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Pod resistance of cocoa clones to black pod disease and antifungal properties of phytoextracts against *Phytophthora megakarya*

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Abstract

In Cameroon, cocoa production is limited by constraints amongst which diseases such as black pod caused by *Phytophthora megakarya* which is the most aggressive in the country (80% losses). This study was aimed at determining the clonal resistance of six cocoa clones (IMC67, SCA12, TAFO79/501, TAFO79/467 ICS40, SNK13) both in field and Laboratory conditions, and to evaluate the antifungal activity of extracts from three plant species (*Allium sativum*, *Zingiber officinale*, *Thymus algeriensis*) and a product of the African traditional pharmacopoeia (African Panaxia) on the growth of *Phytophthora megakarya*. The pathogen was isolated on V_8 medium and inoculated on pods. Disease incidence was evaluated in the cocoa plantation of the Mengang Production and Experimentation Center. Results show that, clones SCA12 and TAFO79/467 were the least susceptible to black pod disease in field and Lab conditions. African Panaxia, aqueous and ethanolic extracts of *T. algeriensis* induced total inhibition of *P. megakarya* at 62.50; 125.0 and 30.0 $mg.ml^{-1}$, respectively. African Panaxia and ethanolic extracts of *T. algeriensis* had a fungicidal effect starting at 62.50 and 30.0 $mg.ml^{-1}$, respectively while the aqueous extract of *T. algeriensis* had a fungistatic effect at 125.0 $mg.ml^{-1}$. The study shows that clones SCA12 and TAFO79/467 are more tolerant to cocoa black pod. Moreover, due to their fungicidal effect against *P. megakarya*, African Panaxia and *T. algeriensis* extracts could be integrated into a management program against cocoa black pod disease.

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Introduction

The cocoa tree (*Theobroma cacao* L., Malvaceae) is a perennial plant, cultivated for its beans used as raw material for the manufacture of chocolate, cosmetics, and starch. Introduced in Cameroon by the Germans in 1892, cocoa cultivation was carried out by more than 400.000 farmers on at least 400.000 ha by 1992 (Losch *et al*., 1992). By 2013, Cameroon ranked 5th in the world and 4th in Africa as a cocoa producing country (ICCO, 2013). It is a major export cash crop in Cameroon, where annual production is around 232 000 t. Despite the interest in cocoa cultivation, yields remain low due to constraints amongst which diseases such as black pod caused by *Phytophthora megakarya*.

This disease can cause up to 80% loss when environmental conditions are favorable for its development (Dooh *et al*. 2015). In certain production zones, losses due to the disease can reach 70 to 100% when no control measure is in place (Ndoumbè-Nkeng, 2002). Several management practices, viz. physical, chemical, regulatory (control by regulatory agencies, plant quarantine and certification agencies), cultural and biological control methods have been used against cocoa black pod disease in the world.

In Cameroon, chemical method is the widely used and more than 10 fungicides are homologated each year against cocoa black pod disease. However, 77% of farmers do not respect the official spray recommendations for chemicals (Mahob *et al*., 2014).

The inappropriate application of fungicides has not only caused environmental pollution through the accumulation of chemical residues, soil and groundwater contamination, but also the appearance of more resistant strains of these phytopathogenic agents (Triki *et al*., 2012) and have resulted in various human diseases (Griffiths, 1981). The frequent use of fungicides, cause hazardous effects on plant products, consumers and the environment. Hence strong regulatory actions have been imposed on their use. Recently, Cameroon government banned any chemical with Metalaxyl active ingredients, from being marketed and/or from circulation in the country.

The ban was attributed to the fact that metalaxyl has the propensity to induce some long-term health hazards such as cancer (MINADER, 2017). These health and environmental concerns have stimulated the development of alternative pesticides such as botanical or plant extracts (El-Wakeil, 2013). In fact, some plant extracts have fungistatic or fungicidal properties that could be exploited as alternatives to chemicals against plant diseases. Moreover, in fulfilment of the quality requirements that must be met by commercial cocoa in international trade to preserve the health of consumers, the ideal for cocoa production would be to reduce as much as possible the use of chemical pesticides.

This could be by using natural substances against phytopathogens and/or by exploitation of clonal resistance of host plant varieties or cultivars. In this light, this study was conducted with the objective of evaluating clonal resistance and antifungal activity of plant extracts against cocoa black pod disease.

