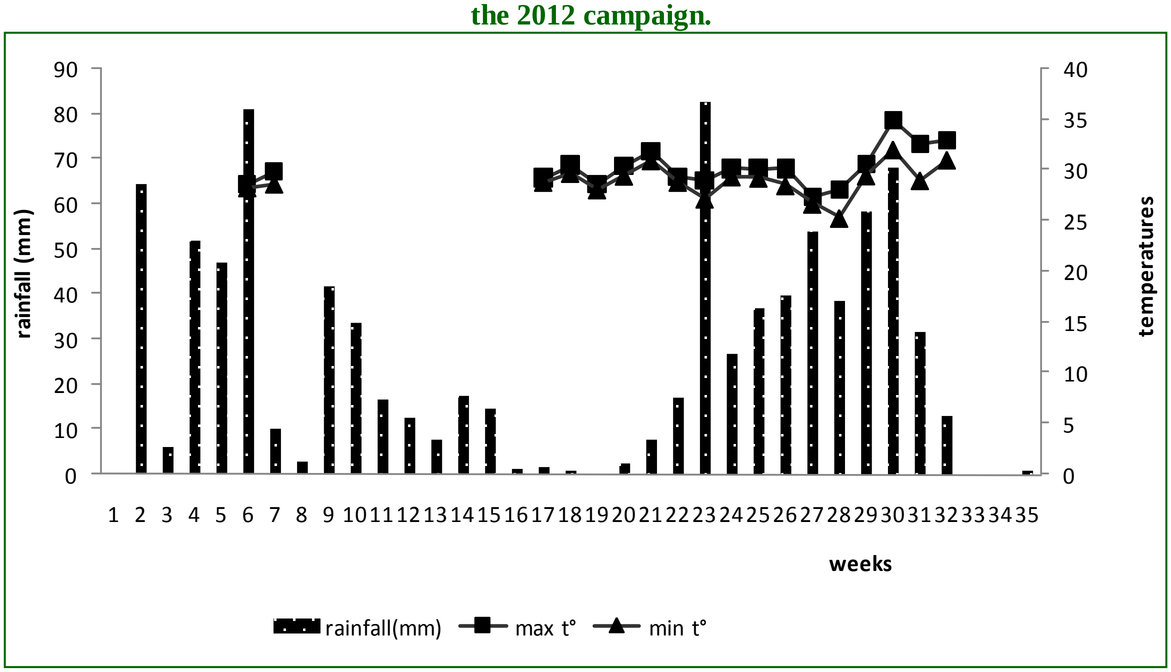


Fig. 5: Weekly rainfall, minimum and maximum temperatures in the locality of Mbalmayo during the 2012 campaign.



Effect of the four treatments on the disease incidence during 2011 and 2012 campaign

The results showed a significant effect of the treatments on the disease incidence (P< 0.05) during the two years campaigns. The rate of black pod rot was higher during 2011 season in all treatments. There was no significant difference (p=0.146), between the control (73.95 %) and the ME (67.6 %) while the treatment with the AqE (53.65 %) showed similar efficacy than treatment with the RD fungicide (36.22 %) (p=0.50). The results highlight the potential of AqE treatment to reduce the disease in the field in comparison to the control treatment (Table 1).

Table 1. Incidence of black pod disease in the different treatments in 2011 and 2012 at the last week of observation.

Treatments	Rate of pod rot (%)	
	2011	2012
Control[a]	73.95 a	54.03 a
AqE[b]	53.65 bc	14.95 b
ME[c]	67.6 ab	28.72 a
RD[d]	36.92 c	10.51 b

***** Values followed by the same letter are not significantly different at 5%; [a] Untreated control plots; [b] Plots treated with aqueous extract; [c] Plots treated with methanol extract; [d] Plots treated with Ridomil Gold Plus.

