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Essentials Oils (*Ocimum gratissimum* and *Cymbopogon citratus*) Against *Pseudotheraptus devastans* in Coconut Plantations at Marc-Delorme Station in Ivory Coast

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Abstract: The bug *Pseudotheraptus devastans* is one of main insect pests in Ivory Coast. The objective of this study was firstly to evaluate the incidence of *Pseudotheraptus devastans* attacks on coconut palm plants of two varieties (GOA and NJM), and secondly to study the insecticidal activity of essential oils (*Ocimum gratissimum* and *Cymbopogon citratus*) on adults of *Pseudotheraptus devastans*. Bunches of nuts aged 2 to 4 months showed the highest attack rate in the GOA variety. On other hand, all the nuts aged 1 to 7 months were all attacked in the NJM variety. As for the drop rates, bunches aged 1 to 4 months recorded the highest drop rates for both varieties. It appears that the NJM variety is more sensitive to bites unlike that of GOA which was more tolerant. Mortality rates varied with applied concentrations and observation time. Thus, the essential oils extracted from the two aromatic plants *Ocimum gratissimum* and *Cymbopogon citratus* have insecticidal properties on the adults of *Pseudotheraptus devastans*. This insecticidal effect was highlighted by the LC50, therefore determining the essential oil of *Ocimum gratissimum* as the most toxic with an LC50 of 1.41% unlike *Cymbopognon citratus* which recorded a value of 7.76%. These biopesticides can be used to protect coconut palms against insect pests.

Keywords: *Cocos nucifera*, Bug Attacks, Fall Rate, Biopesticides

1. Introduction

The coconut palm is an oleaginous perennial plant belonging to the Arecacae family, native to the basins of the Pacific, Indian and Atlantic oceans [1]. *Cocos nucifera* L., coconut palm cultivation is practiced in the coastal regions of the intertropical zone [2]. Coconut is used in various fields, particularly in industrial and artisanal fields [3]. World production is estimated at 5.8 million tons of copra. Coconut cultivation covers an area of 11.8million hectares spread over 93 countries [4] of which 4.3% goes to Africa [5]. In Ivory Coast, the area of the coconut grove is estimated at around 50,000ha [6] of which 85% is located on the coast, from Assinie to San-Pedro [7], with an annual production of 65,000 tonnes of copra [5]. According several authors, Ivory Coast is the leading African country exporting coconut products (copra, oil, nuts, etc.) [8, 9]. It is therefore one of the main cash crops of the populations of this region, ensuring the subsistence of more than 20,000 families [10, 11]. However, the economic stake represented by this crop can be compromised because it is confronted with many diseases including the fatal yellowing discovered in the department of Grand-Lahou [6], and is also the target of many pests. Among these pests, special attention is paid to a DISTANT Heteroptera *Pseudotheraptus devastans*. In Ivory Coast, since 1973, this insect has caused major damage in coconut groves [12]. This insect from the Coreidae family, also called the coconut palm bug, limits the development of the coconut palm [13]. This species attacks some crops such as Manihot esculenta cassava. *Pseudotheraptus devastans* is the insect vector of canker "cassava candle disease" [14]. Larvae and adults bite young nuts less than 9 months old near floral parts [15]. Their stings cause the lysis of the affected tissues, leading to the premature fall of these nuts or their deformation [16, 17]. Attacks by *P. devastans* can lead to a drop in production ranging from 50 to 80% [13]. Faced with the threat posed by *P. devastans*, two control methods are used to reduce its harmfulness. These are chemical control with synthetic insecticide and biological control [18]. Chemical control consists of repeated monthly treatments of endosulfan. Although significantly causing the reduction of populations of *P. devastans*, it can generate resistance in them [12]. In addition, endosulfan, subsequently used, has been on European Union POPs (Persistent Organic Pollutants) list since May 5, 2011 [19, 20]. This pesticide therefore constitutes a threat to the health of populations and the protection of the environment. As for biological control, it was done using the red ant *Oecophylla longinoda* Latrille (Hymenoptera: Formicidae) which are natural predators [13]. For this reason, a plot colonized at 70% by *oecophylla*, with a homogeneous distribution, is effectively protected against *P. devastans* [21, 22]. However, this method is sometimes limited because black ants of the genus *Camponotus sp* and the rain disturb their pullulating. The innumerable consequences linked to the use of chemical insecticides, such as their toxicity, the disruption of the biological balance of ecosystem and development of resistant strains, combined with the limits of biological control with *Oecophylla*, impose on the search for new alternative methods of controlling this pest. The use of biopesticides for the control of insects pests is recommended for better respect for biocenoses and environment [23]. This study proposes to test the effectiveness of essential oils of local aromatic plants *Ocimum gratissimum* and *Cymbopogon citratus* on the coconut bug. Thus, the objective of this work is to develop an effective and environmentally friendly method of biological control, in order to reduce the harmfulness of the pest *P. devastans* for an improvement in coconut production in Ivory Coast. This will specifically involve determining the current level of infestation of *Pseudotheraptus devastans* on two varieties of coconut palm and evaluating the insecticidal potential of essential oils on adults of *Pseudotheraptus devastans*.