Materials and methods

Plant and fungal material

The plant material consisted of healthy pods of six cocoa clones namely: SNK13, TAFO79/467, TAFO79/501, SCA12, ICS40, and IMC67 from the Mengang Production and Experimentation Center (CEPEM); rhizomes of *Zingiber officinale*, cloves of *Allium sativum*, leaves of *Thymus algeriensis* and African Panaxia. African Panaxia is a locally manufactured product made of medicinal plants and used to control many human diseases in Cameroon; it was bought on the local market.

The composition of African Panaxia is as follows: ginseng roots (30%), *Ocimum grastissimum* (20%), Quinquifolium (25%), *Aloe vera* (20%) and water (5%). The fungal material consisted of pure *P. megakarya* cultures isolated from pods showing symptoms of black pod disease in the centre region of

Cameroon. The physical characteristics of plant organs, plant powder and African Panaxia used to prepare the different extracts are presented in Fig. 1.

Extraction procedure

The rhizomes of *Z. officinale* and the cloves of *A. sativum* were previously cleared of their superficial envelopes and then cut into thin strips. The rhizomes of *Z. officinale* were dried for 4 weeks under shade, while leaves of *T. algeriensis* and thin strips of *A. sativum* were dried in the oven at 40°C respectively for 5 and 9 days respectively. These organs were then crushed separately and for each of them, 150 g of powder were introduced into a jar with a capacity of 2 liters containing 1 liter of solvent (95° ethanol or distilled water).

The mixture was stirred 3 times per day for 3 days and then filtered with filter paper. The filtrate from the maceration was oven-dried at 40°C (Serferbe *et al.,* 2015).

The aqueous extract was oven-dried at 50°C for 7 days while ethanolic extracts were evaporated on a shaking water bath at 60 rpm at 60°C and crude extracts were separately stored in small containers at room temperature for further experiments (Djeugap *et al.,* 2017).

Isolation and identification of P. megakarya

Isolation of the pathogen was done according to the protocol described by Zhu *et al.* (2001). Infected pods were disinfected with 95° alcohol and then a scalpel was used to remove the superficial layer of lesions on the pods. Fragments from the growth front of the lesion were removed and deposited on sterilized Petri dishes each containing 20 ml of water agar.

These inoculated dishes were sealed using film paper and incubated at 21±1°C in the dark. Pathogen identification was based on microscopic description of sporangia and mycelial structures with reference to fungal systematics documents (André and Barbara, 2001). The developed mycelial fragments were then removed and sub cultured on V_8 medium supplemented with antibiotics (Piramicin : 0.4 ml/L, Ampicillin : 2.5 ml/L, Rifampicin : 1 ml/L, Para chloronitrobenzen : 100 mg/L, Hymezazol : 0.5 ml/L) and incubated at 21±1°C in the dark for 3 days. Successive subcultures on the medium made it possible to obtain pure cultures of the pathogen which were stored at 4°C. Identification of the fungus was done under an ordinary microscope (Olympus BH_2) using identification keys in mycology (André and Barbara, 2001).

Clonal resistance

Clonal resistance was evaluated both in field and Lab conditions. In the field, 4 cocoa trees from each clone were randomly selected from CEPEM cocoa farms. The incidence of black pod (I) was estimated on each clone according to the formula: I (%) = (number of infected pods / total number of pods of the plant considered) x 100 (Mfegue, 2012). In the laboratory, healthy pods of the clones studied, with approximatively the same morphology, size and weight were collected in CEPEM cocoa farms and placed carefully in labeled polyethylene bags and taken to the laboratory.

These healthy pods were washed with tap water and soaked in 3% sodium hypochlorite solution for 3 min. They were then rinsed three times for 15; 10 and 5 minutes with sterilized distilled water and dried on blotting paper. A cylindrical orifice 5 mm in diameter and 0.5 cm deep was created in the middle of the cocoa shell with a flamed cutter. Mycelia disks were obtained using a cookie cutter of 5 mm diameter and taken from the margin of 10 days-old culure of *P. megakarya*. Mycelia disks were deposited into each orifice and then covered with hygrophilic cotton soaked in sterile distilled water to maintain moisture. Each inoculated pod was placed in a sterile polyethylene bag and incubated at 21 ± 1°C. Five pods (replicates) were considered per clone.

The length (L) and the width (l) of lesion growth (cm) were measured every 2 days after inoculation (DAI) using a flexible and transparent graduated ruler. The lesion developed on the infected pods having an

elliptical shape, and its surface (S) was calculated according to the following formula by Cilas and Despréaux (2004): $S = \pi.L.l /4$.