Statistical analysis of the rate of black rot revealed a significant treatment effect (p<0.05) and comparison of averages highlighted two different statistical groups. Black pod rot was moderate during the 2012 campaign compared to the previous year. Once again, there was no significant difference between treatment with the methanol extract and the control (p=0.334)**.** The treatment with the aqueous extract (14.95 %) showed no significant difference (p= 0.50), compared to treatment with the Ridomil (10.51 %) (Table 1).

Incidence of tree height on the black pod disease during the two campaigns

Analysis on the incidence of black pod rot at different heights, H_1 and H_2, revealed significant interaction height × treatments (p<0.001) during the two campaigns, suggesting that the incidence for each height is likely to vary from one treatment to another. Subsequently, analysis to assess the effect of height on pod rot incidence thus was performed treatment by treatment (Table 2). Statistic analysis revealed that all the pods was attacked in the same way at all heights meaning that no height was susceptible to the disease with all treatment (p=0.266). Except in plot treated with Ridomil where Height H_1 show low incidence (12.50 %) compared to H_2 (51.36 %).

The effect of "height" of cocoa on the influence of black pod rot was no significant for any of the treatments during the 2012 campaign (p=0.665). Observed in all treatments, pods are attacked in the same way at all heights which means that no height of cocoa was susceptible to black pod rot (Table 2).

Table 2. Incidence of black pod disease in the different two heights (H_1 and H_2) in 2011 and 2012 campaign at the last week of observation.

Treatments	Rate of pod rot (%)			
	2011		2012	
	H_1 * *	H_2 * * *	H_1	H_2
Control[a]	68.75 a	76.56 a	55.24 a	52.68 a
AqE[b]	45.16 a	43.59 a	19.54 a	11.43 a
ME[c]	41.88 a	73.10 a	41.58 a	28.06 a
RD[d]	12.50 a	51.36 b	7.46 a	14.00 a

***** For each treatment and same year, values followed by the same letter in row are not significantly different at 5%; * * 0 to 1.5 m from soil; * * * 1.5 to 3 m from soil; [a] Untreated control plots; [b] Plots treated with aqueous extract; [c] Plots treated with methanol extract; [d] Plots treated with Ridomil Gold Plus.

Incidence of the developmental stage on black pod disease during 2011 and 2012 cacao season

Analysis on the incidence of black pod rot at different developmental stages S_1 and S_2 of cocoa pods showed a highly significant interaction, treatment × stage in 2011 (p<0.0001). For this reason, an analysis to assess the effect of developmental stage on the incidence of pod rot was performed for each treatment. The results revealed a significant effect of development stage of pods on the disease incidence (Table 3). In all experimental plots treatments, the disease incidence is significantly higher in the "immature pod" stage compared to the "cherelle" and ripe stages for 2011 season (p=0.001). But, in the AqE plot, ‘’cherelle’’(0.00 %) showed low significant incidence compared to ripe pod (0.94 %). The black pod disease incidence on the various developmental stages indicated that losses due to the disease were higher at the “immature pod” stage in control, AqE, ME, and RD plots. In all treatments experimental plots in 2012 season, the disease incidence was significantly higher in the “immature pod” stage (S_1) compared to the “cherelle” stage (S_2) (p=0.002). The ripe pod stage was least susceptible to black pod with extracts. The black pod disease incidence on the various developmental stages indicated that losses due to the disease were higher at the “immature pod” stage in control, AqE, ME, and RD plots (Table 3).

Table 3. Incidence of black pod disease at different stages of development (S_1, S_2 and S_3) during 2011 and 2012 campaign at the last week of observation.

Treatments	Rate of pod rot (%)					
	2011			2012		
	S_1 *	S_2 * *	S_3 * * *	S_1	S_2	S_3
Control[a]	6.25 b	67.71 a	0.50 c	2.44 b	51.59 a	0.11 c
AqE[b]	0.00 c	53.65 a	0.94 b	1.09 b	13.87 a	0.02 c
ME[c]	1.74 b	65.86 a	0.59 c	1.04 b	27.69 a	0.02 c
RD[d]	0.00 b	36.92 a	0.15 b	1.82 b	8.70 a	0.03 c

For each treatment and same year, values followed by the same letter in row are not significantly different at 5%; * “cherelles”; * * immature pod; * * * ripe pod; [a] Untreated control plots; [b] Plots treated with aqueous extract; [c] Plots treated with methanol extract; [d] Plots treated with Ridomil Gold Plus.

