



Aqueous extracts effects of seeds of *Thevetia peruviana* and *Azadirachta indica* on the development of *Phytophthora megakarya* in locality of Biakoa (Cameroon)

S. B. Mboussi^{1*}, Z. Ambang^{1,2} et J. P. Ngoh Dooh^{1,3*}.

¹ Laboratoire de Biotechnologies Végétales, unité de Phytopathologie et de Microbiologie de l'Université de Yaoundé I.

² Faculty of Management and Economics Sciences, University of Yaounde II.

³ Department of Biological Sciences, Faculty of Sciences, BP 814, University of Maroua.

*corresponding authors: ndjuliopat@yahoo.fr, smboussi@gmail.com

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ABSTRACT

Objective: The objective of this work was to evaluate, the impact of aqueous extracts of seeds of “yellow oleander” *Thevetia peruviana* (AETP), *Azadirachta indica* (AEAI) “neem” and Ridomil Gold Plus 66WP in black pod disease in Cameroon during two consecutive years.

Methodology and results: A completely randomize bloc device containing four treatments (control, AETP, AEA and Ridomil[®] and three replications was used in the farm. Extracts were applied twice in a month at the dose of 16.67 g/l while Ridomil[®] was applied one time a month at the dose of 3.33 g/l to the heights H1 and H2. Disease distribution across various pod distribution development stages showed that mature pods were the most susceptible to *P. megakarya*. A significantly difference is observed between the different treatments on all parameters evaluated. The disease rates were 7.92 %, 5.38 %, 8.94 % and 36.24 % in the plots treated with fungicide, AETP, AEA and untreated respectively, in 2013, and 10.95 %, 7.85 %, 16.21 % and 37.83 % in the same plots in 2014. The major results obtained compared to the rate of rot showed that AETP was more efficient than Ridomil[®] and AEA during these campaigns.

Conclusion and application of results: This study showed that aqueous extracts are promising and could be an effective and cheap formulation for the biological control of black pod disease. They have to be applied twice in a month at the dose of 16.67 g/l.

Keywords: Black pod disease, biological control, plants extracts, Ridomil[®], neem, yellow oleander.

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 cosmetics, chocolate and starch. In Cameroon, cocoa is one of the major export crops among others. From the social perspective, the economy of more than 400,000 families is based on cocoa (Anonymous, 2009). Although the global

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. The ICCO (International Cocoa Organisation) estimated that productions should reach 4.4 million tons by 2018. 71 % of world production came from Africa (2,813,000 tons), of which 225 000 tons against 16 % for Cameroon 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 *Phytophthora*. *P. megakarya* proves to be the most aggressive fungal species that attack cocoa in Cameroon (Nyassé, 1997). Its attack causes losses that can reach more than 80 % when environmental conditions are favourable 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. The control of these pests is achieved at the cost of frequent phytosanitary measures. Several control recommendations exist : Cultural practices such as regular removal of diseased pods and modification of the microclimate, creating unfavourable conditions for disease development, chemical control based mainly on the use of synthetic pesticides is effective (Ndoumbe-Nkeng and Sache, 2003). Unfortunately, chemical control 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 like the most cost effective and reliable method for control. The clones

MATERIAL AND METHODS

Obtaining the plants extracts: The mature fruits of *Thevetia peruviana* and *Azadirachta indica* were collected from different locations of the city of Yaoundé and Maroua. The seeds obtained from the fruits were dried in the laboratory at room temperature, weighed and then crushed using a hand mill (brand: "Victoria"). To obtain the aqueous extract, 250 g of the pulp, was wrapped in muslin fabric and soaked directly in 5 L of sterile distilled

developed with this gene are tolerance 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; Deberdt *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; Imitiaj *et al.*, 2005; de Oliveira *et al.*, 2012; Pohe *et al.*, 2013). Seeds, leaves, fruits and roots of *Thevetia peruviana* and *Azadirachta indica* are considered as potential sources of biologically active compounds, such as insecticides (Reed *et al.*, 1982; Ambang *et al.*, 2005), rodenticides (Ahmed *et al.*, 1985; Oji and Okafor, 2000; Javed *et al.*, 2008; Kosma *et al.*, 2011), fungicides (Gata-Goncalves, 2003; Ambang *et al.*, 2010; Ngoh Dooh *et al.*, 2015), virucides (Tewtrakul *et al.*, 2002) and bactericides (Saxena and Jain, 1990; Reddy, 2010). *Thevetia peruviana* and *Azadirachta indica* have already shown their effectiveness in reducing the inoculum pressure as well as the incidence of black pod rot (Ngoh Dooh *et al.*, 2015; Pohe *et al.*, 2013). The objective of this work was to evaluate, the impact of aqueous extracts of seeds of yellow oleander (*Thevetia peruviana* (Pers.) K. Schum), *Azadirachta indica* and Ridomil[®] in black pod disease and dynamic of pod production.

water for 12 h. The next day the pulp in the muslin was gently squeezed to extract the maximum amount of product (Stoll, 1994; Ngoh Dooh, 2014a), which was applied directly in the field.