Antifungal activity of extracts

The antifungal activity of plant extracts were evaluated according to the agar dilution method on V_8 medium (Sharma and Trivedi, 2002). For this purpose, concentrations of 15.63; 31.25; 62.50; 125.0 mg/ml and 7.50; 15.0; 30.0; 60.0 mg/ml were used respectively for the aqueous and ethanolic extracts. African panaxia was tested at the same concentrations like the aqueous extracts.

The V_8 medium supplemented with different concentrations of the plant extracts was prepared by adding the appropriate amounts of the extracts and one drop of Tween 80 to the medium followed by manual stirring in an Erlenmeyer flask in order to disperse the extracts in the medium. A volume of 15 ml of the medium were poured into each Petri dish. A 5 mm diameter mycelial fragment taken from the growth front of *P. megakarya* pure cultures aged 10 days was deposited in the center of each Petri dish. The negative control (no extract, no fungicide) and positive control (no extract but with the fungicide Mancozeb) were inoculated following the same procedure.

The concentration of the fungicide was 1 $mg.ml^{-1}$ as prescribed on the label. The dishes were incubated at 21±1°C for 7 days. Three repetitions were considered per concentration. Radial growth diameters were measured daily from two orthogonal lines drawn on the reverse side of the Petri dishes intersecting at the point of deposition of the explant.

The radial growth (C) of the pathogen was evaluated by the relation $C = (d_1 + d_2 - 2d_0) / 2$ (Djeugap *et al.* 2011) where d_0 is the diameter of the explant, d_1 and d_2 are the two diameters of the mycelium measured on the Petri dishes. Percent inhibition (% I) of mycelial growth was calculated according to Dohou *et al.* (2004) formula as follows: $\% I = 100 \times (Dt-Dx)/Dt$ where Dt is the mean diameter of the culture of the negative control (without extract, nor Mancozeb), and Dx, the mean diameter of the culture with extract or Mancozeb. The nature of the toxicity of the extracts was evaluated by removing the explants from the Petri dishes with complete inhibition, then transplanted on the freshly prepared V_8 medium without extract nor fungicide for 8 days to see if fungal growth resumed or not.

Data analysis

The incidence of black pod of cocoa, surface of lesions (cm^2) after pod inoculation in the Lab, growth inhibition percentages, Equivalent concentration of the inhibition of 50% (EC_{50}) and 90% (EC_{90}) of the growth of the pathogen were processed using the Microsoft Excel and submitted to ANOVA test using the SPSS 20.0 software. When the variance homogeneity test (Levene and Brown-Forsythe tests) was significant, means were separated using the Student test at 5%.

Results

Clonal resistance to cocoa black pod

In CEPEM plantation, no tested clone developed a total resistance vis-à-vis the black pod disease of cocoa. The different clones showed partial level of resistance. Clones SCA12 and TAFO79/467 were tolerant to black pod while clones ICS40 and IMC67 were the most susceptible during the fifth week of observation. Disease incidence was 25.70 and 25.48% for clones SCA12 and TAFO79/467, respectively after five consecutive weeks. Clone IMC67 recorded the highest disease incidence (50.21%) at the same period of observation (Fig. 2).

In Lab conditions, all pods inoculated with pure culture of *Phytophthora megakarya* developed the disease compared to the control. Lesions were observed from the 4th day after inoculation (DAI), in all the pods of the clones considered but absent on the control (Fig. 3). Lesion sizes of cocoa black pod were significantly lower ($P<0.05$) in clones SCA12 (103.24 cm^2) and TAFO79/467 (101.97 cm^2), at 10th DAI. In contrast, lesion size was higher in clone ICS40 (259.17 cm^2) (Table 1).

Table 1. Lesion size (cm²) of black pod disease on different cocoa clones at 4 and 10 day after inoculation (DAI) of *P. megakarya*.

Cocoa clones	Surface of the lesion (cm²)*	
	4th DAI	10th DAI
Control	0.0 ± 0.0 c	0.0 ± 0.0 d
TAFO79/501	8.45 ± 1.59 b	217.09 ± 16.17 b
TAFO79/467	8.29 ± 2.57 b	101.97 ± 11.10 c
IMC67	12.60 ± 2.24 a	220.91 ± 16.93 b
SCA12	10.87 ± 1.91 a	103.24 ± 11.18 c
ICS40	10.50 ± 2.93 a	259.17 ± 15.26 a
SNK13	11.34 ± 0.91 a	225.59 ± 17.55 b

*Mean in each column followed by the same letter do not differ statistically (Student test, P <0.05); control = pod inoculated with culture medium without the pathogen.

