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Variability of polyphenolic extracts from different oil palm trees and evaluation of their effect on *Coelaenomenodera lameensis* (Coleoptera, Chrysomelidae) larvae

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ABSTRACT

Coelaenomenodera lameensis is an insect and the major pest of the oil palm. In case of strong attack, it causes up to 50% loss of production. Larval development of *C. lameensis* is more pronounced on sensitive palm tree *Elaeis guineensis* originating from La Mé, Yocoboué and Deli compared to tolerant palm trees *Elaeis oleifera* originating from Central American. The objective of this work is to study the variability content of polyphenols from different palm trees and their effect on the larvae of *C. lameensis*. To do this, the extracts from the leaflets of these palms were analyzed by HPLC and tested on larvae of the leaf miner. Considering the results, HPLC analysis has revealed additional peaks polyphenols characterizing trees palm *Elaeis oleifera* tolerant, the retention time of 22.9, 26.4 and 30.4 min. Furthermore, bio-assays conducted on the larvae of *Coelaenomenodera lameensis* showed differential mortality of these larvae following the origin, the time and the concentration of applied chemical extracts. Indeed, this result indicates that the molecules in the three characteristic peaks are probably potential polyphenols and are in charge of tolerance to *C. lameensis*.

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Keywords: *Elaeis guineensis*, *Elaeis oleifera*, leaf miner, HPLC, polyphenols.

INTRODUCTION

Growing palm tree (*Elaeis guineensis*) has a socio-economic (Djegui and Daniel, 1996) and cultural (Amoussou, 1967) importance in the Gulf of Guinea. In Benin, this activity is done by the majority of people in the South to an average production of

50,000 tons of palm oil (FAOSTAT, 2008; Kimble et al., 2008) and is for the Beninese economy, an opportunity in terms of foreign exchange earnings. In 2002, the plantations of Pobè station (a Benin Southeast city) underwent high pressure due to the overgrowth of *Coelaenomenodera lameensis*

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(*C. lameensis*) that caused severe defoliation of palms (Coffi, 2003). The distribution of populations of this pest related to this type of defoliation is based on the origins of the palm tree (Philippe, 1990, 2003). Consequently, this defoliation varies in terms of the origins of the *Elaeis guineensis*. The study of the development of the leaves miner, *C. lameensis* Berti, previously conducted (Coffi, 2006 ; Fagbohoun, 2009) has revealed that: the mortality of larval phases is more pronounced from certain origins than others ; the complete phenol concentrations detected in the leaflets of different origins of *Elaeis guineensis* (*E. guineensis* or *Eg*) vary with the resistance or susceptibility of the plant.

Many control methods are used to reduce the population of this pest: biological control, selection of resistant varieties and chemical control by terrestrial, air or systemic way (Mariau et Lecoustre 2004; Yawson et al., 2009; Coffi et al., 2009; Niamouké et al., 2011). Only chemical control can now maintain populations of this insect to a tolerable economic threshold by the injection of systemic insecticides in the trunk of the palm tree (Mariau, 2001). Unfortunately, this chemical control has limitations. Ground spraying is only applicable to non-hilly areas extending up to 500 ha (Philippe et Mariau., 1983). It is not suitable for small agricultural areas exploited by farmers of our region. In addition, the active material is heat labile and would probably deteriorated at 60 °C (Philippe, 2003) and its repeated use may cause resistance problems and the destruction of the parasite and predator associated with pest (Mariau, 2001). Added to this, are the difficulty of application, the high cost, the unavailability of products and facilities that make this chemical control less risky for human health and environment.

In terms of biological control, Mariau et al. (1978) found that the main pest of *C. lameensis* larvae, belong to the family of Eulophidae but its action is insufficient to limit the growth of these insects population. On the contrary, isolated virus from dead larvae in the galleries, which are responsible

for 50-60% of larval mortality, may play an important role in the fight against the propagation of *C. lameensis* limiting (Marie and Miguel, 2008). However, mortality rates lower than 98.6% can involve instability of the insects' population and then may cause an outbreak. Nevertheless, according to Lattanzio et al. (2006), Khanna et Kannabiran (2007), phenolic compounds seem to play an important role in resistance to certain pests. Besides, the knowledge of the biochemical and molecular mechanisms involved in the interaction of palm/pest couple could help the development of control strategies for stimulating defense mechanisms of the plant (Métraux and Raskin, 1993; Lattanzio et al., 2006).

