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Effects of Essential Oils of *Eucalyptus citriodora* (Myrtaceae) and *Lippia multiflora* (Verbenaceae) on the Survival of Coconut Palm Bug, *Pseudotheraptus devastans* in Côte d'Ivoire

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Abstract

Pseudotheraptus devastans (Heteroptera: Coreidae) is a coconut pest in Côte d'Ivoire. Various larval stages and adults cause deformation of young nuts, sometimes leading to them falling off. The search for a strategy to protect these nuts involved evaluating the efficacy of essential oils of *Eucalyptus citriodora* and *Lippia multiflora* on *P. devastans*. Present study was carried out at the coconut research station of the Centre Nationale de Recherche Agronomique of Côte d'Ivoire. The tests were carried out by spraying EOs at different concentrations on *P. devastans* larvae and adults. Lethal concentrations causing the death of 50% (LC_{50}) and 90% (LC_{90}) of the populations tested were determined for each of the EOs. EOs had variable effects on the two developmental stages of the insect. Oil extracted from *E. citriodora* was more toxic, with respective values of LC_{50} = 0.04%, LC_{90} = 0.30% for larvae and LC_{50} = 1.24%, LC_{90} = 2.43% for adults, compared with that extracted from *L. multiflora* (LC_{50} = 0.17%, LC_{90} = 6.43% for larvae and LC_{50} = 3.51%, LC_{90} = 27.57% for adults). Its use in the field can, therefore, be suggested for the control of this coconut pest.

Keywords: Coconut coreid bug; Essential oils; *Eucalyptus citriodora*; *Lippia multiflora*

Introduction

Côte d'Ivoire coconut production is estimated at 124,810 tonnes per year from an estimated area of 38,531 hectares (FAOSTAT 2021). 80% of which is located on the coast from Assinie to San-Pedro (Konan *et al.* 2013). Coconut cultivation provides income and food security for more than 2000 families along Côte d'Ivoire's coastline (Assa *et al.* 2006). However, these benefits are under threat because the coconut palm is subject to heavy pest pressure. The main pests include *Pseudotheraptus devastans* Distant, a Heteroptera of the Coreidae family (Julia and Mariau 1978). This insect, the coconut palm bug, bites immature nuts to feed during its larval and the adult stages. Their bites cause the fruit to fall prematurely or become deformed (Vossen and Chipungahelo 2007; Doh *et al.* 2014). These attacks can lead to a loss of 50 to 80% of fruit production (Allou *et al.* 2006). In view of its threat posed, various control methods have been proposed to reduce its harmfulness. These include chemical control using synthetic insecticides and biological control using a natural predator (Allou *et al.* 2010). However, these control methods have shown their limitations. Chemical control leads to the development of resistant strains linked to the use of chemical insecticides that are harmful to the environment and to the natural enemies of *P. devastans.* An alternative to chemical control that is less restrictive in its use could be envisaged by exploring the anti-parasitic activity of local plants. The insecticidal properties of more than 2000 plant species (Philogène *et al.* 2002) have been proven on several insect pests, such as the aphthous effect of *O. basilicum* EOs on *Aphis gossypii* Glover (Akantetou *et al.* 2011), the efficacy of pepper extract on *Brassica oleraceae* L. and *Phaseolus vulgaris* L. (Fening *et al.* 2014), the efficacy of extracts from *Azadirachta indica* and *Jatropha curcas* on *Plutella xylostella* (Diabaté *et al.* 2014), effect of *L. multiflora* extracts on *Helicoverpa armigera* (Traore *et al.* 2015), effect of eucalyptus and lippia EOs on cotton parasites (Kobenan *et al.* 2018),

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insecticide effect of castor and ginger extracts on *P. devastans* (Doh *et al.* 2021). The aim of this study, search for alternative control for *P. devastans,* is to evaluate the effect of EOs from the leaves of *E. citriodora* H (Myrtaceae) and *L. multiflora* M. (Verbenaceae) on *P. devastans* larvae and adults in a semi-controlled environment.

Materials and Methods

Study site

The study was carried out at the CNRA Marc Delorme coconut research station located in Port- Bouët, 12 km from the Bassam motorway (Côte d'Ivoire). It is located between 05°14' and 05°15' north latitude and 03°54' and 03°55' west longitude (Koffi *et al.* 2017). Average monthly temperatures vary little, with a monthly average of 26.7°C. The average monthly relative humidity recorded during the trial was 87.11% with an average monthly rainfall of 149.90 mm.