2. Material and Methods

2.1. Presentation of Study Site

The Marc Delorme Research Station (3°14' and 3°15' North latitude and 3°54' and 3°55' West longitude) is located in Port-Bouët. Its total area is 998 ha including 806 ha of area planted with coconut palms. This station was created in 1949 by the Research Institute for Oils and Oilseeds (RIOO). She houses the international collection of coconut trees for Africa and the Indian Ocean which is classified as "international heritage by the United Nations Food Fund (FAO). The climate of the Marc Delorme station is tropical with two dry seasons and two rainy seasons [24]. The average annual rainfall is 1475 mm for annual temperature of 26.4°C [18, 25]. The soils consist of Tertiary sands, very poor in exchangeable cation, but also characterized by a low percentage of organic matter (± 1%) and total nitrogen (0.5 to 0.4%).

2.2. Sampling Device for Pseudotheraptus devastans

The samples were carried out in two plots: 081 (19 years old) and 062 (12 years old) hosting respectively the "Nain Jaune Malaysia" (NJM) and "Grand Ouest Africain" (GOA) varieties. Each of these plots has 15 rows respectively, with 26 trees per row. To assess the incidence of *P. devastans*, thirty coconut seedlings were randomly selected for each variety. The choice of these plots was made according to three criteria in particular, the strong presence of the pest Pseudotheraptus devastans, the absence of *Oecophylla* nests on the coconut palms and the absence of chemical treatment.

2.3. Incidence of Pseudotheraptus devastans Attacks in the Plots and Variables Measured

The surveys were carried out for three months, from February to April 2021, during the dry season. Using a scale, bunches of coconuts, 1 to 7 months old from each selected tree, were observed over a period of three months (one observation per month). The total number of nuts from these different diets was counted while taking into account the nuts marked by bites of P. devastans. Similarly, on the coconut tree that supports the regime, the age of the regime and the date of observation have been inscribed. The results of the observations were recorded on a survey sheet taking into account the number of the tree and the line. The attack rate (1) and the falling rate (2) of the coconuts were calculated according to the following formulas:

$$Attack\ rate = \frac{number\ of\ coconut\ attacked}{number\ of\ coconut\ observed} \quad (1)$$

$$Falling\ rate = \frac{number\ of\ coconuts\ attacked\ and\ dropped}{number\ of\ attacked\ coconuts} \quad (2)$$