Experimental design: The study was conducted in a smallholder's plantation located in Biakoa, in the Central Region of Cameroon for two consecutive years (2013 and 2014). This area is located in the agro-ecological zone

with humid bimodal rainfall pattern. This plantation had not been treated during the two 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 1.5 × 1.5 m to 2.5 × 2.5 m. Trees were on an average 50 years old and intercropped with other wild and domesticated fruit trees (coconut palms, avocado pear and plum/safou trees). The tree height is about 4-8 m and shading was dark. The experimental design used was randomized complete design. It included three blocks. Each block contained four treatments (control, aqueous extract of *Thevetia peruviana* (AETP), aqueous extract of *Azadirachta indica* (AEAI) and Ridomil[®] in the elementary plots. Each elementary plot contained 25 cacao trees randomly chosen. Each cocoa tree had a label that indicated 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. On each tree, two heights (H) were defined as follows: H₁ from 0 to 1.5 m above the ground and H₂ 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. The diseased pods were removed. 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).

Application of extracts and Ridomil[®]: The solutions were prepared by mixing the obtained extracts in 15 litres of water. Ten (10) g of soap (Total clean) used as wetting agent was added to the obtained liquid (Stoll, 1994). For AEAI and AETP, dose was one, which presented the best result to the laboratory (25 mg/ml). To the field it became

RESULTS

Dynamics of the pod production: The weekly potential production per treatment or plot was estimated over the two consecutive years corresponding to 2013 and 2014 seasons (fig.1). In 2013, the pod production was observed during the whole campaign. The production was higher in AEAI plot with a production peak reaching 1937 pods compared to the other plots. Production began at

16.67 g/l. 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 Side lever knapsack sprayer (SLK, Brand Matabi) to a height of about 4 to 5 meters at a rate of 15 l/153 m². The aqueous 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 (AETP and AEAI) and once every four weeks for Ridomil Gold 66WP in each block.

Epidemiological observations: The observations were recorded from the first week of treatments (July 2013 to November 2013) for the first campaign and (June 2014 to November 2014) for the second campaign. Healthy fruits at different heights and at various developmental stages including immature pods (10 to 20 weeks in age), mature pods (more than 22 weeks in age) and ripe pods were counted on all replicates each week. The ripe fruit was counted harvested or marked to avoid counting it twice.

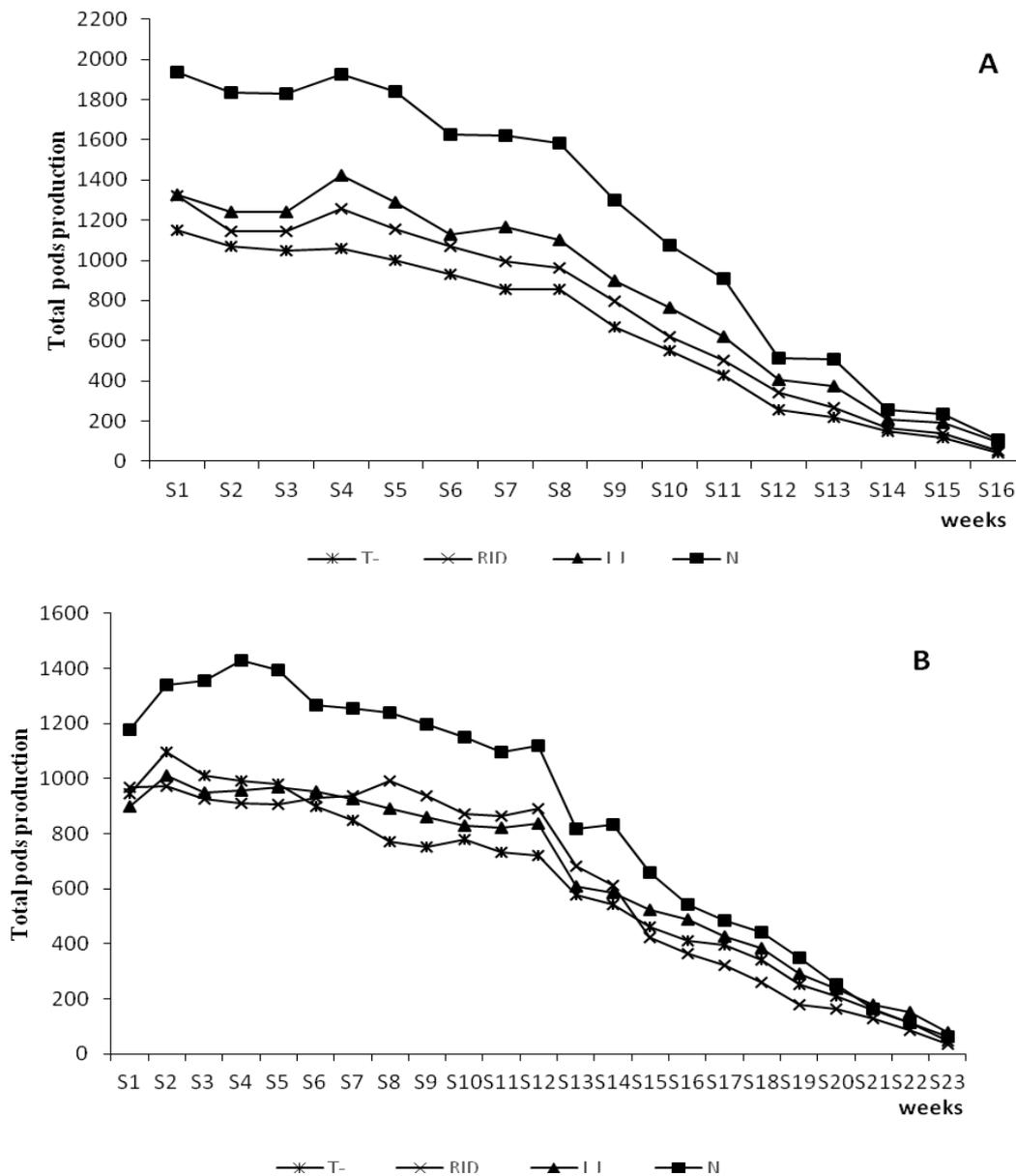
Statistical analyses: The calculation method for the weekly pod rot rate (TPt) was adapted from the formula used by Berry and Cilas (1994) and De Jésus (1992) as follows:

