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Bioinsecticidal Activity of Eucalyptol and 1R-Alpha-Pinene Rich Acetonic Oils of *Eucalyptus saligna* on *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae)

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Abstract: Exploration of plants, animals, microbes and their products for green pesticides have been the mainstay of modern agriculturalists. Plant bioactive oils have been utilized in formulation of green pesticides, which are less toxic, least deleterious on biocontrol agents, readily biodegraded and have no or few ecological effects as compared to synthetic equivalents. This study evaluated the bioinsecticidal activity of Eucalyptol and 1R-Alpha-Pinene rich acetonic essential oils of dry leaves of the Sydney Blue gum (*Eucalyptus saligna* Smith) on common granivorous maize weevil. Fresh leaves of *Eucalyptus saligna* were harvested and the essential oils extracted from its dry powder by hydrodistillation. The chemical composition of the essential oil was analyzed by tandem GC/MS. Twelve components were identified, and the main components were Eucalyptol (34.36%) and 1R-alpha pinene (17.92%). Acetonic essential oils of 2µL, 4µL, 6µL and 8µL in 1ml acetone were used in contact toxicity, fumigant and repellent bioassays. In contact toxicity, 8µL/ml oil exerted a rapid effect on the weevils giving 100% mortality in the first day of exposure; 6µL/ml achieved 100% mortality 96 hours after treatment. Both fumigant and repellent activities showed a gradual increase in insect mortality and repellency with time of exposure as the concentrations of the essential oils were increased; 100% repellency was achieved at 8µL in the second hour and this remained constant with time. The lowest repellency of 20% was obtained at 2µL. In fumigant toxicity, the highest mortality was 70% recorded at 8µL on the third day of exposure. The results indicated that the acetonic essential oil of dry Eucalyptol and 1R-Alpha-Pinene rich *Eucalyptus saligna* leaves can be developed into a bioinsecticide for controlling maize weevil as a repellent, toxicant and fumigant candidate.

Keywords: Insecticide, Bioactive, Fumigant, Contact Toxicity, Repellent, Extraction

1. Introduction

Insects tops the list as the most significant group of crop pests of worldwide economic importance [1]. Insects existed since the earliest of times and evolved to stay and compete with humanity. The insidious threat, nuisance and detrimental action of insects such as maize weevils, locusts, cotton boll weevils, rice weevils, bean weevils, termites, aphids and moths on field and post field crops and crop products cannot be underscored as is always reflected by man's constant attempts to eliminate them. For quite a spell of time, various strategies, including but not limited to, incantations, plant-smoked fumigations, traps, scarecrows, physical picking, crop rotation as well as use of chemical concoctions against

pests have been attempted by man [2]. The maize weevil, *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae) is a cosmopolitan destructive post-harvest pest of cereals and cassava adjunct in Eastern Africa. The grain weight loss is estimated at 20-90% on average for untreated stored maize [3] and the damage severity is multifactorial, depending on the nature of the storage structures and the physical and chemical properties of the target produce. More so, pest damage of maize has been linked to aflatoxin. This is exacerbated by the fact that maize weevils house the fungi *Aspergillus niger, A. glaucus, A. candidus, Penicillium islandicum, P. citrinum, Paecilomyces, Acremonium, Epicoccum, F. semitectum* and yeasts [3]. Heavy infestation by adults and larvae of maize weevil inflicts intolerable postharvest losses. Usually, the weevils chew into cereals and the larva and pupa remain camouflaged in the grain bores. They hollow initially uninfested cereals and severe infestations is manifested by spotted grain hulls accompanied by powdery white frass. Elimination can be by fumigation, hand picking of weevils from the produce, planting resistant maize cultivars, segregation, good store hygiene and timely harvesting. Inert dust (ash and clay) can kill maize weevils; parasitoids: *Anisopteromalus calandrae, Cephalonomia tarsalis, Lariophagus distinguendus, Theocolax elegans* are efficient in early infestation phases. The fungus, *Beauveria bassiana* is another alternative weevil bioinsecticide for stored maize while *Bacillus thuringiensis* is efficient against the matured weevils. Reduced oxygen and carbon dioxide filled atmospheres regulate weevil population in stored grains. Deep freezing for several days and heating for hours are also practical [3]. Many pesticides can be used to eliminate maize weevils, some of which are obtained from nature. Nature has been a veritable evaluated pool of insecticides [4-6] with various nature-based compounds finding applications as templates for the synthesis of pesticides [7-19]. Plant essential oils for centuries have been devotedly studied to replenish and replace synthetic insecticides [20-23] with recently ousted efficacy. Due to their detrimental environmental effects such as ozone layer depletion, greenhouse gas emissions, lethality to non-target organisms, non-biodegradability and high costs of purchase and application, commercial insecticides such as Dichlorodiphenyltrichloroethane (DDT) are being completely banned or otherwise discouraged [24]. Resistance to the once efficacious synthetic pesticides has also been unprecedentedly reported in pest management and consequently the costs of crop production have been frustrating due to the backlashes pesticides [25]. There is, therefore, need to investigate the essential oils of *E. saligna* for a biodegradable, effective, cheaper and locally available bioinsecticide.