2.4. Evaluation of Effects of Essentials Oils on P. devastans in the Laboratory

2.4.1. P. devastans Rearing and Capture Device

The breeding equipment is made up of cages, trapezoidal in shape, 60 x 47 x 33 cm in size, made up of a metal support covered with a transparent white fabric (Figure 1). They served as breeding devices (in semi-natural conditions) and product testing (in the laboratory) on *P. devastans*. Using pruning shears, the cobs of the coconut bunch on which the cages were placed were pruned, and the adults and larvae of P. devastans were captured in cylindrical boxes of 5.5 cm base in diameter and 10 cm in height, fitted with lids. The observation and measurement equipment consists of an aluminum ladder to access the crown of the coconut palms. The Bresser magnifying glass, with a magnification of 10 x 30, was used to observe the eggs and to determine the sex of the adults of *P. devastans*. Then, these P. devastans larvae and adults were transferred to cages containing new diets with healthy 4-month-old young nuts. The adults are then placed in pairs, until the eggs are obtained, followed until the adult stage. The larvae, for their part, are followed until their imaginal moult.

Figure 1. *Rearing cage for P. devastans.*

2.4.2. Preparation of Solutions

The different concentrations of essentials oils of *O. gratissimum* and *C. citratus* (Figure 2) were obtained by dilution with acetone (solvent) then homogenized using a magnetic stirrer. From preliminary laboratory tests, each essential oil was diluted to 1/10th with acetone. Next five concentrations 0.50; 1; 2; 3; 4 and 5% were obtained and tested on insects. The products obtained with the essential oils were homogenized in vials covered with aluminum foil to protect them from light. The two essentials oils were provided by the National Center for Agronomic Research (CNRA) of Bouaké (Ivory Coast). Distilled water served as a blank control. The insecticid Callifan Super 40 EC, a pyretrinoid composed of 20 EC of neonicotinoid acetamiprid and 20 EC of bifenthrin served as a positive control. According to the manufacturer's recommendations, 5ml of this synthetic insecticide was diluted in 1.6l of distilled water. The concentration used for testing was 4.74×10^{-4} g/ml. Using a hand sprayer (at a jet of 0.02 ml of the product), the adults of P. devastans trapped in the different coconut regimes were treated with the different products.

a: Ocimum gratissimum, b: Cymbopogon citratus

Figure 2. *Aromatic plant.*

2.4.3. Performing Biological Tests

The bunches of coconuts of the two varieties studies, GOA (Figure 3a) and NJM (Figure 3b) aged 1 to 7 months were used for the biological tests. During the tests, the essentials oils and the insecticide were applied, using a hand sprayer, to the insects, i.e. 10 insects for each dose of product. The dead insects were counted at 24; 48 and 72 hours after treatment. Three repetitions were performed for each concentration. Any individual who moves with great difficulty and unable to coordinate their movements and difficulty getting back on their stomach was considered dead. Thus, any individual that moves and moves normally was considered alive (Doffou, 2013). When dead insects were observed among the controls, the corrected mortality rate (MC) was calculated by the formula of Abbott (1925).

$$Mr = \frac{Mortality\ rate\ in\ treated - Mortality\ rate\ in\ control}{100 - Mortality\ rate\ in\ control} \times 100 \quad (3)$$

With Mr: Corrected mortality rate.

a: GOA variety, b: NJM variety

Figure 3. *Four month coconut diet.*

2.5. Data Analysis

Data processing was performed using Statistica software version 7.1. The one-factor analysis of variance (Anova 1) as well as Fisher's LSD test at the 5% threshold made it possible to compare the rates of fall, attack and mortality rates according to the insecticidal activity of the two essential oils. The determination of the lethal concentrations, namely the LC50, was made using the Win DL 32 software.

3. Results and Discussion

3.1. Incidence of Attacks of Pseudotheraptus devastans on Coconut

With Grand Ouest Africain (GOA) variety, plans aged 2; 3 and 4 months old presented significant attack rates which vary from 13.68 to 21.68%. As for the bunches of walnuts aged 1 month, the attacks were very low. On 5-7 month old diets, the attack rate is zero. A statistically significant difference in attack rate was observed at the 5% threshold ($p < 0.031$) according to Fisher's LSD test (Figure 4).