$$TPt = \frac{Pt}{Pt + MSt + Clnt} \times 100$$

Where:

t is treatment; Pt is number of rotten pods in treatment t; Clnt 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 modified formulae of TPt permits to calculate the incidence at different developmental stages and 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 on rotten pods at different heights and at various developmental stages of pod per year using SPSS 18.0 (the generalized linear model). Student Newman Keul (SNK) test at 5% were used to compare the averages.

the same time in all the plots (fig.1A). In 2014, Plot treated with Ridomil[®] presented low pod production with of about 990 pods and plot treated by AEAI had the higher production with a peak of 1428 pods compared to the other plots (fig.1B). The potential production in the first season was higher compared to the second season.



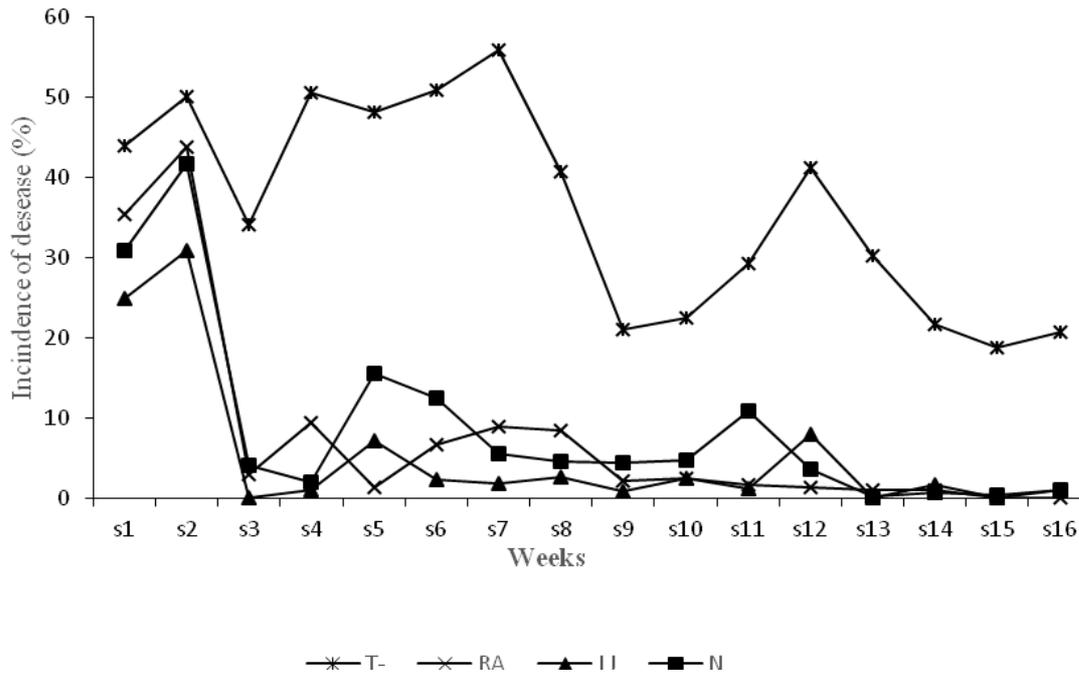
T- : Untreated control plots; Rid: Plots treated with Ridomil Gold Plus; LJ: Plots treated with AETP; N: Plots treated with AEA I
Fig.1: Total pods production per plots treated with AETP, AEA I, Ridomil and untreated (control) in year 2013 (A) and 2014 (B).

Dynamics of the pod rot progression a descriptive study of the weekly progression of black pod disease depending on weather conditions during the 2013 campaign: The weekly progression of black pod disease during the 2013 campaign showed on all the different curves, many exponentials phases of the disease appearance. These exponentials phases corresponded to

the weeks with the higher rainfall. These weeks are those of the months of July, August and October. The black pod was higher during the all campaign on the control. However, small values: 20.98 % (end of August-beginning beginning of September) and 18.78 % (middle of November) were observed due to small number of pods on the trees and small rainfall (fig.2). In the others

treatments AEAI, AETP and Ridomil^R the disease was reduced during all the campaign since the third week. At the fifth week, treatment with AEAI showed an exponential phase of 15.6 % compared to 7.15 % and 1.28 % for AETP and Ridomil^R respectively. The results obtained on all climate data showed that the 2013 campaign had had less rain. Rainfall was about 1496.6

mm (fig. 3). The average of temperature obtained was 25.2°C. Minimum temperatures coincided with the highest rainfall months (October) and maximum temperatures coincided with the month of August. These climatic variations are responsible for the less brown rot in all treatments during the 2013 campaign compared to the 2014 cocoa season.