Antifungal activity of plant extracts

Mycelial growth inhibition of *P. megakarya* increases with concentration of both aqueous and ethanolic extracts. Total growth inhibition (100%) of the pathogen was observed with aqueous extracts at the concentration of 62.5 mg.ml^{-1} and 125mg.ml^{-1}, respectively for African Panaxia and *T. algeriensis*. Their efficiency were statistically similar to that obtained with the reference fungicide Mancozeb. At the highest concentration tested (125 mg/ml), the growth inhibition of the fungus was lower (47.82%) in aqueous extract of *A. sativum* (Table 2).

Table 2. Effect of aqueous extracts on the growth inhibition (%)* of *P. megakarya*.

Concentrations	*Allium sativum*	*Zingiber officinale*	*Thymus algeriensis*	African Panaxia
Control (-)	0.0 ± 0.0^{c}	0.0 ± 0.0^{f}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
15.63mg/ml	39.68 ± 2.25^{b}	51.39 ± 1.82^{e}	37.73 ± 3.34^{c}	83.13 ± 2.48^{c}
31.25 mg/ml	40.67 ± 3.92^{b}	61.31 ± 1.57^{d}	40.87 ± 4.47^{c}	95.04 ± 1.50^{b}
62.5 mg/ml	42.66 ± 2.25^{b}	70.83 ± 1.79^{c}	66.27 ± 5.83^{b}	100.0 ± 0.0^{a}
125 mg/ml	47.82± 4.91^{b}	90.67 ± 1.82^{b}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
Mancozeb	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}

*Mean in each column followed by the same letter do not differ statistically (Student test, P <0.05); Control (-) = negative control (distilled water).

Regarding the ethanolic extracts, total growth inhibition (100%) was obtained at low concentration compared to aqueous extracts. In fact, total growth inhibition of the pathgen was obtained with ethanolic extract of *T. algeriensis* at 30 mg/ml. Like in aqueous extracts, there was no total growth inhibition with ethanolic extracts of *Z. officinale* and *A. sativum*. Ethanolic extract of *A. sativum* was the less efficient (Table 3).

Table 3. Effect of ethanolic extracts on the growth inhibition (%)* of *Phytophthora megakarya*.

Concentrations	*Allium sativum*	*Zingiber officinale*	*Thymus algeriensis*
Control (-)	0.0 ± 0.0^{e}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
7.5mg/ml	42.26 ± 1.19^{d}	84.52 ± 5.46^{c}	65.87 ± 2.25^{c}
15 mg/ml	44.64 ± 2.57^{d}	89.09 ± 3.44^{bc}	91.47 ± 3.59^{b}
30 mg/ml	48.61 ± 1.82^{c}	89.29 ± 1.57^{bc}	100.0 ± 0.0^{a}
60 mg/ml	54.56 ± 1.91^{b}	91.27 ± 2.24^{b}	100.0 ± 0.0^{a}
Mancozeb	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}

*Mean in each column followed by the same letter do not differ statistically (Student test, P <0.05). Control (-) = negative control (distilled water).

Values of EC_{50} and EC_{90} were higher for aqueous extracts than for ethanolic extracts. EC_{50} and EC_{90} for aqueous extract of African Panaxia, ethanolic extracts of *Z. officinale* and *T. algeriensis* were the lowest and did not differ statistically at 5%. Values were 0.19 and 29.18 mg/ml; 2.0 and 33.56 mg/ml; 1.32 and 24.97 mg/ml, respectively. Aqueous and ethanolic extracts of *A. sativum* gave the highest EC_{50} and EC_{90} (Table 4).

Table 4. Equivalent concentration ($mg.ml^{-1}$)* for 50% (EC_{50}) and 90% (EC_{90}) inhibition of the radial growth of *Phytophthora megakarya* by plant extracts.