1.1. Eucalyptus saligna and Its Phytochemical Composition

Eucalyptus saligna Sm is a sturdy tall forest tree reaching 30-55m heights. It regenerates through its epicormic buds on the trunk and lower branches and thus is available throughout the year [26]. Studies on phytochemicals in the essential oils of *E. saligna* has been undertaken in various parts of the world with varying results (Table 1).

Table 1. *Reported literature on phytochemicals in E. saligna.*

Yield	Phytochemicals identified	Authors
0.19%,	∝-pinene, *p*-cymene, γ-terpinene	Batista-Pereira *et al* [27, 28]
0.38%	∝-pinene, 1,8-cineole, o-cimene, ∝-terpineol	Bett *et al* [29]
	p-cymene, ∝-terpineol, ∝-camphonellal, 1,8-cineole, ∝-pinene	Estanislau *et al* [30]
0.30%	∝-thujene, 1,8-cineole	Oyedeji *et al* [31]
0.40%	∝-pinene, *p*-cymene,∝-pinene oxide	Sartorelli *et al* [32]
0.78%	1,8-cineole, limonene, *p*-cymene	Cimanga *et al* [33]
1.42%	Pinene	Filomeno *et al* [34]
-----	1,8-cineole	Gillij *et al* [35]
0.50%	1,8-cineole, p-cymene, ∝-pinene	Mossi *et al* [36]
----	1,8-cineole, limonene	Toloza *et al* [37]
0.36%	1,8-cineole, *p*-cymene,γ-terpinene, ∝-pinene	Lucia *et al* [38, 39]

1.2. Pesticidal Studies Done on E. saligna

Bioactive oils of *Cupressus lusitanica* and *E. saligna* were screened for phytochemical composition, repellent and pesticidal potential on *Tribolium castaneum, Acanthoscelides obtectus, Sitotroga cerealella* and *S. zeamais* by Bett *et al* [29]. *C. lusitanica* had majorly umbellulone (18.38%) and ∝-pinene while *E. saligna* was as reported in Table 1. The insecticidal assay revealed that *A. obtectus* and *S. cerealella* were markedly affected by the oils, with reported lethal concentration (LC_{50}) values of 0.05-0.11% v/w for contact toxicity and 4.07-7.02μl/L in fumigant toxicity. *T. castaneum* had repellency from 65-92.5% whereas the other pests recorded repellency below 30% with appreciable reduction with exposure time [29]. Probing research on bioactive oils of *E. dunnii, E. saligna, E. benthamii, E. globulus and E. viminalis* for possible cidal and repellent potential against *S. zeamais* was done by Mossi *et al* [36]. In contact cytotoxicity, *E. globulus* and *E. viminalis* oils registered 100% mortality in 24hours at 0.16 and 0.23 μ L/cm^2. Regression analysis computed LC_{50} values of 0.08, 0.10, 0.16, 0.25 and 0.79μL/cm^2 for *E. viminalis, E. globulus, E. dunnii, E. saligna* and *E. benthamii* respectively with a correlation between 1,8-cineole content and LC_{50}.

Larvicidal potential of *E. grandis and E. tereticornis, E. grandis* and *E. camaldulensis* hybrid essential oils and essential oils of *E. gunnii*, *E. tereticornis, E. grandis, E. camaldulensis, E. dunnii, E. cinerea, E. saligna, E. sideroxylon, E. globulus ssp. globulus, E. globulus ssp. maidenii, E. viminalis* were evaluated against *Aedes aegypti* by Lucia *et al* [38]. Promising results

