



Insecticidal efficacy of the essential oils of *Eucalyptus saligna* and *Steganotaenia araliacea* and their major constituent to *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

Efficacité des insecticide à base des huiles essentielles d'*Eucalyptus saligna* et de *Steganotaenia araliacea* et leurs composés majeurs à l'égard de *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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Runing Title: Toxicity of two essential oils to *Sitophilus zeamais*

Abstract:

Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) is a dominant insect pest of stored maize in Cameroon and other tropical countries. Synthetic residual chemical insecticides, which are widely used to control the weevil have proven to be devastating to the environment, thus creating a window for the search of environmentally-friendly alternative control options. The objective of this work was to evaluate the insecticidal and repellent effect of essential oils of *Eucalyptus saligna* (Myrtaceae) and *Steganotaenia araliacea* (Apiaceae) and their constituent to *S. zeamais*. For adult toxicity bioassay, weevils were exposed to the essential oils and pure compounds, which were applied on filter paper in a petri dish at the dosages 0.19, 0.39, 0.78 and 1.56 $\mu\text{L}/\text{cm}^2$. Mortality was recorded every day for six days. The repellence of the four test substances was evaluated at five rates (0.026, 0.052, 0.156, 0.260 and 0.519 $\mu\text{L}/\text{cm}^2$) in a choice bioassay on filter paper. All the test substances caused significant dose-dependent mortality to *S. zeamais*. But for α -pinene, all the test substances caused 100% mortality to the weevil. 6-d LC_{50} were 0.07, 0.37, 0.37 and 0.43 $\mu\text{L}/\text{cm}^2$ for α -copaene, *S. araliacea*, *E. saligna* and α -pinene, respectively. *E. saligna* oil evoked the highest repellency of 86%, while the lowest (31%) was recorded by α -pinene. Therefore, the essential oil of *E. saligna*, which demonstrated a high level of repellency and the pure compound, α -copaene with higher toxicity, have great potential as effective and safer alternative for the management of adult *S. zeamais*.

Key words: Essential oil, pure compounds, repellence, toxicity, stockage

Résumé

Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) est un insecte ravageur dominant du maïs stocké au Cameroun et dans d'autres pays tropicaux. Les insecticides chimiques résiduels de synthèse, qui sont largement utilisés pour la lutte contre le charançon du maïs, se sont montrés dévastateurs de l'environnement, donnant ainsi la voie à la recherche d'autres moyens alternatifs de lutte, respectueux de l'environnement. L'objectif de ce travail était d'évaluer l'efficacité insecticide et répulsive des huiles essentielles de *Eucalyptus saligna* (Myrtaceae) et de *Steganotaenia araliacea* (Apiaceae) ainsi que leurs constituants à l'égard de *S. zeamais*. Pour le test de toxicité sur adulte, les charançons ont été exposés aux huiles essentielles et aux composés purs, qui ont été appliqués sur du papier filtre dans des boîtes de Pétri aux doses de 0,19, 0,39, 0,78 et 1,56 $\mu\text{L}/\text{cm}^2$. La mortalité a été enregistrée chaque jour pendant six jours. La répulsion des quatre substances testées a été évaluée à cinq différentes concentrations (0,026, 0,052, 0,156, 0,260 et 0,519 $\mu\text{L}/\text{cm}^2$) dans un essai de choix sur papier filtre. Toutes les substances testées ont entraîné une mortalité significative, dose-dépendante de *S. zeamais*. Excepté l'alpha-pinène, toutes les substances testées ont causé une mortalité de 100% du charançon de maïs. Les concentrations létales CL_{50} à 6 jours d'exposition étaient de 0,07, 0,37, 0,37 et 0,43 $\mu\text{l} / \text{cm}^2$ pour respectivement l'alpha-copaène, *S. araliacea*, *E. saligna* et l'alpha-pinène. L'huile de *E. saligna* a provoqué la répulsion la plus élevée (86%) et celle la plus faible (31%) par l'alpha-pinène. Par conséquent, l'huile essentielle de *E. saligna* qui a démontré un haut degré de répulsion, et le composé pur, l'alpha-copaène, présentant une toxicité plus élevée, ont un grand potentiel en tant qu'alternative efficace et moins dangereuse pour la gestion de l'adulte de *S. zeamais*.