T- : Untreated control; plots; RA: Plots treated with Ridomil Gold Plus; LJ: Plots treated with AETP; N: Plots treated with AEAI.
Fig.2: Weekly progression of black pod disease per plots treated with LJ, N, Ridomil and untreated (control) in the 2013

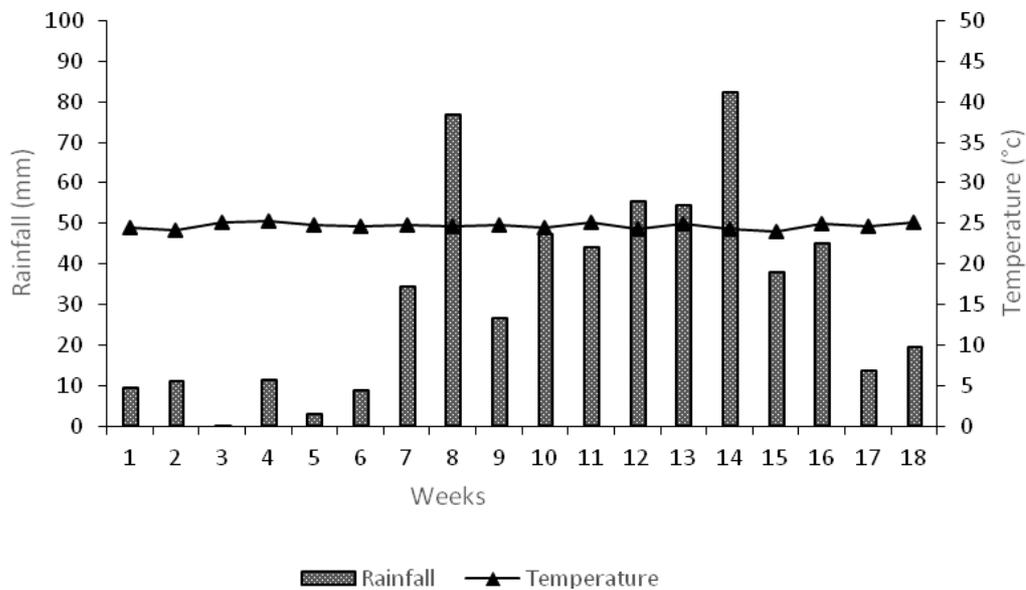
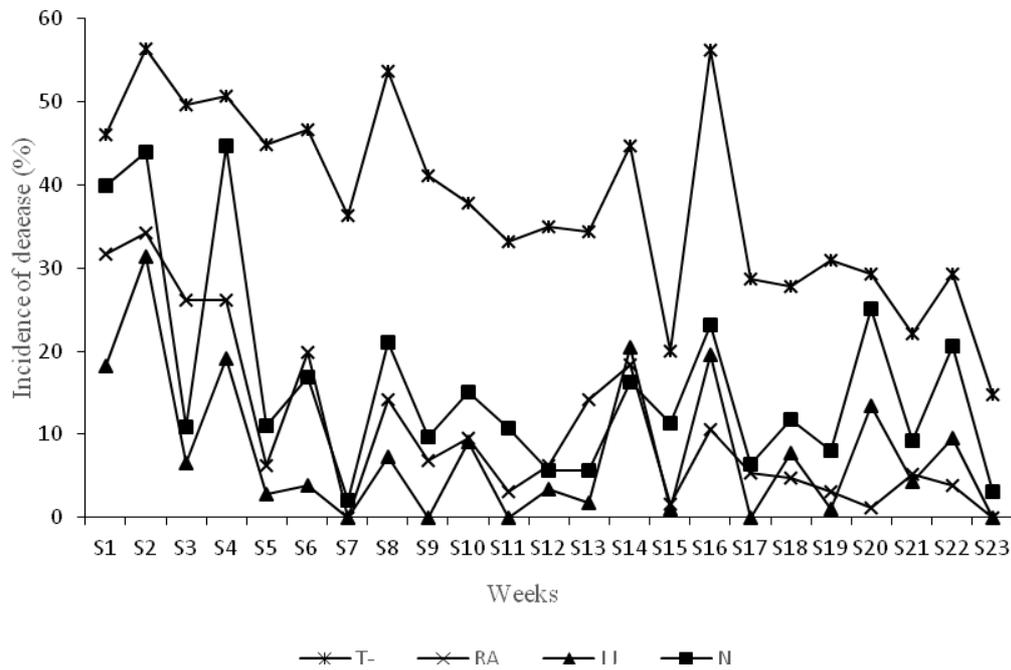


Fig. 3: Weekly rainfall and temperature in the locality of Biakoa during the 2013 campaign