Attacks were significant on all bunches aged 1-7 months during all months of the study with Malaysian Yellow Dwarf (NJM) coconuts. No significant difference in attack rate (Figure 5) was observed for bunches of this variety ($p < 0.734$).

A significant difference at the 5% threshold (p<0.05) in the attack rate was observed at the level of R1; R5; R6 and R7 of the two varieties studied. Attack rates ranged from 17.51 to 33.8% (Figure 5). For R2 schemes; R3 and R4, no significant difference was observed for the two varieties (Figure 4).

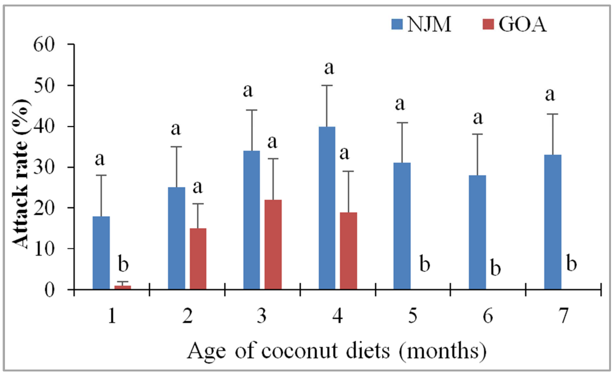

Figure 4. *Average coconut attack rate according to the age of bunches of GOA and NJM varieties.*

Means followed by the same letters are not significantly different at the 5% threshold (LSD Fisher test).

3.2. Coconut Drop Rate by Variety

With Grand Ouest African (GOA) variety, coconut drop is observed from the 1st to 4th month with fall rates varying from 47.06 to 13.4% (Figure 6). From the 5th month, no drop in bunches occurs for this variety. Statistical analyzes show a highly significant difference in the drop rate of diets at the 5% threshold (p < 0.00) according to Fisher's test.

However, with Malaysian Yellow Dwarf Coconut (NJM), bunches, aged 2 months, and suffered the most significant drops at 44.7%. They were followed diets aged 1; 3; and 4 months with respective values of 24; 15 and 8%. No fall was observed between the 6th and 7th month (Figure 7). The statistical analyzes showed a highly significant difference in the drop rate of the diets at the 5% threshold (p < 0.001) according to the Fisher test (Figure 5).

On the other hand, a significant difference was observed at the level of the R6 and R7 diets (Figure 5) at the 5% threshold (p < 0.001).

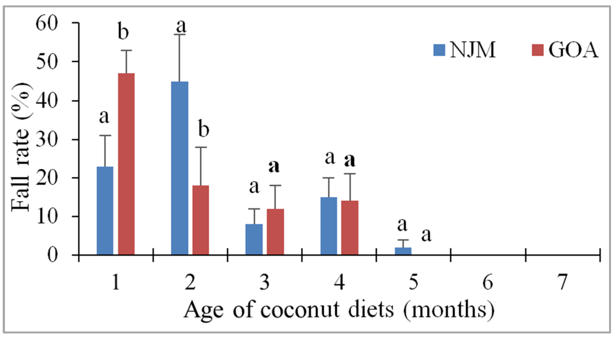

Figure 5. *Average falling rate of coconuts according to the age of bunches of GOA and NJM varieties.*

Means followed by the same letters are not significantly different at the 5% threshold (LSD Fisher test).

3.3. Correlation Between Attack Rate and Fall Rate on Two Varieties

Clusters of 1 to 4 month old coconuts of the GOA variety were all attacked, with the highest attack rate recorded by 3 month old coconuts and the lowest rate by 1 month old coconuts. In addition, falling occurs during all four months. The highest drop rate was obtained in the first month. From the 5th month, no attack and no drop were observed in the coconut clusters (Figure 6). The correlation test between attacks and falls is not significant (r = 0.077; p = 0.870).