Essential oils

EOs extracted from the leaves of *E. citriodora* H. (Myrtaceae) and *L. multiflora* M. (Verbenaceae), two (02) local aromatic plants, were used. These EOs were supplied by the Unité de Recherche Industrielle sur les Biopesticides of the Université Felix Houphouët Boigny (Côte d'Ivoire).

Extraction and analysis of essential oils

EOs of *E. citriodora* and *L. multiflora were* obtained from fresh leaves by saturated steam distillation using a Clevenger-type device for 2 h (Oussou 2009).

The composition of the oils obtained was determined by gas chromatography (GC) with a flame ionisation detector (FID) coupled to a mass spectrometer (MS). The gas chromatographic analyses were carried out using a Perkin Elmer auto-system, equipped with a two-column splitter injector and two flame ionization detectors. The identification of compounds in the EOs of *E. citriodora* and *L. multiflora was* based on a comparison of their retention times (RT) with those of reference compounds contained in a database (Kassi *et al.* 2020).

Mass rearing

Mass rearing of *P. devastans* was carried out in plot 060 on coconut bunches aged between 2 and 6 months placed in cages at an average temperature of 26.39 ± 1.50°C with an average relative humidity of 87.85 ± 2.15%. Pairs of *P. devastans* were placed in a cage containing a diet of immature coconuts on coconut palms to produce sufficient larvae and adults for various tests.

Preparation of concentrations

EOs of *E. citriodora* and *L. multiflora* was diluted with distilled water and tween 20. For each of the EOs, extract diluted 1:5$^{\text{ème}}$ (5% solution) was used to prepare different concentrations tested: 0.01, 0.05, 0.1, 0.5 and 1% on larvae and 1, 2 and 5% on adults. An insecticide containing 40 g of active ingredient/kg (20 g of acetamiprid and 20 g of bifenthrin) was used as reference control, with 10.4 mL diluted in 1 L of distilled water at the recommended dose (125 mL for 12 L of water).

Performing toxicological tests

During these tests, the EOs were applied to *P. devastans* larvae and adults using a hand- held sprayer. Stage 3 larvae (Fig. 1) from the above pre-defined rearing were used for the various treatments. Ten (10) larvae were placed in different cages containing immature nut diets as food. These larvae were also treated with each concentration of EOs. A batch of 10 stage 3 larvae was used for each control (reference control and blank control). Three replicates were made per concentration. Ten adults (Fig. 2) were placed in different cages (Fig. 3) for each concentration of EOs. A batch of 10 adults was used as a control (chemical control and water control) for each treatment.

Determining mortality rates

Dead insects were counted 24, 48 and 72 h after treatment. Insects that were immobile, visibly moribund, and unable to fly normally were considered dead. For each batch of larvae and adults, average mortality rates were calculated and corrected using Abbott's formula (1925).

$$\mathrm{MC}\,(\%) = \frac{\mathrm{Mt} - \mathrm{M0}}{100 - \mathrm{M0}} \times 100$$

Mc: Corrected mortality rate; Mt: Mortality observed at the concentration in question; Mo: Mortality observed in the white control.

Data analysis

An analysis of variance or ANOVA was performed using R software on the corrected insect mortality rate according to the treatments (EOs and concentrations). Where there was a significant difference between the treatments, the means were compared using the Newman Keuls test with a threshold of 5%.

Using WinDL 32.0 software (CIRAD, Montpellier, version 1998), the lethal concentrations causing the death of 50% (LC50) and 90% (LC90) of the populations tested were determined for each of the EOs, two days (48 h) after treatment.

Results

Chemical analysis using gas chromatography/mass spectrometry (GC/MS) identified 22 compounds in the EOs of *E. citriodora* (Table 1). These had percentage content greater than or equal to 0.057%. Examination of Eucalyptus EOs

Fig. 1: Stage 3 larva of *Pseudotheraptus devastans*

Fig. 2: Adult *Pseudotheraptus devastans*

Fig. 3: Breeding cage

showed that it is predominantly oxygenated monoterpene (91.8%). Citronellal (69.77%) is the majority compound. As for the EOs of *L. multiflora,* 18 compounds were identified (Table 2). Examination of the EOs also indicated that it is predominantly oxygenated monoterpene (82.0%), with 1.8 Cineol (41.4%) being the majority compound.