Descriptive study of the weekly progression of black pod disease depending on weather conditions during the 2014 campaign: The weekly progression of black pod disease during the 2014 campaign showed again on all the different curves, many exponentials phases of the disease appearance. The disease was too high during this campaign. On the first weeks of observations (1 to 4), disease was high in the control and AEAI treatments. At the 16th week, exponentials phases of 56.31 %, 23.13 %, 19.5 % and 10.52 % were obtained respectively on the control, AEAI, AETP and Ridomil^R treatments (fig.4). On

AETP and Ridomil^R treatments, the disease curves were lower than those of control and AEAI during all the campaign. The 2014 campaign had had more rain than the previous season. Rainfall was about 1861.8mm throughout the campaign. The average of temperature obtained was 24.5 °C. This temperature was less than the previous season. Temperature fluctuations were low during this season (Fig. 5). In this campaign, the climatic variations are responsible for the highest brown rot in all treatments.



T- : Untreated control; plots; RA: Plots treated with Ridomil Gold Plus; LJ: Plots treated with AETP; N: Plots treated with AEAI. **Fig.4:** Weekly progression of black pod disease per plots treated with LJ, N, Ridomil and untreated (control) in the 2014.

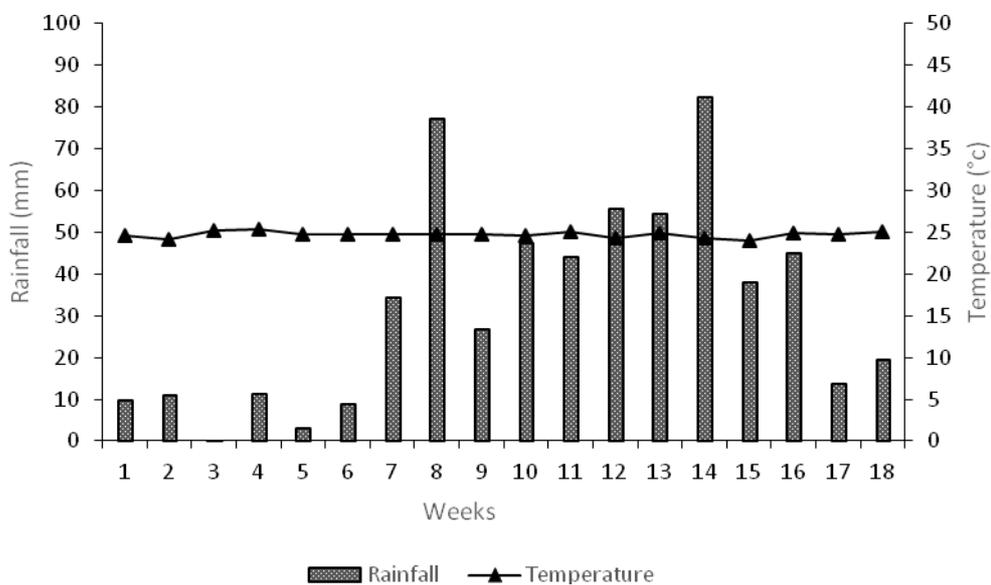


Fig. 5: Weekly rainfall and temperature in the locality of Biakoa during the 2014 campaign

Effect of the extracts on the disease incidence during 2013 and 2014 campaigns: The results showed a significant effect of the treatments on the disease incidence ($P < 0.05$). The rate of brown rot was less during

the 2013 season in all treatments than the 2014 season. There is a significant difference between the control and the three others treatments (AEAI, AETP and Ridomil^R while these three treatments showed similar efficacy

(table 1). The results highlight the potential of AEAJ and AETP treatments to reduce the disease in the field in comparison to the control treatment.

Table 1: Incidence of black pod disease in the different treatments in 2013 and 2014 campaigns

Treatments	Rate of pod rot (%)	
	2013	2014
Control ^a	36,24 a	37,83 a
Ridomil ^R ^b	7,92 b	10,95 b
AETP ^c	5,38 b	7,85 b
AEAI ^d	8,94 b	16,21 b

* Values followed by the same letter are not significantly different at 5%

a Untreated control plots ; b Plots treated with Ridomil Gold Plus; c Plots treated with AETP; d Plots treated with AEAJ.

Incidence of tree height on the black pod disease during 2013 and 2014 campaigns: Analysis on the incidence of brown rot at different heights H1 and H2 revealed significant interaction height × treatments. Subsequently, analysis to assess the effect of height on the incidence of decay was performed for each treatment (table 2). Observations reveal that not all the pods are attacked in the same way at all heights. A significant difference (P< 0.05) is observed between the two heights. This is meaning that the height H1 is the more susceptible

height to the development of the disease. There was an effect of "height" in the development of the disease. In 2014, the results on the incidence of brown rot at different heights H1 and H2 indicate that, the effect of "height" of cocoa on the influence of black pod rot was significant (P< 0.05) for any of the treatments. Observed in all treatments, pods are attacked in the same way at all heights, which means that no height of cocoa was susceptible to brown rot during this campaign (table 2).