reported for *E. dunnii, E. gunnii, E. tereticornis, E. camaldulensis and E. saligna* registered LC_{50} of 25.2, 21.1, 22.1, 26.8 and 22.2 mg/L. The investigation unveiled a correlation between cytotoxicity effect and bioactive components *p*-cymene and 1,8-cineole. Some species (*cinerea, globulus ssp. maidenii, globulus ssp. globulus, sideroxylon, viminalis, grandis, tereticornis, grandis and camaldulensis*) yielded 1,8-cineole rich bioactive oils that were deficient in *p*-cymene and thus had larvicidal activity less than 50% after 24 hours of 40mg/L exposure [38, 39]. The vapor of the oils demonstrated efficacy against *A. aegypti* adults. The fumigant toxicity was expressed as knockdown effect time (KT_{50}) and ranged from 4.2-12.0 minutes with the most promising results obtained from oil of *E. viminalis*. The investigations further noted a linear correlation between 1,8-cineole and toxicity levels [39].

Monoterpenoids have been credited for their fumigant toxicity against storage pests [40,41] with lethality reported to be via acetylcholinesterase inhibition in insects [42]. The repellent activity of *E. saligna, E. camaldulensis, E. globulus* and *E. citriodora* oils were also assayed against *S. zeamais*. Y-shape olphatometer bioassay was used. The oils were solvated in hexane and at the highest concentration, *E. camaldulensis* and *E. citriodora* oils presented the best repellent activity (74.35% and 69.15%) than *E. globulus* (53.68%) and *E. saligna* (40.5%). The repellent activity observed for *E. camaldulensis* oil was markedly higher than that observed for the control. There is no available published data on the insecticidal potential of acetonic essential oils of *E. saligna* on maize weevils. This study reported the bioinsecticidal potential of acetonic essential oils of *E. saligna* on the granivorous maize weevils.

2. Materials and Methods

2.1. Sampling and Sample Preparation

Healthy mature fresh leaves of *E. saligna* were harvested from representative trees in Kyambogo University forest in the west end part of the University and taken to Department of Biology, Kyambogo University where it was identified. The leaves were then transported to the Chemistry laboratory where it was shade-dried for a fortnight prior to essential oil extraction. The dry leaves were cut into small sizeable pieces using a pair of scissors. Exactly 200g of the sizeable dry leaves were weighed using an analytical balance and soaked in distilled water for extraction. Maize weevils (*Sitophilus zeamais*) were obtained from Banda maize flour stores and the colony was mass reared on whole maize grains in glass containers at optimal growth conditions (ambient temperature of 25–28°C, 60–70% relative humidity) in the Chemistry laboratory for three days prior to the assays.

2.2. Phytochemical Analysis

The fine leaf powder was submitted to hydrodistillation using a modified Clevenger-type apparatus for 6 hours. The oil was dried using anhydrous sodium sulfate and stored in amber bottles in a refrigerator at 4 ℃. Separation and identification of the volatile constituents of the essential oil was carried out using a tandem gas chromatography-mass spectrometer with a chromatograph hooked to a mass selective detector equipped with a capillary column. The GC settings were as follows: the column temperature was held at 50℃ for 2min, then increased at 2℃/min to 250℃ and held there for 2min, and then increased at 10 ℃/min until the final temperature reached was 250℃, where it was held for 5min. The injector temperature was maintained at 250℃. Helium was used as the carrier gas at a flow rate of 1.0mL/min. Constituents were identified by comparison of their retention indices with those reported in the literature.

2.3. Contact Toxicity Assay

Different concentrations of the essential oil was prepared by diluting 2µL, 4µL, 6µL and 8µL of the oil in 1ml of acetone. Exactly 12g of the maize grains were weighed and each aliquot of the acetonic essential oil was mixed with the maize grains in petri dishes and thoroughly stirred to allow for homogeneity of oil on the treated grains. Each experiment was replicated three times and averaged. A control was set up by mixing 12g of maize grains with 1ml of acetone alone. Both essential oil and acetone treated maize grains were air dried for 20 mins to get rid of the solvent. The grains were thereafter infested with ten *S. zeamais* adults per petri dish and each petri dish was covered. The number of dead weevils in each petri dish were counted every day for five consecutive days.