Table 1: Chemical composition of the essential oil of *Eucalyptus citriodora*

Terpene groups	Chemical Compounds	Percentage content (%)
Hydrocarbon monoterpenes	*α*-pinene	0.08
	β-pinene	0.43
	Limonene	0.25
	Total	0.76
Oxygenated monoterpenes	1,8 Cineol	0.14
	Cis-Rose oxide	0.27
	Trans-Rose oxide	0.13
	Isopulegol	4.66
	Citronellal	69.77
	Neoisoipulegol	0.24
	α-Terpineol	0.33
	Citronellol	10.63
	Geranial	0.32
	Isopulegyl acetate	0.16
	ρ-Menthane-3,8- diol	2.76
	Citronellyl acétate	2.16
	Jasmone < (Z)->	0.23
	Total	91.8
Hydrocarbon sesquiterpenes	*β*-Caryophyllene	1.34
	α-Humulene	0.10
	Bicyclogermacrene	0.07
	Total	1.51
Oxygenated sesquiterpenes	Spathulenol	0.07
	Caryophyllene oxide	0.22
	Total	0.29
Other compound	2,6- Dimethyl-5- heptenal	0.91
	Total	95.27

Table 2: Chemical composition of *Lippia multiflora* essential oil

Terpene groups	Chemical Compounds	Percentage content (%)
Hydrocarbon monoterpenes	*α*-pinene	2.7
	β-pinene	1.0
	Limonene	1.0
	Sabinene	5.0
	Myrcene	0.6
	α-Phellandrene	1.1
	β-Phellandrene	
	Total hydrocarbon monoterpenes	11.4
	1.8 Cineol	41.4
Oxygenated monoterpenes	Trans-*β*-Ocimene	
	p-Cymene	2.0
	Octene-1-ol-3	
	Linalool	2.3
	Terpineol-4	
	α-Terpineol	11.9
	Neral	9.0
	Geranium	13.9
	Germacreme D	1.5
	Geraniol	
	Total oxygenated monoterpenes	82.0
	Total	93.4

Effects of essential oils on the mortality of *Pseudotheraptus devastans*

Essential oil of *Eucalyptus citriodora*: EOs of *E. citriodora* at concentrations of 0.01 to 1% resulted in a mortality rate varying from 20 to 96.6% in 24 h, from 23.3 to 100% in 48 h and from 26.6 to 100% in 72 h on larvae. On the other hand, chemical control had a mortality rate of 100% for the observation periods and 0% for the water control. Statistical analysis revealed insignificant differences in the average mortality rate between concentrations of 0.5, 1% and chemical control, whatever the observation period, but a significant difference with concentrations of 0.01, 0.05 and 0.1%, as well as water control (Table 3). For adults, *E. citriodora* EOs caused a mortality rate at concentrations of 1 to 5% ranging from 23.33 to 90% in 24 h, 26.67 to 100% in 48 h and 30 to 100% in 72 h. Statistical analysis revealed no significant difference in the average mortality rate between the 2 and 5% concentrations and the chemical control, regardless of the observation periods, but a significant difference with the 1% concentration and the other doses, as well as the water control (Table 4). Mortality of *P. devastnas* larvae and adults was significantly dependent on concentration (*P = 0.00*). In fact, the mortality of the insects tested changed as the concentration of EOs increased.

Lippia multiflora essential oil

The results of the tests on the insecticidal activity of the EOs of *L. multiflora* indicate, at concentrations of 0.01 to 1%, a mortality rate of larvae varying from 13.33 to 63% in 24 h, and from 16.67 to 73.33% in 48 h and 72 h. On the other hand, the chemical control obtained a 100% mortality rate for the observation periods and 0% for the control with water. As for the adults, at concentrations of 1 to 5%, *L. multiflora* EOs caused a mortality rate ranging from 6.67 to 60% in 24 h, from 23.33 to 60% in 48 h and from 23.33 to 70% in 72 h. Statistical analysis revealed a significant relationship between concentrations and mortality rates for *P. devastans* larvae and adults (Table 5-7). Over the three observation periods, analysis of variance of mortality rates as a function of concentrations showed a very highly significant effect of this EOs on the survival of the pest (*P = 0.00*).

Lethal concentration of *Eucalyptus citriodora* and *Lippia multiflora* essential oils

Lethal concentrations causing the death of 50% (LC 50) and 90% (LC 90) of the populations tested were determined for each of the EOs. The oil extracted from *E. citriodora* was more toxic, with respective values of: LC 50 = 0.04%; LC 90 = 0.30% for larvae and LC 50 = 1.24%, LC 90 = 2.43% for adults compared to that of *L. multiflora* (LC 50 = 0.17%, LC90 = 6.43% for larvae and LC 50 = 3.51%, LC 90 = 27.57% for adults).