Table 2: Incidence of black pod disease in the different tree heights (H1 and H2) in 2013 and 2014 campaigns

Years	Treatments	Rate of pod rot (%)	
		H1 (0 to 1.5 m from soil)	H2 (1.5 to 3 m from soil)
2013	Control ^a	39.17 a	34.06 a
	Ridomil ^R	16.02 a	5.94 b
	AETP ^c	15.88 a	3.55 b
	AEAI ^d	13.03 a	6.61 b
2014	Control ^a	34.64 a	39.45 a
	Ridomil ^R ^b	8.56 a	10.47 a
	AETP ^c	9.29 a	7.40 a
	AEAI ^d	16.92 a	15.90 a

* Values followed by the same letter are not significantly different at 5 %

a Untreated control plots ; b Plots treated with Ridomil Gold Plus; c Plots treated with AETP; d Plots treated with AEAJ.

Incidence of the developmental stage on the black pod disease during 2013 campaign: Analysis on the incidence of brown rot at different developmental stages S1, S2 and S3 of cocoa pods showed a treatment × stage interaction highly significant in 2013 (P<0.0001). For this reason, an analysis to assess the effect of stage on the incidence of decay 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 "mature pod" stage compared to the "immature pod" stage and the "ripe pod" stage for 2013 season. The black pod disease incidence on the various developmental stages indicated that losses due to the disease were higher at the "mature pod" stage in control, AEAJ, AETP and Ridomil^R plots.

Incidence of the developmental stage on the black pod disease during 2014 campaign: Analysis on the incidence of black pod at different developmental stages S1, S2 and S3 of cocoa pods showed a treatment × stage

interaction highly significant in 2014 ($P < 0.0001$). An analysis to assess the effect of stage on the incidence of decay was performed again for each treatment. The results revealed a significant effect of developmental stage of pods on the disease incidence (Table 3). In all treatments experimental plots, the disease incidence is

significantly higher in the “mature pod” stage (S2) compared to the “immature pod” stage (S1) and the “ripe pod” stage (S3) for 2014 season. The black pod disease incidence on the various developmental stages indicated that losses due to the disease were higher at the “mature pod” stage in control, AEAI, AETP and Ridomil[®] plots.

Table 3: Incidence of black pod disease at different stages of development (S₁, S₂ and S₃) in 2013 at the last week of observation.

Years	Treatments	Rate of pod rot (%)		
		S ₁ (immature pod)	S ₂ (mature pod)	S ₃ (ripe pod)
2013	Control ^a	11,69 b	52,16 a	0,73 c
	Ridomil [®] b	3,82 b	4,22 a	0,00 c
	AETP ^c	2,36 b	3,16 a	0,07 c
	AEAI ^d	4,10 b	4,47 a	0,22 c
2014	Control ^a	10,63 b	27,96 a	0,69 c
	Ridomil [®] b	2,01 b	8,47 a	0,10 c
	AETP ^c	1,47 b	6,53 a	0,13 c
	AEAI ^d	3,45 b	12,82 a	0,50 c

* For each treatment, values followed by the same letter in row are not significantly different at 5% level

a Untreated control plots ; b Plots treated with Ridomil Gold Plus; c Plots treated with AETP; d Plots treated with AEAI.

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 (*Phytophthora megakarya*) and the environment (abiotic and biotic factors, including our extracts of *Thevetia Peruviana* and *Azadirachta indica*). Temperature and rainfall data compared to the decay exchange 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 2014 (1861.8 mm rainfall) than in 2013 (1496.6 mm of precipitation). These results confirm those obtained by Ndoumbe-Nkeng *et al.* (2002); Ngoh Dooh *et al.* (2015) 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 2014 campaign. Weak

precipitation recorded in 2013, could also explain the low incidence obtained during this campaign. On the other hand, this incidence in 2013 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 2014 campaign, disease was higher but moderate with an incidence rate of 37.83 %; 10.95 %; 7.85 % and 16.21 % respectively for treatments control, Ridomil[®] AETP and AEAI. This result can be explained partly by the fact that the inoculum pressure was reduced during the previous season. Generally, treatments with extracts made from the seeds of *Thevetia peruviana* and *Azadirachta indica* 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 AEAI and AETP treatments compared with Ridomil[®] despite the very different values. However, these tree treatments were significantly different compare to the control. In the 2013 campaign, the incidence of black pod disease was found a significant difference from each other in the two heights. In all treatments, the H1 got the highest incidence of black pod rot than the H2. This result is close to that obtained by Silatsa (2006) who, at the height H1 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 H1). According to Ndoumbe-Nkeng *et al.* (2004), fruits, in cocoa tree height H2 are contaminated from water flowing on to 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 (fruits in the H1 of the tree). During the year 2014, the incidence of black pod disease was found not significantly different from each other in height (H1 and H2) in all treatments. This result could be explained by the practice of agro-technical methods (cultural methods) that create unfavourable conditions for the development of the pathogen so that the pressure of the inoculum was reduced. This result is close to that obtained by Ngoh Dooh *et al.* (2015) who, at the heights H1 and H2 of the cocoa plants got the same incidence of black pod rot. The incidence of black pod rot was higher in developmental stage S2 (mature pod) fruits in all treatments during the two study seasons compared to S1 stage (immature pods) and S3 (ripe pods). 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 immature pods are formed early in the season when environmental conditions are not favourable enough for the development and expansion of the pathogen responsible of black pod disease opposite to the mature pod which are formed when the environmental conditions are favourable for the development and expansion of the pathogen. This result is not similar to that obtained by Deberdt *et al.* (2008) and Ngoh Dooh *et al.* (2015) who in these stages found a very high rate of decay stage S1 (immature pods). These results are also in opposition to 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)