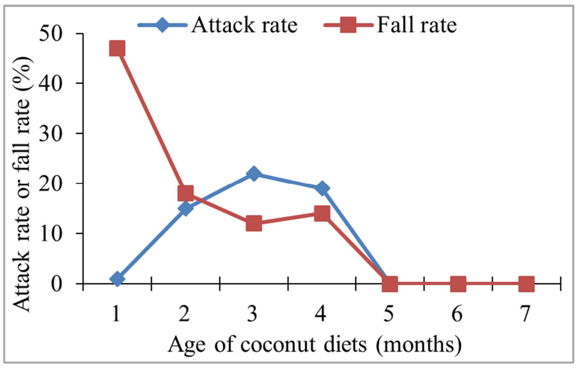

Figure 6. *Correlation between attack and drop rate of coconuts of the GOA variety.*

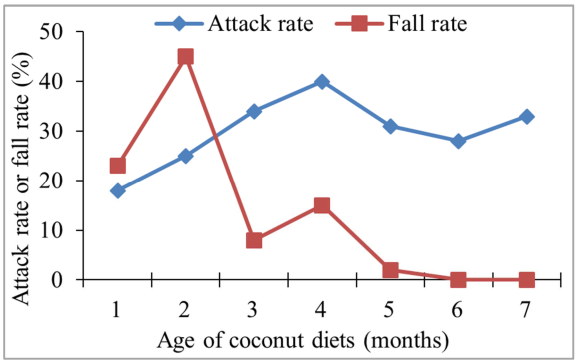

Figure 7. *Correlation between attack and drop rate of coconuts of the NJM variety.*

With the NJM variety, attacks ranged from the 1st to the 7th month while falls lasted from the 1st to the 5th month with the highest fall rate recorded in R2. In addition, attacks are higher than falls in this variety (Figure 7). The correlation test between attacks and falls was not significant (r = 0.458; p = 0.301).

3.4. Effect of Essential Oils on Pseudotheraptus devastans Adults

3.4.1. Mortality Rate of P. devastans According to Essential Oil of Ocimum gratissimum

The essential oil of *Ocimum gratissimum* was effective against adults of *Pseudotheraptus devastans* although the mortality rate recorded was not maximal (53.33%). The mortality rates of *P. devastans* adults generated by the different concentrations (0.5, 1, 2, 3, 4 and 5%) of *O. gratissimum* essential oil varied respectively from 20 to 53.33% during the

72 hours of this test. Except for the concentrations of 0.5 and 2%, all concentrations tested showed an increasing mortality rate over time. As for the positive control, it allowed the maximum mortality of the insects after 72 hours. The analysis of variance, followed by Fisher's LSD test at the 5% threshold, showed the existence of a significant difference between the concentrations according to the three dates of observation (p = 0.001; Table 1).

Table 1. *Average cumulative mortality of Pseudotheraptus devastans adults according to the concentration of essential oil Ocimum gratissimum applied.*

Tested Produts	Dose (%)	Time after contact (h)		
		24 h	48 h	72 h
Essential oil	0,5	20 ± 0 c	20 ± 0 c	20 ± 0 bc
	1	40 ± 0 b	40 ± 0 b	46,7 ± 6,6 b
	2	53,3 ± 6,6 a	53,6 ± 6,6 b	53,3 ± 6,6 b
	3	20 ± 0 c	33,3 ± 6 bc	53,3 ± 17 b
	4	26,7 ± 6 bc	46,7 ± 6,6 b	53,3 ± 6,6 b
	5	33,3 ± 6 bc	33,3 ± 6 bc	40 ± 1,5 b
Negative control (water)	0	0 ± 0 c	0 ± 0 c	0 ± 0 d
Positive control (Callifan 40 EC)	$4,7.10^{-4}$	40 ± 0 a	60 ± 0 a	100 ± 0 a
Significance	p	0.001	0.001	0.001

Anova test followed by Fisher's LSD test at the 5% threshold. Mean values followed by the same letter on the columns are statistically equal.