Discussion

The results of the analysis of the chemical composition of the EOs revealed that the extracts of *E. citriodora* and *L. multiflora* consisted predominantly of monoterpene compounds. This chemical characteristic is consistent with EOs as described by Kanko (2010). These oils are highly concentrated in oxygenated monoterpenes, as the results of analyses by Oussou *et al.* (2008) have also shown. The dominant compound was citronellal in the EOs of *E. citriodora*. These results corroborate those obtained from the work of Tolba *et al.* (2015) who revealed that the dominant compound in the EOs of *E. citriodora* was citronellal. 1, 8-cineole was determined to be more dominant in the EOs of *L. multiflora*. This compound is one of twelve (12) described by Adou *et al.* (2011) across sixteen regions of Côte d'Ivoire. However, the chemotype studied is different from that of Kobenan (2020). Indeed, this author identified *a*-phellandrene (39.01%) as the majority compound and the absence of 1,8-cineole. This difference would be due to the difference between the localities where the plants were collected. The chemotype used in this study originated in central Côte d'Ivoire, while the chemotype used in this study originated in northern Côte d'Ivoire. Aromatic compounds such as carvacrol and thymol were completely absent from our chemotype, despite being highly represented in the EOs of plants from the east and south of Côte d'Ivoire according to Soro (2016). The presence of carvacrol in *L. multiflora* extract has been reported by numerous authors in several countries (Viana *et al.* 2000; Abena *et al.* 2003; González-Trujano *et al.* 2017). This compound was considered characteristic of the EOs of this aromatic plant.

Table 3: Chronological evolution of the average mortality of *Pseudotheraptus devastans* larvae as a function of the concentration of essential oil of *Eucalyptus citriodora*

Concentration of essential oil (%)	Average mortality (%)		
	24 h	48 h	72 h
0.01	20 ± 10 **c**	23.33 ± 11.55 **d**	26.67± 5.77 **d**
0.05	30 ± 10 **c**	43.33 ± 5.77 **c**	53.33 ± 5.77 **c**
0.1	56.67 ± 11.55 **b**	66.67 ± 5.77 **b**	70 ± 10 **b**
0.5	93.33 ± 5.77 **a**	96.67 ± 5.77 **a**	100 ± 0 **a**
1	96.67 ± 5.77 **a**	100 ± 0 **a**	100 ± 0 **a**
Control (chemical)	100 ± 0 **a**	100 ± 0 **a**	100 ± 0 **a**
Control (water)	0 ± 0 **d**	0 ± 0 **e**	0 ± 0 **e**
Meaning (p)	0	0	0

Mean ± standard deviation. Within the same column, means marked with the same letter do not differ statistically at the 5% threshold (Newman Keuls test)

Table 4: Chronological evolution of the average mortality of *Pseudotheraptus devastans* adults as a function of the concentration of essential oil of *Eucalyptus citriodora*

Concentration of essential oil (%)	Average mortality (%)		
	24 h	48 h	72 h
1	23.33 ± 15.27 **b**	26.67 ± 11.55 **b**	30 ± 10 **b**
2	86.67 ± 15.27 **a**	93.33 ± 5.77 **a**	100 ± 0 **a**
5	90 ± 10 **a**	96.67 ± 5.77 **a**	100 ± 0 **a**
Control (chemical)	100 ± 0 **a**	100 ± 0 **a**	100 ± 0 **a**
Control (water)	0 ± 0 **c**	0 ± 0 **c**	0 ± 0 **c**
Meaning (p)	0	0	0

Mean ± standard deviation. Within the same column, means marked with the same letter do not differ statistically at the 5% threshold (Newman Keuls test)

Table 5: Chronological evolution of the average mortality of *Pseudotheraptus devastans* larvae as a function of the concentration of essential oil of *Lippia multiflora*

Concentration of essential oil (%)	Average mortality (%)		
	24 h	48 h	72 h
0.01	13.33 ± 5.77 **d**	16.67 ± 5.77 **d**	16.67 ± 5.77 **e**
0.05	23.33 ± 11.55 **cd**	26.67 ± 5.77 **d**	30 ± 10 **d**
0.1	40 ± 17.32 **bc**	50 ± 10 **c**	53.33 ± 5.77 **c**
0.5	50 ± 20 **b**	63.33 ± 11.54 **b**	63.33 ± 11.55 **bc**
1	63.33 ± 5.77 **b**	73.33 ± 5.77 **b**	73.33 ± 5.77 **b**
Control (chemical)	100 ± 0 **a**	100 ± 0 **a**	100 ± 0 **a**
Control (water)	0 ± 0 **d**	0 ± 0 **e**	0 ± 0 **f**
Meaning (p)	0	0	0