Discussion

This study is an experiment in the field under actual farming conditions starting from a laboratory test (Ambang et al., 2010; Ngoh Dooh et al., 2014b). The study helps to better understand the environmental parameters prevailing in the cocoa plantations, while emphasizing the components of epidemiological triangle namely the host plant (*Theobroma cacao* L.), the pathogen (*P. megakarya*) and the environment (abiotic and biotic factors, including our extracts of *T. peruviana*). Temperature and rainfall data compared to exchange pod rot rate have shown that, the wide variation in rainfall during the two campaigns revealed an existing relationship between the fluctuations in rainfall and severity of the impact of black pods rot of cocoa. Indeed, it was found that the disease and rainfall was higher in 2011 (1532.6 mm rainfall) than in 2012 (889.9 mm of precipitation). These results confirm those obtained by Ndoumbe-Nkeng et al. (2002) who showed that water (rainfall), temperature and moisture were essential for the dissemination and germination of spores of *Phytophthora megakarya* especially in high rainfall areas in Cameroon. Ndoumbe-Nkeng et al. (2009) also showed that the factors that most influence the black pod rot are rainfall and to a lesser extent, the maximum temperature. These variables of precipitation could therefore explain the higher impact during the 2011 campaign. Weak precipitation recorded in 2012, could explain the low incidence obtained during this campaign.

On the other hand, this higher incidence in 2011 could be explained by the fact that the site was abandoned for several years and therefore was not subject to the fungicide. Furthermore, during the 2012 campaign, disease was moderate with an incidence rate of 54.03 %; 14.95 %; 28.72 % and 10.51 % respectively for treatments control, AqE, ME and RD. This result can be explained partly by the fact that the inoculums pressure was reduced during the previous season by the extracts used. A significant year × treatment interaction was revealed. Generally, treatments with extracts made from the seeds of *Thevetia peruviana* have behaved differently in the presence of the agent *P. megakarya* from one year to another. During the two study campaigns, the incidence of black pod disease, was not significantly different in the ME treatment compared with control, despite the very different values. This result can be explained by a low concentration of the methanol extract or poor formulation of this extract. This inefficiency of the ME in the field could also be explained by factors such as, UV radiation (products applied in the field, are directly subjected to UV radiation and therefore photolytic decomposition), the decomposition of substances under the action of microorganisms present in the leaf and pod surface and insufficient resistance to precipitation (Neuhoff et al., 2003).

Whatever the year, the incidence of black pod disease was found not significantly different from each other in height in all treatments. This result could be explained by the practice of agro-technical methods (cultural methods) that create unfavorable conditions for the development of the pathogen so that the pressure of the inoculum was reduced. This result is not close to that obtained by Silatsa **(**2006) who, at the height H_1 of cocoa plants got a very high incidence of black pod rot. Previous work, Muller (1974) and Gregory et al. (1984) showed that rainwater dripping in ensuring the dissemination of propagules of *P. megakarya* throughout the cocoa from the primary inoculum point then by the splashing phenomenon, they ensure even dispersal of propagules in propelling the ground on the nearest ground pods (pods belonging to the height H_1). According to Ndoumbe-Nkeng et al. (2004), fruits, in cocoa tree height H_2 are contaminated from water flowing onto the tree through the flower cushions which are also considered as inoculum points. Then the transmission is in pods by pods through diseased fruits maintained on the tree.