3.4.2. Mortality Rate of P. devastans According to Essential Oil of Cymbopogon citratus

The essential oil of *Cymbopogon citratus* was also effective against adults of *Pseudotheraptus devastans* with a maximum mortality rate of 60%. After 24h, the mortality rates of *P. devastans* adults generated by the different concentrations (2; 3; 4 and 5%) of *Cymbopogon citratus* essential oil were all 20%. This rate increased for all concentrations tested. Nevertheless, the concentrations of 0.5, 2 and 3% resulted in the mortality of 60% of the tested individuals after 72 hours. The concentrations of 1, 4 and 5% resulted in 40, 20 and 26% mortality respectively. The analysis of variance, followed by Fisher's LSD test at the 5% threshold, showed a significant difference between the concentrations according to the three observation dates (p = 0.00; Table 2).

Table 2. *Average cumulative mortality of Pseudotheraptus devastans adults according to the concentration of essential oil Cymbopogon citratus applied.*

Tested Produts	Dose (%)	Time after contact (h)		
		24 h	48 h	72 h
Essential oil	0,5	13,33±6,66 b	40 ± 11 b	60 ± 0 b
	1	0 ± 0 c	40 ± 0 b	40 ± 0 bc
	2	20 ± 0 b	46 ± 6,66 ab	60 ± 0 b
	3	20 ± 0 b	40 ± 0 b	60 ± 0 b
	4	20 ± 0 b	20 ± 0 c	20 ± 0 c
	5	20 ± 0 b	26 ± 6,66 c	26,7 ± 6,6 c
Negative control (water)	0	0 ± 0 c	0 ± 0 c	0 ± 0 d
Positive control (Callifan 40 EC)	$4,7.10^{-4}$	40 ± 0 a	60 ± 0 a	100 ± 0 a
Significance	p	0.001	0.001	0.001

Anova test followed by Fisher's LSD test at the 5% threshold. Mean values followed by the same letter on the columns are statistically equal.

3.4.3. Lethal Concentration (LC50) of Essential Oils

The lethal concentrations causing the death of 50% (LC50) of the populations tested were determined for each essential oils. According to the results recorded (Table 3), it is the extracts of *O. gratissimum* which presented the lowest LC50 (1.41%). On the other hand, values from 1 to 7 times higher (7.76%) were obtained with the extracts of C. *citratus.*

Table 3. *Lethal concentrations of essential oils on Pseudotheraptus devastans adults after 48 h of exposure.*

Essentials oils	N	Chi2	ddl	Pente	CL50 (%)
O. gratissimum	180	5,01	4	-0,41	1,41±03
C. citratus	180	3,24	4	-0,37	7,76±02

LC50: Lethal concentration killing 50% of the population, N: Number of individuals tested ddl: degree of freedom