Extracts	Plants	EC_{50}	EC_{90}
Aqueous extracts	African Panaxia	0.19 ± 0.13^{e}	29.19 ± 2.64^{c}
	Zingiber officinale	14.81 ± 0.84^{d}	124.11 ± 21.47^{b}
	Thymus algeriensis	32.35 ± 2.02^{c}	112.55 ± 16.57^{b}
	Allium sativum	127.72 ± 22.12^{a}	1151.14 ± 135.12^{a}
Ethanolic extracts	*Zingiber officinale*	2.0 ± 1.2^{e}	33.56 ± 4.45^{c}
	Thymus algeriensis	1.32 ± 0.4^{e}	24.97 ± 4.9^{c}
	Allium sativum	40.11 ± 7.20^{b}	137.58 ± 29.56^{b}

*Mean in each column followed by the same letter do not differ statistically (Student test, $P < 0.05$).

Fungicidal and fungistatic activity of plant extracts

There was no fungicidal activity among aqueous extracts of *A. sativum*, *Z. officinale* and *T. algeriensis* at the concentrations tested. However, aqueous extract of *T. algeriensis* develop a fungistatic effect on the growth of *P. megakarya* at 125 $mg.ml^{-1}$. Aqueous extract of African Panaxia present a fungicidal activity on the growth of the pathogen at 62.5 and 125 $mg.ml^{-1}$. Conversely, there was a fungicidal activity with ethanolic extracts of *T. algeriensis* at 30 and 60 $mg.ml^{-1}$. Aqueous extract of African Panaxia and ethanolic extract of *T. algeriensis* were therefore the most effective while both aqueous and ethanolic extracts of *A. sativum* and *Z. officinale* were the least effective (Table 5).

Table 5. Fungicidal and fungistatic activity* of plant extracts against *Phytophthora megakarya*.

Concentrations	*Allium sativum* extract		*Zingiber officinale* extract		*Thymus algeriensis* Extract		African Panaxia
	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous
7.5 mg/ml	#	/	#	/	#	/	#
15 mg/ml	#	/	#	/	#	/	#
15.63 mg/ml	/	#	/	#	/	#	/
30 mg/ml	#	/	#	/	#	+	#
31.25 mg/ml	/	#	/	#	/	#	/
60 mg/ml	#	/	#	/	#	+	#
62.5 mg/ml	/	#	/	#	/	#	+
125 mg/ml	/	#	/	#	-	#	+

*Activity of the plant extracts : # = extract was not tested at that concentration; / = growth inhibition of the pathogen was no total at that concentration in the presence of the extract; - = fungistatic activity of the extract and + = fungicide activity of the extract.

Discussion

Clonal resistance

It was noted that in field conditions, no clone developed total resistance against the disease. Clone IMC67 (Amazonian group from Perou) was the most susceptible followed by clones ICS40 and SNK13, while SCA12 and TAFO79/467 clones (Hybrids selected in Tafo (Ghana) from the cross between parents NA32 and Pa7) were the most tolerant. These

varied levels of clonal resistance may be due to their phenolic compounds content. In fact, Djocgoue (1998) showed that flavonoid, tannins and lignin content is high in cocoa pod and leaves of resistant clones, than the highly susceptible clones, suggesting their involvement in resistance to *Phytophthora*. An increased level of these specific phenolic compounds among cocoa clones with high resistance levels could be due to reaction of the clones against *Phytophthora* colonization. Moreover, total polyphenols compounds, soluble and insoluble sugars, nitrogen, proteins, flavonoid, tannins and lignin are involved in resistance of cocoa pod to black pod disease caused by *P. palmivora* and *P. megakarya* (Djocgoue, 1998).

Fig. 1. Organs and plant powder used to prepare the different extracts (A: *Zingiber officinale*, B: *Allium sativum*, C: *Thymus algeriensis* and D: African Panaxia).

It was also established that the presence of amino acids and carbohydrates in the cortex of resistant cocoa clones to *P. megakarya*, inhibited expansion of lesions (Omokolo *et al.*, 2002). However, field resistance could be attributed to several traits, such as pod susceptibility, the ability of the pathogen to produce inoculum on the pod, or to tree architecture, as well as the pod-bearing period. In Lab conditions, all pods of cocoa clones inoculated with *P. megakarya* developed the disease compared to negative control, showing that none of them was immune to the pathogen. This fungus is therefore associated with cocoa black pod disease observed. This is in agreement with Mboussi *et al.* (2016) who observed that eight strains of *Phytophthora megakarya* collected in different cocoa production areas in Cameroon caused cocoa black pods in many clones. Cocoa pod of clones SCA12 (belonging to Forastero group from Ecuador) and TAFO79/467 (Amazonian group) were «moderately resistant» revealing good coherence with the field observations. However, the case of "susceptible" clones shows that the black pod disease in the field is not constantly due to the same virulent strain as in laboratory conditions. Other factors could interfere in field conditions and affect disease development (plant physiology, synthesis of defence molecules, etc.). Resistance of some cocoa clones could also be explained by the resistance of the epidermis to the penetration of the fungus, the internal resistance of the cortex or a favoring or disadvantageous action of the clone on the multiplication of the parasite (Cilas and Despréaux, 2004).