The incidence of black pod rot was higher in developmental stage S_2 (immature pods) fruits in all treatments during the two study seasons compared to S_1 stage (cherelles) and S_3 stage. There is a significant difference in the incidence of disease in these three stages of fruit development in all treatments without exception. This result could be explained by the fact that many cherelles are formed early in the season when environmental conditions are not favorable enough for the development and expansion of the pathogen responsible of black pod disease. This result is similar to that obtained by Deberdt et al. (2008) who in these stages found a very high rate of rot pod stage S_2 (immature pods). These results, also confirm those obtained by Blaha and Lotode **(**1976), who demonstrated the influence of the pod physiological stage on the success of the infection, and those obtained by Efombagn et al. (2004) who found the 2-3 month old developmental stage of the pod to be the most susceptible to black pod disease.

The ripe pods were very less affected by black pod rot during the two years of study. This is certainly the cause of their number and their low density compared to immature pods as they were constantly harvested. The envelope hulls of ripe pods, stiffer than immature pods would also explain this observation. This result is similar to that obtained by Deberdt et al. (2008), which had a low incidence of rot on ripe pods.

Similar to the previous studies, the present study has also shown the positive impact of the fungicide treatment on black pod disease (Ndoumbe-Nkeng and Sache, 2003). When compared to the control plot, AqE disease impact was significantly reduced by 20.3 in 2011 and by 25.31 % in 2012. Ridomil Plus Gold 66WP is known to have a direct toxic action against *P. megakarya*, mainly through persistence of metalaxyl, which, after spraying penetrates inside the pods and so prevents the infection. However its marked price is consider being too expensive for smallholders in Africa and its toxicity towards human and environment remains a concern.

The effectiveness of AqE can be explained by the presence of sterols, phenols, essential oils, monoterpenes, coumarins, tannins and sugars which are secondary metabolites strong antifungal power. Gata-Gonçalves et al. (2003) obtained using high performance liquid chromatography (HPLC), numerous secondary metabolites such as sterols, terpenes, and lactones. They showed the efficacy of these compounds on the growth of *Cladosporium cucumerinum*. Similarly, the screening performed by Ngoh Dooh et al. (2014a), revealed the same compounds in the aqueous and methanolic extracts of *Thevetia peruviana* and subsequently their effectiveness against *Colletotrichum gloeosporioides* f.sp. Manihotis. Ambang et al. (2010) have shown the efficacy of extracts from *Thevetia peruviana in vitro* and *in vivo* on the development of *P. megakarya*. Moreover Ambang et al. (2011) showed the effectiveness of the methanol extract of the seeds of *T. peruviana* on the control of leaf spot disease caused by *Cercospora* sp on groundnut.

The plant used in this study belongs to Apocynaceae. The extracts are rich in compounds. This could act against target microorganism in several ways. They can inhibit the growth by acting in metabolic functions like cell division. Others inhibited respiration by blocking ATP production or induced plant resistance (Laurent et al., 2003; Chwaleka et al., 2006).

During cacao season, it is recommended to use 6 packets of Ridomil per ha. Every packet cost 500 Fcfa.

The application of fungicide began on April-may months, and in period of great rainfall of September (SODECAO, personal communication). Smallholders can benefit by using AqE which does not cost anything**.** Seeds of *T. peruviana* can be found anywhere in Cameroon and around some cocoa fields. Smallholders will gain an increase yield and economise by not buying fungicides**.**

Conclusion

The 2011 campaign saw strong precipitation and higher black pod disease compared to the 2012 cocoa season. These different rot rates obtained in the field during the two years demonstrated that, the aqueous extract is more effective than the methanol extract at the doses used. AqE showed the same efficacy than Ridomil (fungicide reference). AqE reduced the impact of this disease by 20.3 in 2011 and by 25.31 % in 2012. These extracts from *Thevetia peruviana* (AqE) appear to be a promising avenue in the IPM against black pod disease. They could be valued and popularized because of their biodegradable nature and also because of the dual economic and environmental benefits they provide for cocoa farmers.