3.5. Discussion

The study made it possible to evaluate the incidence of Pseudotheraptus devastans attacks on the nuts of bunches aged 1 to 7 months of *Cocos nucifera* of the NJM and GOA variety. The average attack rate of *P. devastans* observed during this work is lower than those obtained during the work of Julia and Mariau and that of Allou *et al.* [12, 13]. Indeed, the latter obtained respectively 60 and 39.97%. The difference would be due to settlement of a population of Oecophylles, a natural enemy of *P. devastans* in this research station. In their presence, the attack rate of nuts could decrease to less than 10%. But also, because that work took place in the dry season, this made it possible to record respectively a lower attack rate which is 21.68% for the GOA variety and 38.43% for the NJM variety. This therefore confirms the results of Julia and Mariau which stipulate that the maximum infestation rate occurred in the rainy season and the minimum in the dry season [12]. Concerning the average falling rates, the walnut bunches aged from 1 to 4 months for the GOA and NJM varieties, presented the highest falling rates. This would reflect their high sensitivity to attacks by *P. devastans*. This sensitivity could be explained by the fact that at their youngest age, the nuts present a stage where they are more tender and full of sap [18]. They are, therefore, a food source easy to exploit by these sucking insects. These results are in agreement with those of Yeboue et al. [26]. Indeed, this author confirms that the youngest fruits of a certain number of plant families would constitute an easy source to exploit for the coreids of which *P. devastans* belongs. Also the fall of these nuts would be due to the repeated bites of *P. devastans*. In reality, adult *P. devastans* would inject a toxic substance, thus causing lesions on the cells of the contact zone by the latter, but also due to the weak attachment of the floral parts to the epicarp [15]. As for the bunches of walnuts aged 5 and 7 months, their low fall rate could be explained by the small number of walnuts on these bunches and the firm attachment of the floral parts to the epicarp, but also by their maturation phase which would be very initiated. Indeed, at this stage, the epicarp of the coconuts becomes harder and fibrous. However, the NJM variety exhibited more attacks and drops of the coconuts, unlike the GOA variety. This would be explained by the yellow coloring of the nuts, representing a visual stimulus which, probably, would exert an attractive effect on the insect. Furthermore, the high concentration of kairomones emitted by these nuts could be mentioned [27]. This implies that nut drop from clusters of both GOA and NJM varieties would not necessarily be related to *P. devastans* bites. The data for this work were obtained from February to March (dry season according to the umbrothermal diagram). This aspect could therefore explain that falling nuts would be linked to other external factors such as temperature, humidity, wind, etc.

The results of this study showed that the essential oils of the two aromatic plants *O. gratissimum* and *C. citratus* have insecticidal effects on the adults of *Pseudotheraptus devastans*. After application, they caused the death of the majority of individuals tested depending on the concentrations used and the duration of exposure after treatment. These observations confirm their insecticidal property described by previous work [28]. The LC50 values showed that the essential oil *O. gratissimum* is much more toxic than that of *C. citratus* on *P. devastans*. This remarkable insecticidal potential of *O. gratissimum* would be due to the presence of thymol as recently proven by Kobenan *et al*. on the main cotton pests in Ivory Coast [29, 30]. Other authors such as Cloyd and Chiasson have hypothesized that this oxygenated compound acts directly on the cuticle of insects and mites, especially those with soft bodies, causing its degradation [31]. Thymol would also interfere with the activity of the synapses, which would prevent respiration by suffocation and lead to the death of the insect [32, 33]. As for the essential oil of *C. citratus*, it had a toxicity 7 times lower on *P. devastans* compared to that of *O. gratissimum*. Indeed, the essential oil of *C. citratus* is rich in monoterpenes with a predominance of compounds such as α-citral. The difference in insecticidal activity observed between these two essential oils would therefore be linked to their chemical composition.

4. Conclusion

This study highlighted that 2- to 4-month-old nut diets had the highest attack rate in the GOA variety. In contrast, 1- to 7-month-old nuts were all attacked in the NJM variety. In terms of drop rates, clusters aged 1 to 4 months had the highest drop rates for both varieties. It appears that the NJM variety is more susceptible to bites in contrast to the GOA variety which was more tolerant. This work was able to clearly show that bunch drop of both varieties would not necessarily be related to P. devastans attacks but to other external environmental factors. The results of this work make it necessary to study the impact of other environmental factors on coconut drop. As for the mortality rates obtained with the essential oils, they varied according to the concentrations applied and the time of observation. Thus, essential oils extracted from the two aromatic plants *Ocimum gratissimum* and *Cymbopogon citratus* have insecticidal properties on *Pseudotheraptus devastans* adults. This insecticidal effect was highlighted by LC50 values, thus determining the essential oil of *O. gratissimum* as the most toxic with an LC50 value of 1.41% in contrast to *C. citratus* which recorded a value of 7.76%. This efficiency obtained with essential oils suggests the realization of toxicity studies on mammals in order to popularize these extracts in the fight against this insect.

Conflict of Interest

The authors declare that they have no conflict of interest regarding this article.

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