In such context, phenolic compounds could represent a more efficient alternative, and less toxic compared with the methods, using chemical insecticides. The aim of our study is to evaluate effect of polyphenolic extract from different oil palm tree and analyse their chromatographic profile.

MATERIALS AND METHODS

Samples and extracts preparation

Palm trees older than 8 years grown on Agricultural Research Perennial Plants Center (CRA-PP) Pobè have been chosen. These palm trees are specifically of two types : the miner sensitive *Elaeis guineensis* (Africa) from 3 origins (Deli, La Mé and Yocoboué) and the miner resistant *Elaeis oleifera* from America. Three leaflets are taken from the sheet 17 per vegetal material selected and 10 cm from the medium part of the leaflets are cut, dried in a room away from sunlight and then powdered.

A 200 mg quantity of the powder obtained for each sample is diluted in 10 ml of acetone-water solution (70: 30 v/v) and 0.5% formic acid. The resulting mixture is homogenized for 1 hour then filtered and evaporated (Wagner and Bladt, 2001). Ethyl acetate is added to the obtained product after evaporation and re-evaporated to 1 ml. It is then carefully filtered and injected into the HPLC system.

Biological tests on *C. lameensis* larvae

Biological test was realized according to method described by Coffi et al. (2012). The total polyphenols have been extracted through the same method as above from 50 g of powder per vegetal material selected. The dry resulting after evaporation at each selected vegetal extract is weighed. It is identified as solvent control, non-toxic, can promote complete dissolution of the obtained extract; ethanol to 6.36°. As a matter of fact, 220 larvae of the 2nd and 3rd stages of *C. lameensis* have been reaped and disseminated into 11 Petri dishes with filter paper, 10 larvae per Petri dish. Polyphenols extracts solution at variables concentrations (0.1, 0.2 and 0.4 g/ml) have been placed on filter paper near the mandibles of the larvae and the number of larvae dead or alive after 24 h, 48 h and 72 h and more have been counted to determine the mortality rate of the larvae depending on the days and the concentration of the total polyphenols consumed or which induces mortality to the larvae. Two petri boxes have been used as box-controls, where the larvae have been treated with distilled water and ethanol 6.36°. These experiments have been twice repeated.

Phytochemical analysis

Phytochemical screening has been referred to the exclusive identification of polyphenols in order to confirm their presence and their role in the resistance to pests of the palm tree. It is a qualitative analysis based on chemical reactions or discoloration of precipitation more or less specific to each class of active constituents (Table.1). Details of these tests and the composition of the chemicals have been recovered in the work of Houghton (1998).

HPLC analysis

The High Performance Liquid Chromatography (HPLC) of brand HITACHI (UV Detector L-24000) is used for the analysis of phenolic compounds present in the leaflets of palm trees. For those analyses, we used a new ACE5 C18

column (250 × 4.6 mm, 5 microns). Eluent consisted of acetonitrile-water (992: 8 ml) and formic acid 20 µl carefully filtered with a 5-95% gradient for 46 minutes to a flow rate of 1 ml/min and temperature 30 °C. Spectrometry UV recorded at 280 nm associated. The pressure is maintained at approximately 120 bar.

The standard used is provided by the CIRAD (Centre for International Cooperation in Agronomic Research for Development) and is composed of twenty phenolic compounds. The overlay of chromatograms from the injection of this standard to those of analyzed samples has allowed to have an idea of chemicals present in the leaflets of different origins of palm trees. Finally, the observed profiles have also allowed to continue the study and compare profiles of different samples.

Statistical analysis

The analysis of variance (ANOVA) on repeated quantities of the different results from the biological tests has been performed with the SAS software version 9.1. The mean observed and adjusted following the logarithmic transformation $y = \ln(x + 1)$ (with y : adjusted means; and x the proportions observed) were extracted and served to build curves that illustrate the evolution of mortality rates of larvae following the 3 extracts and different concentrations applied and then, revealed significant differences between the data.