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References

Alverson, W.S., Whitlock, B.A., Nyffeler, R., Bayer, C., Baum, D.A., 1999. Phylogeny of the core Malvales: evidence from *ndhf* sequence data. Am. J. Bot. 86, 1474-1486.

Ambang, Z., Ndongo, B., Essono, G., Ngoh Dooh, J.P., Kosma, P., Chewachong, G.M., Asanga, A., 2011. Control of leaf spot disease caused by *Cercospora* sp on groundnut (*Arachis hypogeae*) using methanolic extracts of yellow oleander (*Thevetia peruviana)* seeds. Australas. J. Crop Sci. 5(3), 227-232.

Ambang, Z., Ngoh Dooh, J.P., Essono, G., Bekolo, N., Chewachong, G., Asseng, C.C. 2010. Effect of *Thevetia peruviana* seeds extract on *in vitro* growth of four strains of *Phytophthora megakarya*. Plant Omics J. 3(6), 204-209.

Anonymous, 2009. Les mesures prises par le gouvernement pour parvenir à une économie durable: cas du Cameroun. Table ronde sur l'économie cacaoyère mondiale, rapport annuel sur la production de cacao, 45 p.

Bautista-Banos, S., Hernandez-Lopez, M., Barrera-Neecha, L.L., 2000. Antifungal screening of plants of the state of Morelos, Mexico, against four postharvest pathogens of fruits and vegetables. Mexican J. Phytopathol. 18, 36-41.

Berry, D., Cilas, C., 1994. Etude du comportement d'une parcelle diallèle 6 X 6 vis à vis de la pourriture brune des cabosses de cacaoyer due à *phytophthora* spp. au Cameroun. Actes de la 11ème conférence internationale sur la recherche cacaoyère, pp 91-96

Blaha, G., Lotode, R., 1976. Un caractère primordial de sélection du cacaoyer au Cameroun: la résistance à la pourriture brune des cabosses de cacaoyer. Cafe Cacao Thé 20, 97-116.

Chwaleka, M., Lalunb, N., Bobichonc, H., Plea, K., Voutquenne-Azabadiokoa, L., 2006. Structure-activity relationships of some hederagenin diglycosides: Haemolysis, cytotoxicity and apoptosis induction. Biochim. Biophys. Acta 1760, 1418-1427.

Coulibaly, J.H., Balerly, C.F., Gene, J., 2002. Cocoa (Chocolate Bean) Growing in the Florida Home Landscape. University of Florida, Ifax Extension HS 1057, 1-8.

De Jesus, A.M., 1992. Exploitation des résultats de l'étude épidémiologique sur la pourriture brune. Course report, CIRAD-CP, Montpellier.

Deberdt, P., Mfegue, C.V., Tondjé, P.R., Bon, M.C., Cucamp, M., Hurard, C., Begoude, B.A.D., Ndoumbe-Nkeng, M., Helbar, P.K., Cilas, C., 2008. Impact of environmental factors. Fungicides and biologicals on cocoa pod production dynamics and black pod diseases (*Phytophthora megakarya*) in Cameroun. Biol. Contr. 44, 149-159.

Despréaux, D., Cambrory, D., Clément, D., Nyassé, S., Partot, M., 1988. Etude de la pourriture brune des cabosses de cacaoyer au Cameroun : définition de nouvelles méthodes de lutte. Xe Conf. Inter. Rech. Cacaoyère., Santo Domingo, Dominican Republic. pp. 407-412.

Efombagn, M.I.B., Marelli, J.P., Ducamp, M., Cilas, C., Nyasse, S., Véfonge, D., 2004. Effect of fruiting traits on the field resistance of cocoa

(*Theobroma cacao* L.) clones to *Phytophthora megakarya*. J. Phytopathol. 152, 557-562.

Gata-Gonçalves, L., Nogueira, J.M.F., Matos, O., Bruno De Sousa, R., 2003. Photoactive extract from *Thevetia peruviana* with antifungal properties against *Cladosporium cucumerinum*. J. Photochem. Photobiol. 70(1), 51-54.