Mots clés : Huile essentielle, composés purs, répulsion, toxicité, stockage

1. Introduction

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a serious economic insect pest of stored maize and other cereals across the tropical world. The poor storage facilities in sub-Saharan Africa favour the infestation and spread of the weevil, while the warm climate supports a higher rate of reproduction and development, rendering *S. zeamais* particularly devastating in this region. The weevil causes direct damage to grains from adult and larval feeding. Additionally, feeding by *S. zeamais* facilitates secondary fungal infection and reduction in quality due to biochemical changes in the grains (Mbata 1992), which diminishes the value of the commodity. Post-harvest maize damage/losses by the weevil ranging from 20 to 90% are common (Giga et al. 1991; Nukenine et al. 2002; Muzemu et al. 2013; Abass et al. 2014), and the severity of damage depends on factors which include storage structures and physical and chemical properties of the produce (Ojo and Omoloye 2016). Preventing infestation from *S. zeamais* remains a huge challenge for small-holder farmers in most countries of sub-Saharan Africa, including Cameroon (Nukenine et al. 2002; Rugumanu et al. 2012). Moreover, the problems have significantly increased in recent years due to the replacement of

local varieties by improved varieties, which are mostly not pest resistant (Suleiman et al. 2016). This is increasing the demand for synthetic insecticides which are commonly used to control insect pests of stored products (Dal Bello et al. 2000; Nwosu et al. 2015). However, inadequate education, haphazard application, lack of protective equipment, overuse, and lack of proper regulations of insecticides in developing countries have resulted in a number of serious drawbacks, such as persistence in the environment, chemical residues in foodstuffs, and adverse health consequences to humans and animals (Khan et al. 1989; Ngowi et al. 2007). Therefore, to obviate the drawbacks of synthetic insecticides, alternative insect control measures, which are environmentally friendly, are necessary.

Plant-based or botanical insecticides may be good alternatives to chemical synthetic insecticides because they are generally more biodegradable and the plant themselves are widely available. Botanicals include whole plant parts, powders, solvent extracts, vegetable oils and essential oils. Whole plant parts are usually less active (Jembere et al. 1995), while the powders, solvent extracts and vegetable oils leave residues on treated grains. Plant essential oils are very volatile with favorable ecotoxicological properties (low toxicity to humans,

degradation, and lower environmental impact), making them suitable to managing insects in organic farming (Chermenskaya et al. 2010; Zanuncio et al. 2016). These oils are plant secondary metabolites and include alkaloids, amides, chalcones, phenols, flavones, lignans, neolignans, or kawapirones, which are important in insect-plant relationships (Isman 2000; Martínez et al. 2015). Against insect pests, essential oils could act as contact poisoners, fumigants, repellents, antifeedants or oviposition inhibitors (Martínez et al. 2018).

Although many studies have demonstrated the good insect control ability of essential oils, registration of these oils has been less successful in many countries. Essential oils are mixtures of many compounds and the Herculean task of determining the safety of each compound must be performed before registration is considered. If the activity of a given essential oil is highly dominated by that of one of its constituents, that compound could more easily be developed into a crop protection product than the oil. In general, the yield of essential oils and other botanicals is usually low and obtaining large enough quantities also requires large quantities of plant materials, thus limiting their large school-scale exploitation. Nevertheless, in subsistence agriculture with small- to medium-sized storage structures and where the plants are locally available, the use of essential oils in the fight against the devastating insects of stocks could be practical as insecticides or repellents. Therefore, studies on essential oils may be more important in stored-grain protection for subsistence agriculture, while their pure compounds may be more important for large-scale grain storage.