2.4. Repellency Assay

The repellent activity of the acetonic essential oils against *S. zeamais* was investigated using a modified area preference method described by Zhang *et al* [43]. The test area consisted of a filter paper cut into two halves. Petri dishes (9cm in diameter) were used to confine the weevils during the experiment. The crude essential oil was diluted in acetone to four concentrations (2µL, 4µL, 6µL and 8µL), and acetone was used as the control. The filter paper was cut into half and 500µL of each concentration was applied separately to half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 500µL of acetone. Both the treated half and the control half were then airdried to evaporate the solvent completely for 30s. A full disk was carefully remade by attaching the tested half to the negative control half with a clear adhesive tape. Each remade filter paper after treatment was placed in a petri dish. Ten insects were released in the centre of each filter paper disk and the petri dish was covered. Three experiments were replicated, repeated three times and averaged. Counts of the weevils present on each strip were made after 1 hour for 5 successive hours. The percentage repellency (PR) of each volatile oil was calculated using the formula given by Abdelghany *et al* [44];

$$\mathrm{PR} = \frac{(Nc-Nt)}{(Nc+Nt)} \times 100\% \quad (1)$$

Where Nc is the number of insects present in the negative control half while Nt is the number of insects present in the treated half.

2.5. Fumigant Toxicity

Fumigant toxicity was tested as described by Liu and Ho [45]. Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil (four concentrations) was prepared in acetone. Different concentrations of 2µL, 4µL, 6µL and 8µL were prepared by diluting each quantity in 1ml acetone. They were then applied on the filter papers separately and air dried for 10 mins. Each dried filter paper was placed at the bottom of the glass jar. Counted 10 adult weevils were placed in muslin clothes each with 12.0g of whole maize grains. The clothes were tightly closed with rubber bands and hung at the center of the jars. The control was performed using a filter paper treated with acetone alone. The experiments stood for five days. Every day, the clothes were removed from the jars and the number of dead insects were counted. Each experiment was replicated three times and averaged. Percentage mortality (PM) was determined from (2):

$$PM = \left(\frac{Nd}{Ni} \times 100\right) \quad (2)$$

Where; N_d is the number of dead insects after a given time of exposure, N_i is the number of insects infested in the treated grains.

3. Results

3.1. Phytochemical Analysis Results

The essential oil was green with a yield of 0.1% (v/w) of dry leaves after two hours of extraction. The phytochemical constituents are shown in Table 2.

Table 2. *Phytochemical constituents of the oil from dry E. saligna leaves.*

Compound	Retention time (Min)
1R-alpha-pinene	9.939
Camphene	10.677
Eucalyptol	15.747
Cyclopentanepropanol,2-methylene	18.408
1,3-Cyclohexadiene	19.740
1,6-Octadien-3-ol	20.547
Fenchol	21.235
Bicyclo [2.2.1] heptan-3-one	24.467
3-Cyclohexen-1-ol	25.580
Cyclohexanol	26.425
Cis-Pinen-3-ol	39.641
Caryophyllene	41.218

3.2. Contact Toxicity Assay

In contact assay, an increase in oil concentration and time of exposure resulted in progressive increase in insect mortality. At 8µL/ml, the oil had an appreciable effect on the insects resulting in 100% mortality within the first day of exposure after treatment. This concentration was found to be more toxic to *S. zeamais* in comparison with less than 10% mortality at 2 and 4µL/ml in the first two days (Table 3). Concentration of 6µL/ml achieved 100% mortality four days after the treatment. (Table 4; Figure 1). The effect of the essential oil was insignificant at 2 and 4µL/ml for the first 2 days. There was no further increase in insect mortality rate at 6 and 8µL/ml after the fourth day of exposure.

Table 3. *Number of dead maize weevils during contact toxicity assay.*

Time (days)	2µL/ml	4µL/ml	6µL/ml	8µL/ml
1	0	0	2	10
2	0	0	4	10
3	2	5	6	10
4	3	6	10	10
5	3	6	10	10

Table 4. *Percentage mortality during the contact toxicity assay.*

Time (days)	2µL/ml	4µL/ml	6µL/ml	8µL/ml
1	0	0	20	100
2	0	0	40	100
3	20	50	60	100
4	30	60	100	100
5	30	60	100	100