Gregory, P.H., Griffin, M.J., Maddison, A.C., Ward, M.R., 1984. Cocoa black pod: a reinterpretation. Cocoa Growers Bull. 35, 5-22.

Holmes, K.A., Schroers, H.J., Thomas, S.E., Evans, H.C., Samuels, G.J., 2004. Taxonomy and biocontrol potential of a new species of *Trichoderma* from the Amazon basin of South America. Mycologic. Progress 3(3), 199-210.

ICCO, 2007. Annual forecasts of production and consumption and estimates of production levels to achieve equilibrium in the world cocoa market. 10 p.

ICCO, 2013. International Cocoa Organization, Quarterly Bulletin of Cocoa Statistics. Vol. XXXVIII (2), 12 p.

Imtiaj, A., Rahman, S.A., Alam, A., 2005. Effect of fungicides and plant extracts on the conidial germination of *Colletotrichum gloeosporioides* causing mango anthracnose. Microbiol. 33(4), 200-205.

Iwaro, A.D., Sreenivasan, T.N., Butler, D.R., Thevénin, J.M., Mooleedhar, V., Bekele, F., Sounigo, O., Umaharan, P., 2000. Rapid screening for *Phytophthora* pod rot resistance by means of detached pod inoculation. In: Proceedings of the CFI/ICCO/IPGRI Projet Workshop on Workin procedures for cocoa germplasm evaluation and selection (Eds.: Eskes, A.B., Efron, Y.). 1-6 February 1998. Montpellier, France. pp.109-111.

Kébé, I.B., 1999. Rapport d'activité 1998. Programme Café Cacao Cola, CNRA, Cote d'Ivoire, 39 p.

Laurent, P., Dooms, C., Braekman, J.C., Daloze, D., Habibjiwan, J.L., Rozenberg, A., Termonia, A., Pasteels, J.M., 2003. Oleamane glycosides in defensive glands of Chrysomelid beetles are produced from dietary B-amyrin. Naturwissenschaften 90, 524-527.

Martin, N.F., Tooley, W.P., 2003. Phylogenetic relationships among *Phytophthora* species inferred sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. Mycologia 95(2), 269-284.

Mpika, J., Ismaël, B., Kébé, L., Irina, S., Druzhinina, Monika Komon-zelazowska, Christian P. Ake, S., 2009. Inhibition de *Phytophthora palmivora*, agent de pourriture brune des cabosses de cacaoyer en Côte d'Ivoire, par *Trichoderma* sp. Scien. Nature 6, 49-62.

Muller, R.A., 1974. Effects on prophylactic measures on dissemination of *Phytophthora palmivora*. In: *Phytophthora* Disease of Cocoa (Ed.: Gregory, P.H.), London. pp.169-178.

Ndoumbe-Nkeng, M., Efombang, M.I.B., Nyassé, S., Nyemb, E., Sache, I., Cilas, C., 2009. Relationship between cocoa *Phytophthora* pod rot disease and climatic variable in Cameroun. Can. J. Plant Pathol. 31, 309-320.

Ndoumbe-Nkeng, M., 2002. Indice des facteurs agro-écologiques sur l'épidémiologie de la pourriture brune des fruits de cacaoyer au Cameroun : contribution à la mise en place d'un modèle d'avertissement agricole. Thèse de Doctorat, Inst. Nat. Agron. Paris-Grignon.151 p.

Ndoumbe-Nkeng, M., Cilas, C., Nyemb, E., Nyassé, S., Bieysse, D., Flori, A., Sache, I., 2004. Impact of removing diseased pod on cocoa black pod cause by *Phytophthora megakarya* and cacao production in Cameroon. Crop Protec. 23, 415-424.

Ndoumbe-Nkeng, M., Sache, I., 2003. Lutte contre la pourriture brune des cabosses du cacaoyer au Cameroun. La défense des végétaux. Phytoma. 562, 10-12.