CONCLUSION

During the two cocoa campaigns studied, results on black pod disease have shown that there is a significant difference between cocoa seasons. The 2014 campaign saw strong precipitation and higher black pod disease compared to the 2013 cocoa season. These different rot rates obtained in the field during the two years demonstrated that, the AETP has the same effectiveness than the AEAI at the dose used. The rot rate found in the

who found the immature pods 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 and mature 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 Silatsa (2006); Deberdt *et al.* (2008); Ngoh Dooh *et al.* (2015), who also 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, disease impact was significantly reduced. 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 considered too expensive for smallholders in Africa and its toxicity towards human and environment remains a concern. The effectiveness of AEAI and AETP 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 Kosma *et al.* (2011) and Ngoh Dooh *et al.* (2014a), revealed the same compounds in the aqueous extracts of *Azadirachta indica* and *Thevetia peruviana* and subsequently their effectiveness against *Radopholus similis* and *Colletotrichum gloeosporioides* f.sp. *Manihotis* respectively. Ngoh Dooh (2006) and Ambang *et al.* (2010) have shown the efficacy of extracts from *Thevetia peruviana* *in vitro* and *in vivo* on the development of *P. megakarya*.

treatments with the two aqueous extract and the Ridomil^R (fungicide reference) showed no significant difference. The extracts from *Thevetia peruviana* and *Azadirachta indica* appear to be a promising avenue in the IPM against brown rot. They could be valued and popularized because of their biodegradable nature and because of the dual economic and environmental benefits, they provide for cocoa farmers.

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REFERENCES

- Ahmed S. and Koppel B., 1985. *Plant extracts for pest control: village-level processing and use by limited-resource famers* paper presented at the AAA annual meeting in Los Angeles. 82 p.
- 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 Bekolo, Ngoh Dooh J.P. et Djlé Bouba, 2005. Effets des extraits des grains de laurier jaune (*Thevetia Peruviana* (Pers) K. Schum) sur les charançons (*Sitophilus zeamais* Motsch) ravageurs des stocks. Biosciences proceedings Vol. 11, 57-83 ISSN 1019-7702.
- 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 Journal*. 3 (6) 204-209.
- Anonyme, 2009. Les mesures prises par le Gouvernement pour parvenir à une économie durable: cas du Cameroun. Table ronde sur l'économie cacaoyère mondiale.
- Anonyme, 2013. Annual forecasts of production and consumption and estimates of production levels to Achieve equilibrium in the world cocoa market. 10 p.
- Bautista-Banos S., Hernandez-Lopez M. and Barrera-Neecha L.L., 2000. Antifungal screening of plants of the state of Morelos, Mexico, against four postharvest pathogens of fruits and vegetables. *Mexican Journal of Phytopathology* 18: 36-41.
- Berry D. et Cilas C., 1994. Etude du comportement d'une parcelle diallèle 6x6 vis à vis de la pourriture brune des cabosses de cacaoyer due à *Phytophthora* spp. au Cameroun. Acte de la 11ème conférence internationale sur la recherche cacaoyère, pp. 91-96.
- Blahe G. et Lotode R., 1976. Un caractère primordial de sélection du cacaoyer au Cameroun: la résistance à la pourriture brune des cabosses de cacaoyer. *Café Cacao Thé* 20, 97-116.
- De Oliveira FQ, Malaquias JB, de Souza Figueiredo WR, de Luna Batista J, Barbosa Beserra E, 2012. Inhibition of fruit infestation by Mediterranean fruit fly using natural products. *African Journal of Biotechnology* 11(74): 13922-13927.
- 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 biological on cocoa pod production dynamics and black pod diseases (*Phytophthora megakarya*) in Cameroun. *Biol. Contr.* 44. 149-159.
- Despréaux D., Cambroy D., Clément D., Nyassé S. et Partot M., 1988. Etude de la pourriture brune des cabosses de cacaoyer au Cameroun: définitions de nouvelles méthodes de lutte. *X^e Conf. Inter. Rech. Cacaoyère*, Santo Domingo, Dominican Republic. Pp. 407-412.
- Efombang M.I.B., Marelli J.P., Duncamp M., Cilas C., Nyassé S. and Vefonge D., 2004. Effects of fruiting traits on the field resistance of cocoa (*Theobroma cacao* L.) clones to *Phytophthora megakarya*. *Journal of Phytopathology* 152: 557-562.
- Gata-Gonçalvès L., 2001. Proprieda des biocides de plantas de Origem Tropical: caso da *Thevetia peruviana* Schum. Ph.D. Thesis. Engenharia Agronomica Quinima. Campo das Cebolas Lisboa. 133 p.
- Gata-Gonçalvès L., Nogueira J.M.F., Matos O. and Bruno De Sousa, Phytoactive extract from *Thevetia peruviana* with antifungal properties against *Cladosporium cucumerinum*. *Journal of photochemistry and photobiology*. 2003 70 (1): 51-54.
- Gregory P.H., Griffin M.J., Maddison A.C. and 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. and Samuels G.J., 2004. Taxonomy and biocontrol potential of a new species of *Trichoderma* from the Amazon basin of South America. *Mycological Progress* 3 (3): 199-210.
- Intiaj A., Rahman S.A., Alaml A., 2005. Effect of fungicides and plant extracts on the conidial germination of *Colletotrichum gloeosporioides*