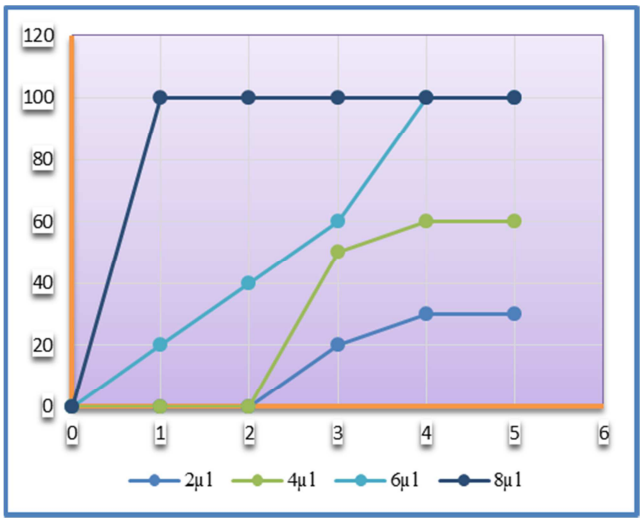

Figure 1. *Percentage mortality during contact toxicity.*

3.3. Repellent Activity

In repellency bioassay, an increase in concentration and time of exposure resulted in a gradual increase in insect repellency within the first 2hours (Table 5). Thereafter, PR remained constant with increase in time of exposure at 4, 6 and 8µL/ml. After the fourth hour, the repellency due to oil of 2µL/ml gradually increased up to 60%. Total (100%) repellency was obtained at a concentration of 8µL in the second hour after which it remained constant (Table 6; Figure 2). The lowest PR of 20% was obtained at 2µL/ml. There were significant differences in repellency of the essential oil against maize weevils at various concentrations tested.

Table 5. *Insects on control and oil treated areas during repellent activity.*

Time	2µL/ml		4µL/ml		6µL/ml		8µL/ml	
(hrs)	Nt	Nc	Nt	Nc	Nt	Nc	Nt	Nc
1	4	6	7	8	3	2	1	9
2	3	7	8	9	2	1	0	10
3	3	7	8	9	2	1	0	10
4	3	7	8	9	2	1	0	10
5	2	8	8	9	2	1	0	10

Table 6. *Percentage repellency during repellent activity assay.*

Time (hrs)	2μL/ml	4μL/ml	6μL/ml	8μL/ml
1	20	40	60	80
2	40	60	80	100
3	40	60	80	100
4	40	60	80	100
5	60	60	80	100

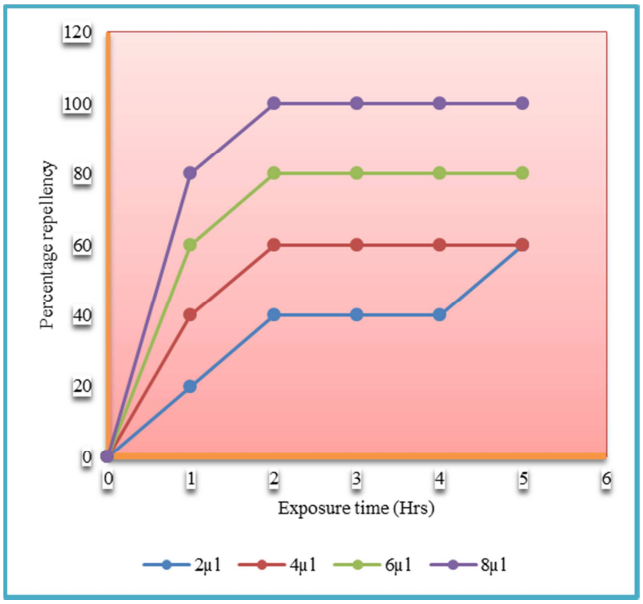

Figure 2. *Percentage repellency during repellency assay.*

3.4. Fumigant Toxicity

In fumigant toxicity, the fumigation effect of the essential oils on adult *S. zeamais* showed a gradual increase in percentage insect mortality as the concentration of the oil increased. The highest mortality was 70% recorded at 8µL/m^2 which was obtained on the third day of exposure. (Table 8; Figure 3). However, 100% (total) mortality was not obtained at all concentrations tested. In fact, no further increase in insect mortality was observed after the fourth day of exposure at all the tested concentrations. There was no mortality noticed at 2µL/m^2 in the first two days of exposure after treatment (Table 7). The lowest percentage mortality obtained was 10% at 4 and 2µL/m^2 in the second and third days of exposure respectively.

Table 7. *Dead maize weevils during fumigant toxicity assay.*

Time (hrs)	2μL/m^2	4μL/m^2	6μL/m^2	8μL/m^2
1	0	0	2	3
2	0	1	4	5
3	1	2	6	7
4	2	5	6	7
5	2	5	6	7

Table 8. *Percentage mortality during fumigant toxicity assay.*

Time (hrs)	2μL/ml^2	4μL/ml^2	6μL/ml^2	8μL/ml^2
1	0	0	20	30
2	0	10	40	50
3	10	20	60	70
4	20	50	60	70
5	20	50	60	70