Eucalyptus saligna Sm. (Myrtaceae) is a tall tree native to Australia, commonly known as blue Sydney blue gum and the leaves are commonly employed in stock protection in Cameroon (Taponjou et al. 2005). The insecticidal activity of the essential oil of this plant has been reported by several authors (Taponjou et al. 2005; Toloza 2006), but studies related to this oil and maize weevil are scanty. However, the composition and the effect of the essential oils depend mainly on the harvesting season, extraction method and geographical sources (Burt 2004; Santogo 2006; Gangue et al. 2017). Although the chemical composition of *E. saligna* varies across sites, the major constituents of the essential oils of its leaves harvested from the same area like that of the present

study were alpha-pine (35,9%), P-cymene (18,9%) and 1,8 cineole (9.6%) (Nukenine et al. 2007). *Steganotaenia araliacea* Hochst. (Apiaceae), a plant indigenous to Africa, is traditionally used for pest control in northern Nigeria (Abubakar et al. 2001) and for nematode control in cattle in Cameroon (Musongong et al. 2004). Petroleum and ethanolic extracts, as well as powders from the leaves of *S. araliacea* showed significant toxic and repellent effect against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Abubakar et al. 2001). The crude extracts of *S. araliacea* showed anthelmintic (Monglo et al. 2006) and antileishmanial activity (Ndjonka et al. 2010). There is lack of information about the insect control properties of *S. araliacea* and the only studies that reported on *S. zeamais* (Nukenine et al. 2007; Nukenine et al. 2013). concerned the leaf powders. The insecticidal properties of the essential oil from the plant is absent in the literature. Nonetheless, Nukenine et al. (2007) found α -copaene (28.5%), B-caryophyllene (9.7%) and delta-cadinene (7.5%) as the major compounds of the essential oil of *S. araliacea* collected from Ngaoundere, where the leaves for the current studies were collected as well. Therefore, in the present studies, the contact toxicity and repellent effects of the essential oils from the leaves of *E. saligna* and *S. araliacea* as well as α -copaene and α -pinene against *S. zeamais* were evaluated.

2. Materials and Methods

2.1 Test insects

Sitophilus zeamais was reared on maize in a controlled temperature and humidity chamber ($25 \pm 1^\circ\text{C}$ and 60 - 5% r.h.) in darkness. Parent adults of the weevil were obtained from laboratory stocks kept for many years at the Institute of Stored Product Protection in Berlin, without exposure to any known insecticide.

2.2 Collection and preparation of plant materials

The leaves of *E. saligna* and *S. araliacea* were collected in May (wet season) of 2017 around Ngaoundere, located in the Vina Division of the Adamawa province (plateau) of Cameroon, with the help of a botanist. Ngaoundere is located on latitude $7^\circ 22'$ North and longitude $13^\circ 34'$ East, at an altitude of 1100 m above sea level. The tropical climate is humid and hot and the agro-ecology is Sudano-Guinean savanna (Anonymous, 1981). The region is characterized by two seasons - a dry

season from November to March and a wet season spanning April to October. Average annual temperature is 22 °C with a maximum of 34 °C in March and a minimum of 12 °C in December or January. Annual precipitation is 1595 mm. The soil is ferrallitic, poor in nutrients and fragile with a pH of 4 - 6 (Anonymous, 1981). The leaves were dried at room temperature for seven days, and then crushed until they passed through a 0.5-mm mesh sieve.

2.3 Extraction of essential oils and procurement of pure compounds

Eight hundred grams of the crushed leaves of *E. saligna* and *S. araliacea*, respectively, were subjected to steam distillation for 6 hours in a Clevenger apparatus. The oil collected was dried over anhydrous sodium sulphate and they yielded 0.42% (*E. saligna*) and 0.09% (*S. araliacea*) (wt/wt) of essential oil. The oil samples were stored in a refrigerator at 4 °C until needed for bioassay. α -copaene ($\geq 90\%$), a major component of the leaf essential oil of *S. araliacea* and (98%), a prominent component of the leaf essential of *E. Saligna* (Nukenine et al. 2007) used for the bioassays were respectively purchased from Aldrich and Fluka, Germany.