Neuhoff, D., Klinkenberg, H.J., Kopke, U., 2003. Nutzung von Pflanzenextrakten zur Kontrolle der Krautfaule (*P. infestans*) im'kologischen Kartoffelbau. In: Tagungsband; 'kologischer Landbau der Zukunft (Ed.: Freyer, B.). Universitat fur Bodenkultur, Wien, 7, 560-559.

Ngoh Dooh, J.P., 2006. Effet des extraits bruts de *Thevetia peruviana* (Pers.) K. Schum. sur quelques souches de *Phytophthora megakarya* Bras. et Grif. *In vitro*. Mémoire D.E.A. Université Yaoundé I. 51 p.

Ngoh Dooh, J.P., Ambang, Z., Tih Ewola, A., Heu, A., Kosma, P., Maho Yalen, E.J., Ghogomu Tih, R., 2014a. Screening and the effect of extracts of *Thevetia peruviana* on the development of *Colletotrichum gloeosporioides*, causal agent of cassava anthracnose disease. E3 J. Agric. Res. Develop. 4(4), 54-65.

Ngoh Dooh, J.P., Ambang, Z., Ndongo, B., Heu, A., Kuate, T. W., 2014b. Effect of extracts of *Thevetia peruviana* (Pers.) K.Schum on development of *Phytophthora megakarya* causal agent of black pod disease of cocoa. J. Appl. Biosci. 77, 6564–6574.

Nyassé, S., 1997. Etude de la diversité de *Phytophthora megakarya* et caractérisation de résistance du cacaoyer (*Theobroma cacao* L.) à cet agent pathogène. Thèse de Doctorat Inst. Nat. Polytech., Toulouse. 133 p.

Oji, O., Okafor, Q.E., 2000. Toxicological studies on steam bark, leaf and seed kernel of Yellow Oleander (*Thevetia peruviana)*. Phytother. Res. 14, 133-135.

Reddy, B.U., 2010. Antimicrobial activity of *Thevetia peruviana* (Pers.) K. Schum and *Nerium Indicum* Linn. Internet J. Pharmacol. 8(2), 1-5.

Reed, D.K., Freedman, B., Ladd, T.L., 1982. Insecticidal and antifeedant activity of neriifolin against Codling moth, Striped cucumber beetle and Japanese beetle. J. Econ. Entomol. 75(6), 1093-1097.

Saxena, V.K., Jain, S.K., 1990. *Thevetia peruviana* kernel oil: a potential bactericidal agent. Fitoter. 61(4), 348-349.

Silatsa, D.M., 2006. Etude épidémiologique de la pourriture brune des cabosses de cacaoyer sous l'influence des agents de lutte biologiques. Mémoire D.E.A. Université de Yaoundé I. 49 p.

Stoll, G., 1994. Protection naturelle des végétaux en zonetropicale.CTA. Agrecol.pp.95-99.

Tewtrakul, S., Nakamura, N., Hattori, M., Fugiwara, T., Suparita, T., 2002. Flavonone and flavonol glycosides from leaves of *Thevetia peruviana* and their HIV-1 reverse transcriptase and HIV-1 integrase inhibitory activities. Chem. Pharm. Bull. 50(5), 630-635.

Tondjè, P.R., Begoudé, A.D., Samnels, G.J., Hebbar, P.K., Fokou, J., 2005. Biological control of the cocoa black pod disease causative agent *Phytophthora megakarya* with *Trichoderma asperellum*. In: Proceedings of the 14th International Cocoa Research Conference, 2003 (Eds.: Cocoa Producers'Alliance). Accra, Ghana, Vol. II. pp. 677-682.

Whitlock, B., Bayer, C., Baum, D., 2001. Phylogenetic relationships and floral evolution of the Byttnerioidae (Sterculiaceae or Malvacecae) based on sequences of the chloroplast gene, *ndhF*. Sys. Bot. 26(2), 420-437.