RESULTS

Phytochemical analysis

Phytochemical screening achieved on the different origins of palm trees showed a differential presence of polyphenols with increasing visibility starting from sensitive origins (Eg-La Mé) to tolerant's (*E. oleifera*). Indeed, the results were more positive to the tolerant (*E. oleifera*) palm tree, than sensitive (Eg-Deli) and very sensitive (Eg-La Mé) palm tree (Table 2). However, this analysis revealed the presence of tanins, anthocyanins, flavonoids and leuco-anthocyanins, in the

three characterized leaflets oil palms. In addition yield extractions obtained from *E. oleifera* were higher than those of sensitive and very sensitive palm tree (Table 3).

HPLC analysis

Comparison of two extracts from miner sensitive Eg-La Mé and Eg-Yocoboué and the extract from miner tolerant or non attacked variety *E. oleifera*, shows a difference in the observed profiles (Figure 1). Indeed, the non-attacked variety (*E. oleifera*) possesses three interesting peaks at 22.9, 26.4 and 30.4 min that do not exist in the miner sensitivities profiles. As for the comparison of polyphenolic extract of miner tolerant *E. oleifera* and that from miner sensitive (Eg-Deli), the difference is also at two peaks 26.4 and 30.4 min (Figure 2). However, Eg-Deli extract has a same peak with *E. oleifera* at 22.9 min. In addition, the chromatographic profiles of polyphenols extracted from sensitive origins Eg-La Mé and Eg-Deli are virtually identical (Figure 3).

Biological tests on *C. lameensis* larvae

Table 4 shows that the controls such as distilled water and ethanol to 6.36° have no effect on the larvae. In fact, the larvae live and continue their development cycle in the presence of these solvents. This shows that humidity does not have a harmful effect on the larvae. Similarly, Eg-La Mé extract did not induce significant mortality on the larvae (only 30% after 72 h with the higher

concentration). On the contrary, the bio-tests performed with Eg-Deli and Eo extracts, induce an increasingly high mortality of the larvae, day after day. This higher mortality seems to be related with an increase amount of the extracts tested. The best result is obtained with the Eo extract, which after 3 days of exposure induces 90% of larval mortality.

The differential deviation presented by the different curves based on time reflects that the variation in mortality of larvae *C. lameensis* depends on the variation in concentrations of the same sample and different applied extracts (Figure 4). In fact, there is very little variation in mortality of larvae in chemicals extracts from Eg-La Mé compared to the other two (Eg-Deli and *E. oleifera*). In addition to concentration C3, there is a significant difference between the three extracts in relation to the mortality of larvae; *E. oleifera* leading to the highest rate of mortality. However to C1 and C2 for the concentration of extracted polyphenols from Eg-Deli and Eo, the larval mortality rate changes very little. The analysis of variance (ANOVA) confirm that death rate of the larvae of *C. lameensis* varies not only in time but also following extracts and applied concentrations with a risk of 5% error (Table 4). Indeed, statistical analysis revealed highly significant differences between the mortality rate of larva following the time and the three applied concentrations ($F = 3.94$, $DF = 8$, $p < 0.0095$).

Table 1: Specific chemicals and reactions of the phytochemical screening.

Family of compounds	Specific reagent and Reaction
Tannins	$\text{FeCl}_3 \rightarrow$ blue darkening color
Flavonoides	Shinoda (reaction to the Cyanidin) \rightarrow orange-red color
Anthocyanes	Red color in acidic environment and purple blue in alkaline environment
Leuco-anthocyanin	Chlorhydric alcohol ($\text{EtOH } 50^\circ/\text{HCl}_{\text{cc}} 2:1$) \rightarrow cherry red color

Table 2: Results of phytochemical screening.

Species	Titles	Polyphenols				
		Catechic Tanins	Gallic Tanins	Flavonoids	Anthocyanins	Leuco- anthocyanins
<i>E. oleifera</i>	Not attacked / tolerant	++	++	++	++	++
Eg-Deli	Less attacked / sensitive	+	+	++	+	++
Eg-La Mé	more attacked/ sensitive	+	+	+	+	++

+: positive; ++ strongly positive.

Eg : *Elaeis guineensis*; Eo: *Elaeis oleifera***Table 3:** Variation of yields following the origin of extracts.