2.4 Toxicity tests

Filter paper toxicity bioassay (Nenaah 2014) was used to evaluate the contact + fumigant toxicity of *E. saligna* and *S. araliacea* essential oils, as well as α -pinene and α -copaene to adult *S. zeamais*. Four concentrations (7.5, 15, 30 and 60 μ L) of each test substance in 1 mL of acetone were applied to Whatman no. 1 filter papers (7 cm diameter, surface 38.5 cm²) corresponding to dosages 0.19, 0.39, 0.78 and 1.56 μ L/cm². Controls received 1 mL of acetone. Each treated filter paper was placed in the bottom of a glass Petri dish (7 cm diameter). After the acetone was allowed to evaporate for 4 min in a fume hood, groups of 20 insects (7-14 days old) of mixed sex were separately placed on each treated filter papers. Each Petri dish was then covered with an original lid and wrapped with linear low density-polyethylene film.

Treated and control (acetone only) weevils were held at the same conditions used for insect rearing. Mortalities were determined daily until the sixth day after treatment. All treatments were repeated three times. Adults were considered to be

dead if appendages did not move when they were prodded with a fine brush.

2.5 Repellency tests

The area preference test described by McDonald *et al.* (1970) was used to evaluate the repellent action of α -pinene, α -copaene and the essential oils of *E. saligna* and *S. araliacea* on *S. zeamais*. Test arenas consisted of 7 cm Whatman no. 1 filter paper cut in half (19.25 cm²). Different test solutions were prepared by dissolving 0.5, 1, 3, 5 and 10 μ L of each test substance in 1 mL acetone, corresponding to the dosages 0.026, 0.052, 0.156, 0.260 and 0.519 μ L/cm². Each solution was applied to a half filter paper disc as uniformly as possible with a pipette. The other filter paper halves were treated with acetone alone. Chemically treated and control half discs were air-dried for 10 min to evaporate the solvent completely. Full discs were subsequently remade by attaching treated halves to untreated halves with clear adhesive tape. Each remade filter paper disc was placed in 7 cm Petri dish and 20 adult *S. zeamais* of mixed sex were released separately at the centre of the filter paper disc at 25 °C and 60 - 65% r.h. The Petri dishes were then covered and left under the same condition. Each treatment was replicated 4 times. The number of insects present on control (N_C) and treated (N_T) strip were recorded after 2 h exposure. Percent repellency (PR) values were computed as follows:

$$PR = [(N_C - N_T) / (N_C + N_T)] \times 100.$$

The mean repellency values of each essential oil was calculated and assigned to repellency classes (Juliana and Su 1983) from 0 to V: class 0 (PR < 0.1%), class I (PR =0.1-20%), class II (0.1-40%), class III (40.1-60%), class IV (60.1-80%), class V (80.1-100).

2.6 Data analysis

Mortality percentages and percent repellency values were transformed to arcsine square root values for analysis of variance (ANOVA) (SAS Institute, 2003; Zar, 1999). Student Newman-Keuls multiple range test (P = 0.05) was applied for mean separation. The lethal dosages causing 50% (LC₅₀) mortality of *S. zeamais* at 4-, 5- and 6-day exposure periods were calculated by probit analysis (Finney, 1971; SAS institute, 2003). Abbott's formula (Abbott, 1925) was used to correct for control mortality before probit analysis.

3. Results

3.1 Toxicity tests

As expected, significant differences in mortality were recorded among the test substances ($F = 36.69$; $d.f. = 3, 347$; $P = 0.0001$). Mortality increased with ascending concentrations ($F = 109.80$; $d.f. = 4, 347$; $P = 0.0001$) and time post-exposure ($F = 104.61$; $d.f. = 5, 347$; $P = 0.0001$). Data pooled over concentrations and time post-

exposure showed that the pure compounds caused greater mortality of *S. zeamais* than the essential oils. Variations in mortality among the concentrations across exposure periods were wider for the essential oils than for the pure compounds (Figure 1). α -copaene was more toxic than α -pinene, while *S. araliacea* caused a higher mortality of the insect than *E. saligna*. With the highest concentration of $1.56 \mu\text{L}/\text{cm}^2$ of filter paper, 100% mortality was recorded for α -copaene