Antifungal activity of plant extracts

Antifungal tests revealed that, all the aqueous and ethanolic extracts had a high inhibitory effect compared to the negative control.

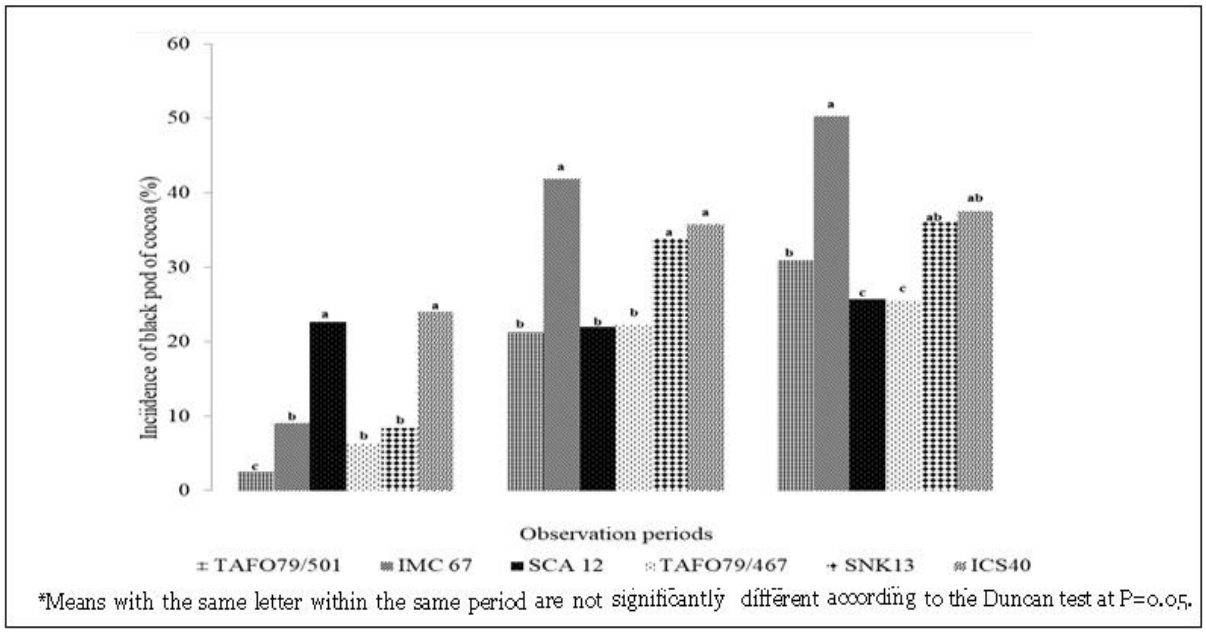

Fig. 2. Incidence of black pod disease per clone in cocoa plantation of the Mengang Production and Experimentation Center.

This inhibitory effect varies with the concentrations, the type of extract and the plant. These differences in the antifungal activity of plant extracts could be attributed to the active ingredients they contain. Indeed, the antifungal activity of *T. algeriensis* extracts could be due to their richness in thymol (5-methyl-1-2-isopropyl phenol) and carvacrol (5-isopropyl-2-methylphenol) which are the main phenolic compounds. Thymol has antispasmodic, expectorant, antiseptic, antimicrobial and antioxidant properties (Ciftci, 2009). This result also corroborates with that of Bessedik (2015) which highlighted the antifungal activity of the essential oil of *T. algeriensis* on the pathogenic fungi of date palms (*Alternaria alternata, Sordaria fimicola, Diplodia phoenicuim, Stemphyluim* sp and *Drechslera spicifera*). High antimicrobial activity against many pathogenic bacteria and fungi was also obtained with essential oil of *T. algeriensis* (Giweli *et al*., 2013).