Material	<i>E. oleifera</i>	Eg-Deli	Eg-La Mé
Intakes (%)	15.54	10.20	9.44

Table 4: Average number and mortality rate of larvae over a period of 3 days and per concentration of the vegetal extract used.

Different origins	Concentration in g/ml								
	24h after			48h after			72h after		
Eg-La Mé (%)	0.1	0.2	0.4	0.1	0.2	0.4	0.1	0.2	0.4
Eg-Deli (%)	0	0	0	0	0	0	30	40	30
<i>E. oleifera</i> %	10	40	50	30	50	50	40	50	60
Controls (distilled water)%	20	50	70	50	50	80	50	50	90
Controls (éthanol 6,36°) (%)	0			0			0		

Table 5: Results of analysis of variance on repeated quantities based on time.

Source	DF	F	Prob.
Time	89.25	2	<0.0001
Time*Extracts	5.4	4	0.006
Time*Concentration	2.73	4	0.0661
Time*Extracts*Concentration	3.94	8	0.0095

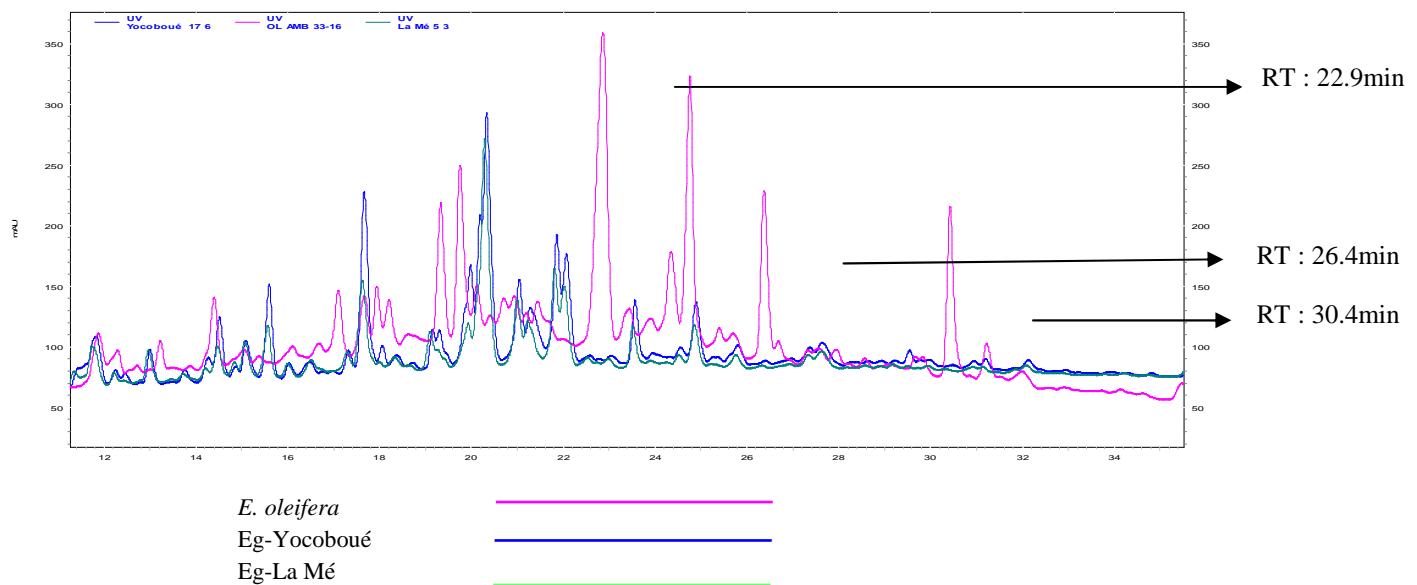


Figure 1: Comparison between sensitive materials (*E. guineensis* Yocoboué, and La Mé) and not-attacked (*E. oleifera*). RT: Retention Time.