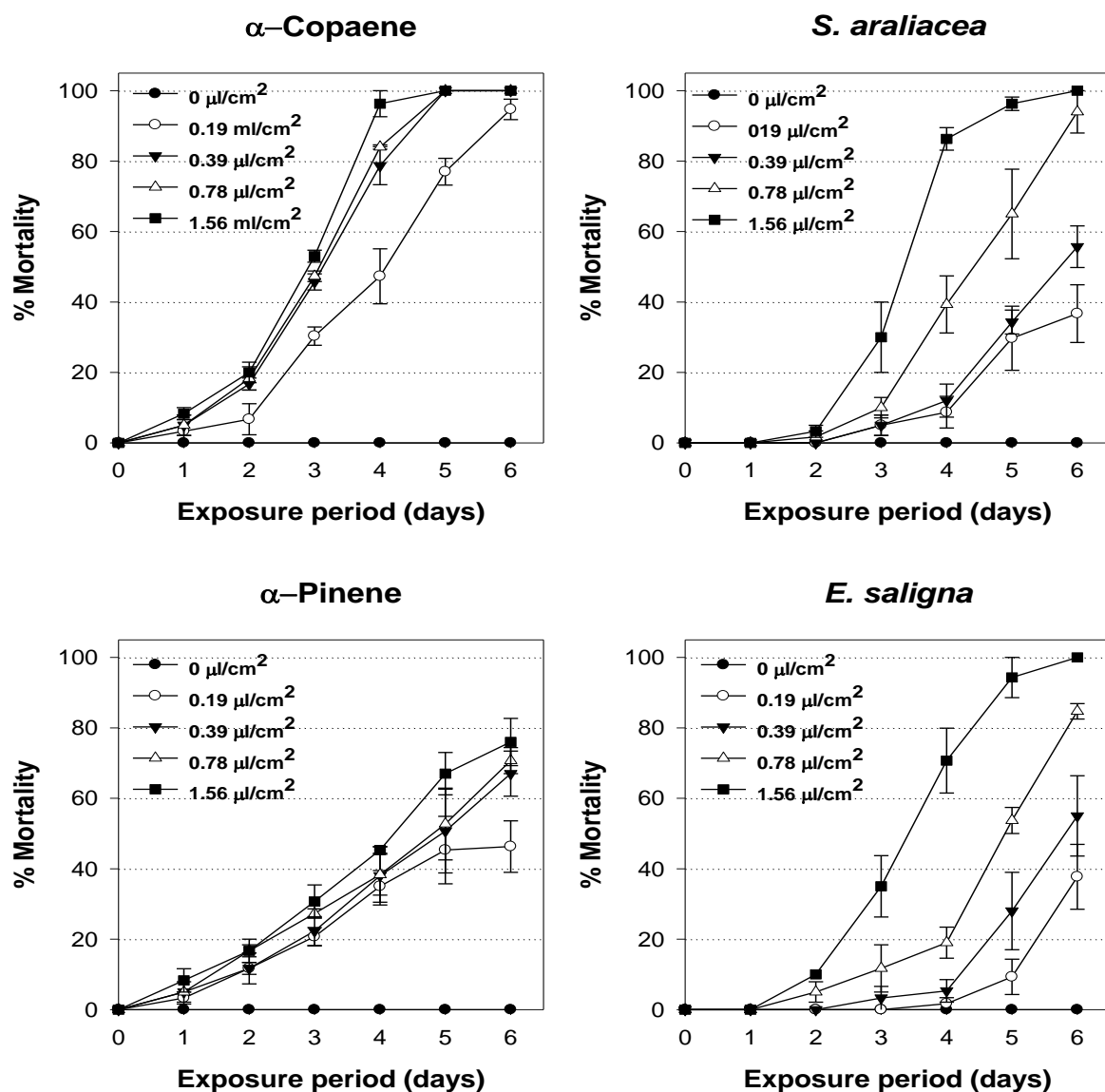


Fig. 1. Percentage adult mortality (mean \pm S.E.) of *Sitophilus zeamais* exposed to essential oils from *Steganotaenia araliacea* (α -Copaene) and *Eucalyptus saligna* (α -Pinene) and their major constituent on filter paper discs for exposure periods ranging from one to six days at 25 °C and 60-65% r.h. in the laboratory