The effectiveness of African Panaxia extract could be due to the mixture of medicinal plants (ginseng, *Ocimum grastissimum*, *Quinquifolium* and *Aloe vera*) with established antimicrobial properties. In fact, ginseng roots contain ginseng saponins which are proven to be the principal and most active constituents (Kim, 2012). *Aloe vera* extract is made of anthraquinones, salicylic acid, saponins, and sterols (Lee *et al*., 2013).

The efficacy of *Z. officinale* against *P. megakarya* observed in this study can be explained by its sesquiterpenes content: zingiberene (32%), β-sesquiphellandrene (11%) and curcumene (8%), responsible for its therapeutic properties (Imtiaj *et al*., 2005). In addition, previous work has shown the efficacy of this plant extract against *Pythium aphanidermatum* (Suleman and Emua, 2009). With regard to the effectiveness of extracts of *A. sativum*, the high sulfur and phenolic compounds content in Alliaceae family are responsible for their antimicrobial activities. Indeed, Triki *et al.* (2012) obtained a total growth inhibition with the same extract against *Rhizoctonia solani*, *Fusarium solani*, *Fusarium oxysporum* and *Verticillium dahliae*. Similarly, the incidence of late blight (*P. infestans*) in tomato was reduced after leaf spraying with garlic juice (Raouf and Khalil, 2001).

The inhibitory effect of *A. sativum* extracts could be attributed to the presence of alliin, a volatile substance that acts on the cellular metabolism of

fungi by oxidizing proteins (Slusarenko *et al*., 2008). The fact that, in this study, the inhibition of *P. megakarya* growth is partial with *A. Sativum* compared to previous studies and with low concentrations of *Z. officinale* and *T. algeriensis* suggests that each fungus reacts specifically to fungicides or any other exogenous factor (Kendrick, 1981).

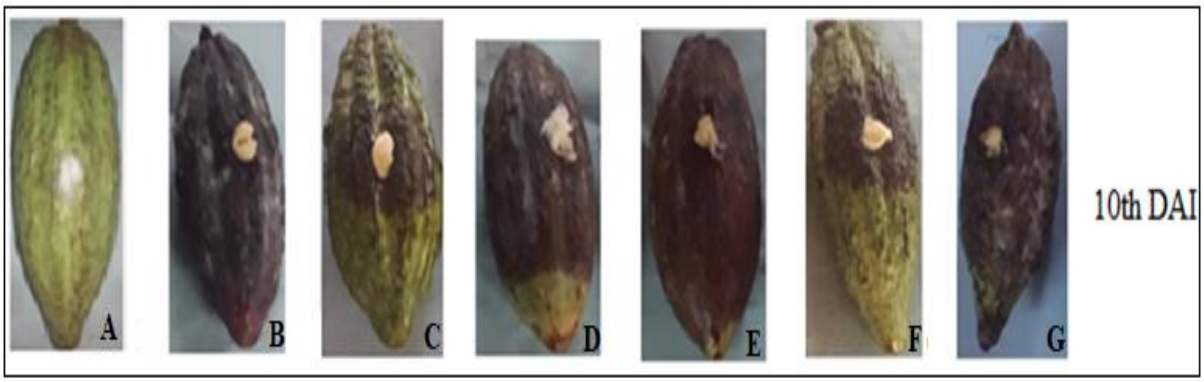

Fig. 3. Evolution of black pod of cocoa on the different cocoa clones after inoculation. A = control, B=SNK13, C= SCA 12, D= TAFO79/501, E=IMC67, F=TAFO79/467, G=ICS40.

Conclusion

Clones SCA12 and TAFO79/467 were more tolerant to black pod disease of cocoa both in Lab and field conditions. Clone ICS40 was the most susceptible. All the plants extracts tested showed significant inhibitory effect compared to the negative control at all the concentrations considered. Some plant extracts or mixtures of plant extracts such as *T. algeriensis* and African Panaxia showed total mycelia inhibition (100%) compared to the reference fungicide (Mancozeb). Aqueous extract of *T. algeriensis* was fungistatic at 125 mg/ml while ethanolic extract of *T. algeriensis* and aqueous extract of African Panaxia were fungicidal from 30 and 62.5 mg/ml, respectively. These plants are widely available and thus could be used in the organic farming environment. This is the first report of *A. sativum, Z. officinale, T. algeriensis* and African Panaxia activity against *P. megakarya*. Further studies are warranted on isolating the active components found in the plant extracts for chemical characterization and their possible use for black pod disease of cocoa management in the field.

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