- causing mango anthracnose. *Microbiology* 33 (4): 200-205.
- Iwaro A.D., Sreenivason T.N, Umaharan P., 1998. *Phytophthora* resistance in cocoa (*Theobroma cacao* L.): Influence of pod morphological characteristics. *Plant Pathology* 46: 557-567.
- Javed N., Gowena S. R., El-Hassana S. A., Inam-ul-Haqa M., Shahinab F. and Pembroke B., 2008. Efficacy of neem (*Azadirachta indica*) formulations on biology of root-knot nematodes (*Meloidogyne javanica*) on tomato. *Crop Protection* 27: 36- 43.
- Kebe L., Koffi N., Konan A., N'Guessan F., Kohi N.J. and Irié B.Z. 2005. Bien cultiver le cacaoyer en Côte d'Ivoire. Direction des programmes de recherché et de l'appui au développement – Direction des systèmes d'information, CNRA, Abidjan, Côte d'Ivoire.
- Kosma P., Ambang Z., Begoude B.A.D., Ten Hoopen G.M., Kuate J. and Amougou A., 2011. Assessment of nematicidal properties and phytochemical screening of neem seed formulations using *Radopholus similis* parasitic nematode of plantain in Cameroon. *Crop protection* 30: 733-738.
- Mpika J., Ismaël B. K., Irina S. D., Monica Komon-zelazowska, Christian P., Ake S., 2009. Inhibition de *Phytophthora palmivora*, agent de la pourriture brune des cabosses de cacaoyer en Côte d'Ivoire par *Trichoderma* sp. *Sciences et Nature* Vol. 6: 49-62.
- Muller R.A., 1974. Effects on prophylactic measures on dissemination of *Phytophthora palmivora*. In: *Phytophthora* disease of cocoa. P.H. Gregory, (ed), London. Pp.169-178.
- 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. and Sache I., 2003. Lutte contre la pourriture brune des cabosses du cacaoyer au Cameroun. *Phytoma*. La défense des végétaux 562, 10-12
- Ndoumbe-Nkeng M., Cilas C., Nyemb E., Nyassé S., Bieysse D., Flori A. and Sache I. 2004. Impact of removing diseased pod on cocoa black pod cause by *Phytophthora megakarya* and cacao production in Cameroon. *Crop Protection* 23: 415- 424.
- Ndoumbe-Nkeng M., Efombang M.I.B., Nyassé S., Nyemb E., Sache I. and Cilas C., 2009. Relationship between cocoa *Phytophthora* pod rot disease and climatic variable in Cameroun. *Can. J. Plant Pathol.* 31: 309-320.
- Ngoh Dooh, 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., 2015. Evaluation de l'effet des extraits de *Thevetia peruviana* (Pers.) K. Schum. sur trois champignons phytopathogènes : *Phytophthora megakarya* Bras. et Grif., *Colletotrichum gloeosporioides* Penz f. sp. Manihotis HENN et *Mycosphaerella fijiensis* Morelet. Thèse de Doctorat Ph/D Université de Yaoundé I, 190 p.
- 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. and Okafor Q. E., 2000. Toxicological studies on steam bark, leaf and seed kernel of Yellow Oleander (*Thevetia peruviana*). *Phytother. Res.* 14: 133-135.
- Pohe J., Agneroh T.A. 2013. L'huile des graines de neem, un fongicide alternatif à l'oxyde de cuivre dans la lutte contre la pourriture brune des cabosses de cacaoyer en Côte d'Ivoire. *Journal of Applied Biosciences* 62: 4644–4652.
- Reddy B.U., 2010. Antimicrobial activity of *Thevetia peruviana* (Pers.) K. Schum and *Nerium indicum* Linn. *The internet Journal of Pharmacology*, 8 (2); 1-5.
- Reed D.K., Freedman B. and Ladd T.L. Insecticidal and anti feedant activity of neriifolin against Coldling moth, Striped cucumber beetle and Japanese beetle. *J. Econ. Entomol.* 75 (6): 1093-1097, (1982).
- Silatsa D. M., 2006. Etude épidémiologique de la pourriture brune des cabosses de cacaoyers

- sous l'influence des agents de lutte biologiques. Mémoire D.E.A. Université de Yaoundé I. 49 p.
- Whitlock B., Bayer C. and Baum D., 2001. Phylogenetic relationships and floral evolution of The *Byttnerioideae* (*Sterculiaceae* or *Malvaceae*). Based on sequences of the Chloroplast gene, *ndhF*. *Sys. Bot.*, 26 (2): 420-437.
- Widmer T. L. and Laurent N. 2006. Plant extracts containing caffeic acid and rosmarinic acid inhibit zoospore germination of *Phytophthora* spp pathogenic to *Theobroma cacao*. *European Journal of Plant Pathology*. 115: 377-388.