within five days of exposure, but within six days for *E. saligna* and *S. araliacea*. Maximum mortality achieved by α -pinene was 76%. Within one day of exposure, the essential oils caused no mortality of the weevils while α -pinene and α -copaene recorded a maximum similar mortality of 8.3%. At the lowest tested concentration of $0.19 \mu\text{L}/\text{cm}^2$ of filter paper, α -copaene, α -pinene, *E. saligna* and *S.*

araliacea caused 94.7%, 46.3%, 37.7% and 36.7% mortality of the weevil, respectively, 6 days after exposure. The LC₅₀ values reduced with time post-exposure, irrespective of the test substance (Table 1). At 6-d post treatment, the pure compound α -copaene had lower LC₅₀ of 0.07 (0.006 – 0.140) $\mu\text{L}/\text{cm}^2$ compared to 0.37 (0.249 – 0.448) $\mu\text{L}/\text{cm}^2$ for α -pinene. At the same time-point, the LC₅₀ for *S. araliacea* and *E. saligna* were 0.37 (0.316 – 0.425) $\mu\text{L}/\text{cm}^2$ and 0.43 (0.326 – 0.442) $\mu\text{L}/\text{cm}^2$, respectively.

Table 1. LC₅₀ values of two essential oils (*Eucalyptus saligna* and *Steganotaenia araliacea*) and their major constituent (α -pinene and α -copaene, respectively) against adult *Sitophilus zeamais* on 7-cm diameter filter paper. LC₅₀ values are followed by 95% fiducial limits

Exposure time (d)	Product	LC ₅₀ ($\mu\text{L}/\text{cm}^2$)	Slope ^a
4	<i>E. saligna</i>	1.19 (1.060 – 1.361)	2.93 (0.27)
	α -pinene	1.54 (0.996 – 4.646)	0.68 (0.20)
	<i>S. araliacea</i>	1.07 (0.965 – 1.196)	3.40 (0.29)
	α -copaene	0.29 (0.214 – 0.354)	1.97 (0.25)
5	<i>E. saligna</i>	0.73 (0.660 – 0.817)	3.15 (0.27)
	α -pinene	0.65 (0.491 – 0.842)	1.17 (0.20)
	<i>S. araliacea</i>	0.53 (0.091 – 1.130)	2.43 (0.40)
	α -copaene	0.14 (0.095 – 0.228)	2.0 (0.32)
6	<i>E. saligna</i>	0.43 (0.326 – 0.442)	2.71 (0.20)
	α -pinene	0.37 (0.249 – 0.484)	1.27 (0.21)
	<i>S. araliacea</i>	0.37 (0.316 – 0.425)	2.76 (0.29)
	α -copaene	0.07 (0.006 – 0.140)	2.06 (0.62)

^a Slope of the probit line followed by SE.

3.2 Repellency tests

Results from the choice bioassay showed that the two essential oils and the two pure compounds were repellent to *S. zeamais* (Table 2). Maximum PR values for *E. Saligna* oil, *S. araliacea* oil, and α -

copaene were respectively 100% for the concentration 0.156 $\mu\text{L}/\text{cm}^2$ of filter paper, 75% for 0.260 and 0.519 $\mu\text{L}/\text{cm}^2$ of filter paper, 50% for 0.026 $\mu\text{L}/\text{cm}^2$ of filter paper and 65% for 0.260 $\mu\text{L}/\text{cm}^2$ of filter paper. *E. saligna* oil evoked the strongest repellency, followed by α -copaene and *S. araliacea* oils, while α -pinene was the weakest ($F =$ Table 2. Mean percent repellency (PR) values (\pm S.E.) and repellency classes (RC) for different dosages of essential oils of *Eucalyptus saligna* and *Steganotaenia araliacea* and their major constituent (α -Pinene and α -Copaene, respectively) against *Sitophilus zeamais* in the choice arena