Mean ± standard deviation. Within the same column, means marked with the same letter do not differ statistically at the 5% threshold (Newman Keuls test)

Table 6: Chronological evolution of the average mortality of adults of *Pseudotheraptus devastans as a* function of the concentration of essential oil of *Lippia multiflora*

Essential oil (%)	Mortalité moyenne (%)		
	24 h	48 h	72 h
1	6.67 ± 5.77 **d**	23.33 ± 5.77 **c**	23.33 ± 5.77 **c**
2	26.67 ± 20.81 **c**	33.33 ± 15.27 **c**	33.33 ± 15.27 **c**
5	60 ± 10 **b**	60 ± 10 **b**	70 ± 10 **b**
Control (chemical)	100 ± 0 **a**	100 ± 0 **a**	100 ± 0 **a**
Control (water)	0 ± 0 **d**	0 ± 0 **d**	0 ± 0 **d**
Meaning (p)	0	0	0

Mean ± standard deviation. Within the same column, means marked with the same letter do not differ statistically at the 5% threshold (Newman Keuls test)

Table 7: Lethal concentrations (LC 50 and LC 90 in %) of essential oils of *Eucalyptus citriodora* and *Lippia multiflora* 48 h after application to larvae and adults of *Pseudotheraptus devastans*

Aromatic plant	Insect development stage	N	Chi2	ddl	Slope (%)	CL_{50} (%)	CL_{90} (%)
*Euc*alyptus *citriodora*		150	0.83	3	0.44	0.04 ± 0.29	0.30 ± 0.14
*Lippi*a *multiflora*	Larvae	150	0.623	3	0.16	0.17 ± 0.67	6.43 ± 0.41
E. citriodora		90	0.62	1	-1.41	1.24 ± 0.11	2.43 ± 0.17
L. multiflora	Adults	90	0.65	1	-1.42	3.51 ± 0.12	27.57 ± 0.38

LC 50: Lethal concentration killing 50% of the population, **LC 90:** Lethal concentration killing 90% of the population, **Insect stage**: Stage of development of the insect, **N**: Number of individuals tested, **ddl**: degree of freedom, λ **2**: Chi2

The bioassays carried out showed that the EOs of the two aromatic plants had an insecticidal effect on the larvae and adults of *P. devastans*. After application, they caused the death of most of the individuals tested, depending on the concentrations used. These observations confirm their insecticidal properties in addition to their antifungal and antimicrobial activities highlighted by previous work (Tolba *et al.* 2015; Tiendrebeogo *et al.* 2017). The high presence of monoterpene compounds in the essences of the two aromatic plants would explain their insecticidal efficacy against *P. devastans* larvae and adults. Indeed, Konstantopoulou *et al.* (1992) revealed that monoterpenes in EOs are toxic to many insects. Abdelkader (2012) has also shown that the insecticidal efficacy of an EOs is due to the nature and chemical structure of its terpene constituents. In general, larvae were more sensitive to the two oils than adults. This low sensitivity may be due to the fact that adults are relatively more resistant to the effects of the extracts than larvae, as reported by Doh *et al.* (2021). *In fact,* the more the larva evolves, the more resistant the cuticle becomes, and also the appearance of adult wings could constitute a barrier.

Determination of the lethal doses of the EOs showed that the EOs of *E. citriodora* was more toxic than that of *L. multiflora*. This could be explained by the significant presence of citronellal and citronellol in the chemical composition of the OEs of *E. citriodora*. This could be explained by the fact that these monoterpenes act on the chitin that makes up the insects' exoskeleton, dissolving it and causing them to die of dehydration. Indeed, the work of Deletre *et al.* (2014) revealed the toxicity of citronellal and geraniol on the whitefly *Bemisia tabaci*.

Conclusion

The EOs extracted from the two aromatic plants *E. citriodora* and *L. multiflora* have insecticidal properties on *P. devastans*. Referring to the lethal concentration values of each of these oils used, the study revealed that the EOs of *E. citriodora* is the most toxic. Field trials should be envisaged to assess its biological efficacy on this pest. This could lead to the practical use of these EOs as an alternative to chemical insecticides in the fight against the *P. devastans* bug in Côte d'Ivoire. Their use would respect the environment and people's health.

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Author Contributions

KJMK conceived this work by producing the toxicological text and writing this manuscript. GECO supervised the field work. KFJMK supervised the extraction of the EOs and the determination of the chemical components. NFH read the manuscript and provided comments. KA and KDK supervised the overall work.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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