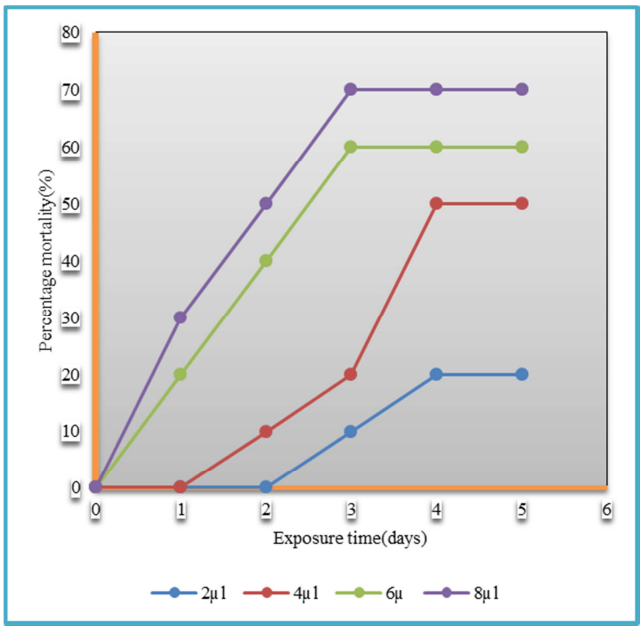

Figure 3. *Percentage mortality during fumigant toxicity assay.*

4. Discussion of Results

The essential oils of *E. saligna* was extracted by preferential hydrodistillation. The essential oil obtained in this study was green with a yield of 0.10% (v/w) of dry leaves after two hours of acetonic extraction. This agrees with the 0.06 to 7.00% yield reported by Gonçalves *et al* [46]. In fact, for *Eucalyptus* species, phytochemical composition of the bioactive oils rest on the species and variety investigated. Moreover, several global studies have reported essential oil yields not more than 7.00% and with varying compositions even for the same species in different locations [27-39] (Table 1).

Results from the study showed that the acetonic essential oils of *E. saligna* leaves exhibited distinct levels of toxicity against the maize weevils at various concentrations used in the bioassays (Tables 3-8). The insecticidal activities of the essential oils could have been due to some of the major chemical constituents which have been reported to have strong toxic and repellent effects against stored product pests [36, 40, 41, 43, 45]. Eucalyptol and 1R-∝-pinene are the major components present in the essential oil obtained which are entirely responsible for the toxicity and repellency against the maize weevils. The presence of more than one compound in the essential oils has been known to be an advantage in pest control as it encourages synergistic insecticidal activity. Compounds present in the essential oil are known to act synergistically against the physiology of many insects [39]. Although the major components are largely responsible for the toxic action, the toxicity and repellency against maize weevils might likely be due to the cumulative effects of the different minor chemical components present in the essential oil. The increase in concentration of essential oil resulted in an increase in mortality rates and repellent effect in all the bioassays

performed. The rapid action of the essential oil inducing as much as 100% mortality in contact toxicity indicated that it could be a potential candidate for use as an insecticide. The monoterpene, eucalyptol has been reported to be effective in insect fumigation toxicity [40]. The insecticidal property of many essential oils is mainly attributed to monoterpenes which are typically volatile and rather lipophilic compounds [41] that can penetrate insects rapidly and interfere with their physiological functions. Due to their high volatility, they have fumigant and gaseous action. Jointly or independently, the compounds present in essential oil contribute to its bioefficacy with a range of effects such as anti-feeding and ovicidal activities [41]. Some researchers have also reported that the essential oils have neurotoxic, cytotoxic, phototoxic and mutagenic action among others in different insect pests [47]. The essential oils act at multiple levels in insects so the possibility of insects generating resistance is probably little. Basing on such sound evidence and the results obtained in the bioassays performed in the study, the essential oil of *E. saligna* could be employed in the control of *S. zeamais* in agricultural produce.

5. Conclusions and Recommendations

Acetonic essential oil of dry Eucalyptol and 1R-∝-Pinene rich *E. saligna* leaves can be developed into a bioinsecticide for *S. zeamais* as a repellent, toxicant and fumigant candidate. Increase in concentration of acetonic essential oil of dry Eucalyptol and 1R-∝-Pinene rich *E. saligna* leaves results in high mortality and repellency. The essential oil should be used with caution to avoid individuals from applying it for long term exposure at relatively high doses. Further research aimed at assessing the effect of the acetonic essential oils prior to use should be done to avoid possible dangers to non-target organisms and the environment. Further research should be done using other solvents such as water, ethanol and methanol. Further research should be done to determine the lethal concentration (LC_{50}) of the acetonic oils on the maize weevils.

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