Dosage ($\mu\text{L}/\text{cm}^2$)	Essential oil		Major compound	
	<i>E. saligna</i>	<i>S. araliacea</i>	α -pinene	α -copaene
0.5-0.026	48 \pm 2.5	23 \pm 4.8	23 \pm 6.3	35 \pm 15.0
1-0.052	93 \pm 2.5	48 \pm 13.1	50 \pm 7.1	53 \pm 13.8
3-0.156	100 \pm 0	38 \pm 11.1	20 \pm 3.9	60 \pm 14.7
5-0.260	98 \pm 2.5	75 \pm 10.4	23 \pm 12.5	65 \pm 19.4
10-0.519	93 \pm 2.5	75 \pm 6.4	40 \pm 9.1	40 \pm 12.3
Mean	86 \pm 4.9a	52 \pm 6.1b	31 \pm 4.3c	51 \pm 6.6b
RC	V	III	II	III

5.81; $d.f. = 4, 72$; $P = 0.0004$). The repellency classes were V, III, III and II for *E. saligna* oil, *S. araliacea* oil, α -copaene and α -pinene, respectively.

4. Discussion

Plant essential oils are worthy of consideration as a natural alternative in the control of stored grain insects and their potential for developing promising insect control agents has been emphasized (see Kumrungsee et al. 2014). These oils could act as contact poisoners (Nenaah 2014; Martínez et al. 2018), fumigants (Nenaah 2014), repellents (Nenaah 2014; Martínez et al. 2018), antifeedants or oviposition inhibitors. The contact toxicity and repellency of two essential oils and one of the constituents from *E. saligna* and *S. araliacea* against the maize weevil, *S. zeamais* were determined from bioassays in the laboratory. *E. saligna* oil and its constituent, α -pinene, as well *S. araliacea* oil and its constituent, α -copaene caused

significant mortality to *S. zeamais*, providing scientific rationale for their use by subsistence African farmers in grain protection against weevils (Tapondjou et al. 2000; Abubakar et al. 2001). The *E. saligna* oil (4-d $LC_{50} = 1.19 \mu\text{L}/\text{cm}^2$) from Ngaoundere, Cameroon, in the present study appears to be less toxic to *S. zeamais* than those from Dschang, Cameroon (3-d $LC_{50} = 0.36 \mu\text{L}/\text{cm}^2$) (Tapondjou et al. 2005) and Brazil (1-d $LC_{50} = 0.25 \mu\text{L}/\text{cm}^2$) (Mossi et al. 2011). These may be due to variation in chemical composition. For instance, the three major components of the *E. saligna* oil from Ngaoundere (α -pinene (35.88%), Cymol (18.86%) and Eucalyptol (9.65%): Nukenine et al. 2007), Dschang (α -pinene (39.5%), Cymol (31.1%) and Eucalyptol (9.8%): Tapondjou et al. 2005) and Brazil (eucalyptol (45.21%), cymol (34.37%) and α -pinene (12.81%): Mossi et al. 2011) were α -pinene, cymol and eucalyptol, but with variation in quantity across locations. More so, the differences in the chemical composition and bioactivity of essential oils over locations are well documented (Burt 2004; Santogo 2006; Gangue et al. 2017). Therefore, for efficient stored grain protection and pest control by essential oils, it is imperative to assess the bioactivity of the oils from the same plant species for different locations.