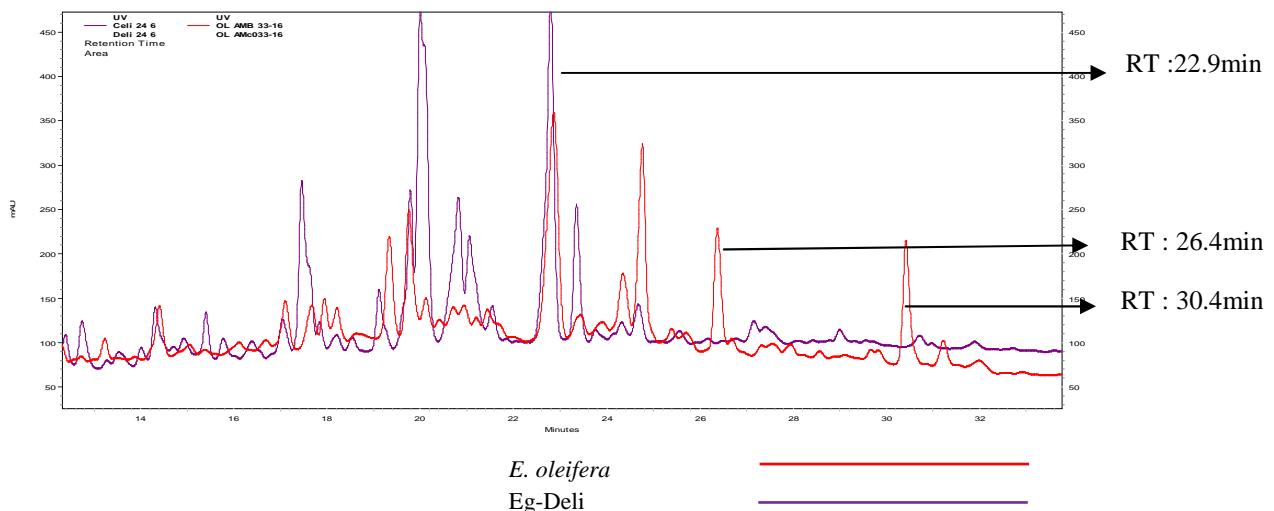


Figure 2: Comparison of tolerant materials (*E. oleifera*) and less attacked (*E. guineensis*, Deli). RT: Retention Time.

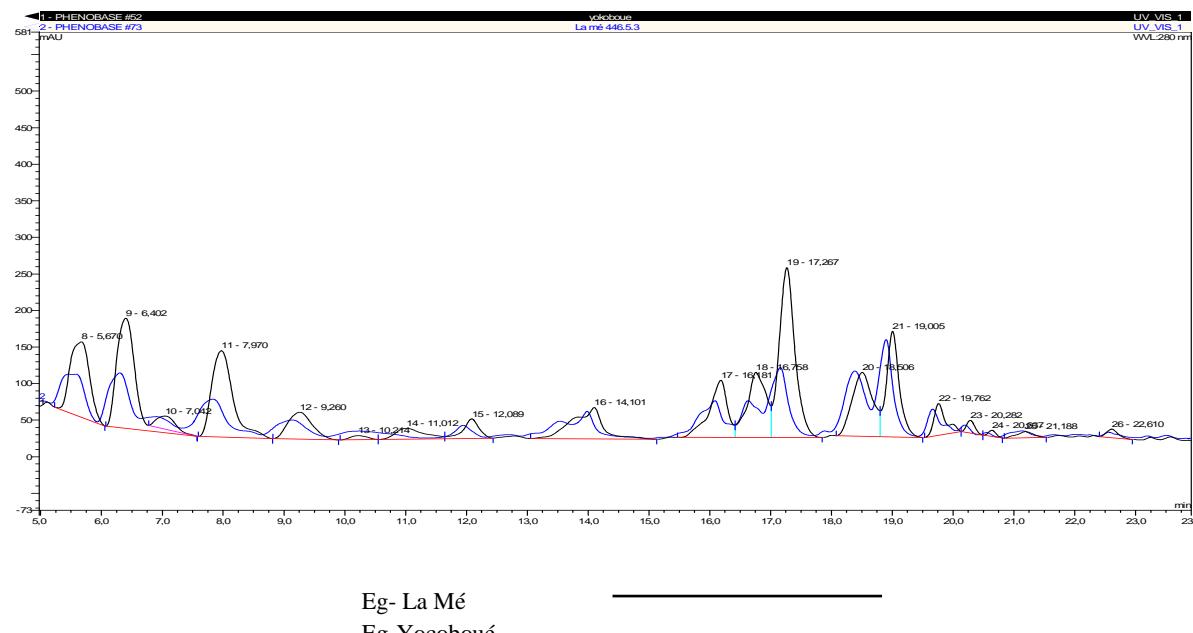


Figure 3: Comparison between Eg from La Mé and Eg from Yocoboué.

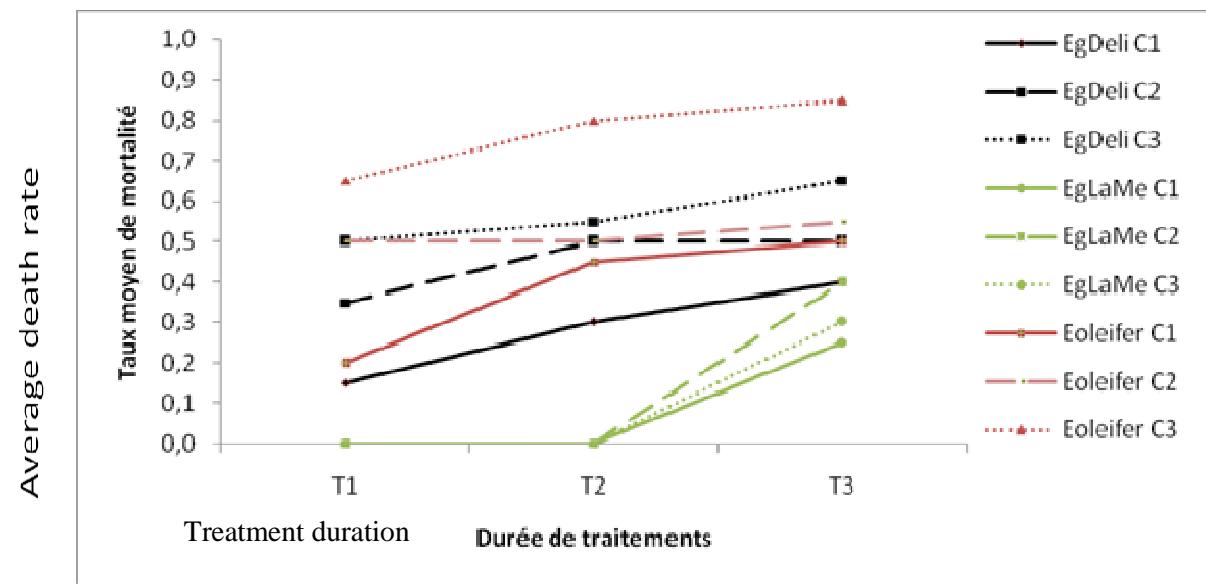


Figure 4: Average rate of larvae *C. lameensis* mortality based on extracts from palm trees of different origins and their processing time. T1 = 24 h; T2 = 48 h and T3 = 72 h, C1: 0.1 g/ml; C2: 0.2 g/ml; C3: 0.4 g/ml.

DISCUSSION

Our study showed us a better larvicidal activity with the polyphenolic extract of *E. oleifera* on *C. lameensis* (90% after three days with the high concentration C3), in comparison with those of Eg-Deli less active (60% after three days with C3) and those of Eg-LaMé, almost inactive (only 30%. % also after three days with C3). These results are comparable with observations made on the field by Coffi et al. (2013) and Konan et al. (2014) which had shown that the leaflets of *E. oleifera* are very unfavorable to the development of the pest. These results correspond according to them, to a different sensitivity against *C. lameensis* which would be related to the various origins of oil palm trees. These various origins which could confer specific characteristics to each type of oil palm tree and thus could explain the different sensitivity against *C. lameensis* (Coffi et al., 2012). Our study clearly showed that the phenolic compounds were involved in this larvicidal activity against *C. lameensis*. Indeed, according to our experimental conditions, all the polyphenolic extracts whatever the origin of the oil palm tree, caused a larval death after 72 h with the higher concentration C3. We thought that chemical analysis of the polyphenolic extracts from different palm tree would make it possible to notice the differences in the compositions in their phenolic compounds and thus could explain the differences observed in the larvicidal activity between the oil palm trees from different origin. The phytochemical screening of the leaflets of the different palm trees confirmed the presence of the principal families of the phenolic compounds (tanins, flavonoids, anthocyanins and leucoanthocyanins). *E. oleifera* seems to contain more phenolic compounds than Eg-La Mé and Eg-Deli (Table 2). However, the phytochemical screening carried out here, is based on colorimetric and precipitations reactions, this can explain the differences observed because the limit of detection of the tests carried out is not well known.