The insecticidal effects of *S. araliacea* is rare in the literature, although extracts from the plant possess anthelmintic (Musongong et al. 2004), antibacterial (Lino and Deograceous 2006), ant-leishmanial (Ndjonka et al. 2010), antiplasmodial (Njan Nloga et al. 2014) and probably other bioactivities. Abubakar (2001) reported the insecticidal efficacy of the petroleum and ethanol extracts of the plant against *Culex quinquefasciatus* Say (Diptera: Culicidae) and *T. castaneum*. The dried pulverized leaf powder of the plant showed good insecticidal efficacy against *S. zeamais* (Nukenine et al. 2007). To the best of our knowledge, studies on the essential oil of *S. araliacea* against *S. zeamais* are absent in the literature. In the present study, where leaves were collected from Ngaoundere, the potency of *S. araliacea* oil (6-d $LC_{50} = 0.37 (0.316 - 0.425 \mu\text{L}/\text{cm}^2)$) towards *S. zeamais* tended to be similar to that of the oil from *E. saligna* (6-d $LC_{50} = 0.43 (0.236 - 0.442 \mu\text{L}/\text{cm}^2)$). Therefore, if well exploited, *S. araliacea* oil could contribute significantly to the protection of stored grains against the infestation of *S. zeamais*.

Alpha-copaene (6-d $LC_{50} = 0.07 (0.006 - 0.140 \mu\text{L}/\text{cm}^2)$) was more toxic to *S. zeamais* than

α -pinene (6-d $LC_{50} = 0.37 (0.249 - 0.484 \mu\text{L}/\text{cm}^2)$). The difference in the potency of the two pure compounds was not surprising, because, although both are terpenes, the former is a sesquiterpene with three isoprene units, while the latter is a monoterpene with two isoprene units. Many studies have concentrated on the bioactivity of α -pinene in general, and against *S. zeamais* in particular (Suthisut et al. 2011; Yildirim et al. 2013). However, studies concerning the insecticidal activity of α -copaene are lacking, and thus should be encouraged, considering its higher level of toxicity compared with α -pinene to *S. zeamais* in the present study. Concerning the mechanism of action, when *S. zeamais* was exposed to higher dosages of the essential oils and the pure compounds in the present study, the insect displayed altered locomotion activity, with hyperextension of the wings followed by paralysis and death, which depicts toxic effect in the nervous system. Martínez et al. (2018) presented different studies, which showed that neurotoxic effect of insects exposed to monoterpenoids can cause blockade of octopamine receptor binding sites. These authors further stated that in this context, octopamine induces hyperextension of the legs and abdomen by increasing the frequency of excitatory postsynaptic potentials received by the appropriate abdominal motor neurons. Octopamine has a broad spectrum of biological roles in insects, acting as a neurotransmitter, neurohormone and circulating neurohormone neuromodulator (Martínez et al. 2018).

Within the dosage range 0.026 – 0.519 $\mu\text{L}/\text{cm}^2$, the essential oils and pure compounds were repellent to *S. zeamais* in a dose-dependent manner, with *E. saligna* much more active than the other products. The insect repellent properties of essential oils from *Eucalyptus* spp. are well documented (Araújo de et al. 2019). Mossi et al. (2011) demonstrated significant repellent activity of *E. saligna* against *S. zeamais*. The *E. saligna* from Ngaoundere in the present study was more repellent to *S. zeamais* than the one from Dschang, Cameroon, which recorded mean repellency of 73% for the concentration range of 0.05 to 0.40 $\mu\text{L}/\text{cm}^2$ (Tapondjou et al. 2005). More so, mean repellency of the oil in our study was 86%, with the dosage 0.156 $\mu\text{L}/\text{cm}^2$ repelling 100% of the weevil. α -pinene evoked the least repellency of 31% towards *S. zeamais*.

In conclusion, the essential oil of *E. saligna* which demonstrated a high level of

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repellency and the pure compound, α -copaene with higher toxicity have great potential as effective and safer alternative for the management of adult *S. zeamais*. However, under subsistence agriculture and the small-scale farmer's level, the two essential oils may be exploited for use against insect infestation of stored maize; they should be less cumbersome to apply than the dried foliage.

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