Chromatograms resulting from HPLC analyses of our different oil palm trees varieties show that those having the same degree of sensitivity to *C. lameensis* (Eg-La Mé and Eg-Yocoboué) have almost the same chromatographic profiles (Figure 4). However, when we compare chromatograms of species which have different sensitivity degrees with the larva, some differences appear. So, between the non-attacked variety (*E. oleifera*) and the attacked variety (Eg-La Mé and Eg-Yocoboué) we notice some differences on their chromatograms. Indeed, *E. oleifera* has 3 significant peaks at the retention time (RT): 22.9; 26.4 and 30.4 min which don't exist in Eg-La Mé and Eg-Yocoboué, chromatograms (Figure 2). However, chromatogram of Eg-Deli which slightly resists to the pest attacks compared with *E. oleifera* showed only one peak in common with that one, at RT 22.9 min (Figure 3). These results confirm on the one hand the results obtained from phytochemical screening and show on the other hand, the involvement of the compounds corresponding to the peaks observed at RT: 22.9; 26.4 and 30.4 min, in the different sensitivity between the three oil palm trees, against *C. lameensis*. This activity against the pest seems to depend on tested concentrations, because we observed that for *E. oleifera* and Eg-Deli, the increasing of larvae death go with the increasing concentration. However, it is not excluded to evoke in addition to this activity dependent on the concentration, a phenomenon of accumulation, necessary to tend towards the lethal dose when the larvae are in contact over several days with the extract. It is what we observed with Eg-La Mé which, with the strongest concentration of its extract caused finally larval mortality only at the third day and nothing during the two previous days. According to Coffi et al. (2012) and Tano et al. (2011, 2013), accumulation of the active substances within the pest from the first stages (eggs, larvae of first stage) until advanced stages (starting from the larvae of 2nd stage) can lead to the lethal dose causing the death of more than 50% of *C. lameensis*. The same

observations are also made with *E. oleifera* and Eg-Deli which both presented a higher larval mortality the third day compared to the two previous days. Our polyphenolic extracts contain different families of the phenolic compounds but our study could not identify the principal group clearly in charge for the larvicide activity. However, previous study had already highlighted the involvement of the gallic tanins of *Quercus lusitania* (Fagaceae) in its larvicidal activity against *Culex pipiens* an urban harmful effect mosquito (Redwane et al., 2002). Some authors Bernays (1980) and Lawson et al. (1984), had explained larvicidal activity of tanins by making plant not very digestible to the insects whereas the phenolic acids block their digestive enzymes at the stage larval. In addition, phenolic compounds Berenbaum (1983) may induce a toxic direct effect on certain species. Besides, studies of Nezha and Ismail (2000), conducted in Morocco in full olive field, have shown that synthetic phenolic compounds (tyrosol, oleuropein and caffeic acid) contribute to the reduction of the female reproductive potential of psyllid and particularly an increased larval mortality. Separation of the active principles of our three palm trees, research into their mode of action, effect on non target organisms are presently under investigation.

Conclusion

From this study, we can conclude that there is a difference in content of polyphenols of different origins of palm trees produced on the site of Pobè. This work has confirmed that the observed differences in the resistance of palm trees of different origins can be much influenced by the variance of the content of polyphenols. It can be argued that chemicals natural features of the peaks observed in RT: 22.9; 26.4 and 30.4 min are responsible for tolerance observed in *E. oleifera*. On the contrary, guineensis materials presenting the absence of these peaks are more sensitive to insect attack. In fact, palms trees that tolerate *C. lameensis* attack; *E. oleifera*, have an abundance of these chemicals substances and

substances increase the larvae's mortality when considering *E. guineensis* sensitive palm.

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