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Towards Food Security: Essential Oil Components as Protectants Against the Rice Weevil, *Sitophilus Oryzae*

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Abstract

Rice (*Oryza sativa*) is an important staple with high food value for a large part of the world population, but damage caused by rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) is a major constraint to its production. Synthetic chemicals are effective in managing stored produce insect pests, but they leave residues on food substances. Although botanical essential oils have potential in managing insect pests, information is scanty on the use of their constituents against the rice weevil.

Therefore, this study was conducted to evaluate the efficacy of two essential oil constituents: linalool and nerolidol against *Sitophilus oryzae.* Each of the constituents was formulated at 1, 5 and 25 µL/mL, relative to the actellic dust, pirimiphos-methyl (0.015 g) and ethanol-treated control. Treatments were evaluated on rice grains (FARO 60, an Indian variety) infested with *Sitophilus oryzae* under laboratory conditions (27±2^{0}C temperature; 78±5% relative humidity). The median lethal concentration (LC_{50}) of the oil components was evaluated using probit analysis while mortality was assessed following standard procedure. Repellent effect of the oil constituents on *S. oryzae* was determined in an olfactometer bioassay. Data were analysed using Analysis of Variance (ANOVA) and *t*-test analysis while means were compared with the Least Significant Difference at 5% significant level.

Linalool and nerolidol gave LC_{50} values of 3.98 µL/mL and 5.01 µL/mL, respectively, while mortality ranged from 0.00% in the control to 79.72% in treatment with linalool and 58% in nerolidol. Linalool and nerolidol at concentrations 5 and 25 µL/mL were highly repellent ($P < 0.05$) to adult *S. oryzae* in olfactometry test. The use of linalool and nerolidol as essential oil components could be included in design of Integrated Pest Management approach of *Sitophilus oryzae,* thereby reducing the problems caused by synthetic insecticides to human and environmental health.

Keywords : Bio-insecticides; Linalool; Adult mortality; Olfactometry test; Repellent effects

1. Introduction

Many biotic factors including diseases, bacteria, virus, nematodes, weeds, vertebrate pests and insects, are constraints to production of rice (*Oryza sativa* L.), an important staple with high food value for a large part of the world population (Okpile *et al.,* 2021). Field insects such as the rice gall midge, *Orseola oryzivora* (Wood-Mason) and the rice stem borer, *Scirpophaga incertus* (Walker) (Paul, 2007) as well as storage insect pests like *Sitotroga cerealella* (Olivier) and *Sitophilus oryzae* L. (Okpile *et al.,* 2021) are responsible for yield reduction in cultivated and stored rice. The rice weevil, *Sitophilus oryzae* (L.) is a major insect pest damaging rice grains in storage (Nwaubani *et al.*, 2014; Akhtar *et al.,* 2015) with a cosmopolitan distribution, occurring in several agro-ecologies of the world as well.

In Nigeria, towards reducing importation of rice, farmers are currently empowered to increase production of rice grains to feed the teeming population but being damaged by insects such as *S. oryzae* remains a challenge. The damage of stored rice, which usually occurred under both larvae and adult stages, lead to loss in nutritive value, grain weight and inducing secondary contamination by fungi and mites (Ashamo and Ogungbite, 2014). Synthetic insecticides and fumigants are commonly used to control pests due to their availability and rapid actions; however, their overuse, misuse and abuse have resulted in serious environmental consequences thus warranting widespread criticisms. In addition to the risks associated with the use of chemicals on human and environmental health, a number of insect pests has been reported to be resistant to them.

Use of botanicals as pest control agents have constituted alternative strategies to the use of chemical insecticides as plants constitute a rich and cheap source of bioactivities (Wink, 2008; Akinbuluma *et al.,* 2017). Given that many of the plant part powders and their extracts are specific to target insects, have low mammalian toxicity and are eco-friendly, their exploration should be an eloquent indicator towards the development of novel classes of biopesticides for a resilient pest management. Botanical essential oils are among the products that have been used to mitigate the effects of both field and storage insect pests (Marimuth *et al.,* 1997; Isman, 2000; Isman

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and Ahkar, 2007; Aboaba *et al.,* 2019; Akinbuluma 2020) as a potential alternative to synthetic pesticides (Al Dawsari, 2020). Although efforts are directed at investigating plant-derived constituents as useful products in insect-control practices, only little progress is made in the determination of actual compounds responsible for insecticidal activities. There was no recent report on the effects of individual constituents of plant essential oils as biopesticides against *S. oryzae*. So, this study aims to assess the potential of two essential oil components, linalool and nerolidol, relative to a synthetic compound, pirimiphos-methyl on *Sitophilus oryzae.*

2. Materials and Methods

2.1. Study location

This study was conducted between January and April, 2020. All experiments were carried out at the Entomology Research Laboratory and the PEARL Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan, at 27±2^{0}C (temperature) and 78±5% (relative humidity).

2.2. Insects

Infested rice grains were bought from the market and kept in Kilner jars with mesh lids. Emerged adults were sieved out and used to infest clean grains in 3 Kilner jars (200 g/jar). Old insects were removed after 7 days of mating and oviposition and newly emerged adults were used for the experiments (Akinbuluma and Ewete, 2014).

2.3. 3.3 Insecticidal materials and rice variety

Linalool and nerolidol were obtained from the Behavioural and Chemical Ecology unit of International Centre of Insect Physiology and Ecology, Nairobi, Kenya. Rice grains (FARO 60, japonica indica variety) were purchased from the International Institute for Tropical Agriculture, Ibadan, Nigeria. The grains were parboiled, dried and subsequently dehusked.

2.4. Biological assays

2.4.1. Median Lethal Concentration (LC_{50})

Each of linalool and nerolidol was diluted in 95% ethanol to obtain ten concentrations, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 microliter/millilitre (µL/mL) with a control 0 (µL/mL) containing only ethanol. The concentrations were applied topically on the ventral sides of five pairs of adult *S. oryzae* with a micro-syringe. Mortality was recorded after 24 hours and converted to probits, while the logarithms of the concentrations were taken. Probits were plotted against the logarithmic values to obtain a regression line, using Microsoft Excel (DSAASTAT version 1.101). The LC_{50} for each of linalool and nerolidol was determined where the log-dose at the median point was changed to antilogarithm (Finney, 1971; Akinbuluma and Ewete, 2014).

2.4.2. Insect mortality

Thirty grammes (30 g) of rice grains was weighed in each of five 1-L kilner jar, and 0.5 mL of three selected concentrations (1, 5 and 25 µL/mL) of linalool was added to the grains inside each jar and the jars were shaken for about 3 minutes to allow effective coverage of the grain surface with the oils (Aboaba et al., 2019). The set-up included a jar containing ethanol only (negative control) and another jar containing 0.015 g of synthetic chemical, pirimiphos-methyl as the positive control. Thereafter, each jar received five pairs (1♀: 1♂) of 1-2 day-old adult *S. oryzae* and were covered with lid wire mesh. The five treatments were arranged in a Completely Randomised Design (CRD) with 4 replications. The number of adult *S. oryzae* that died was recorded every other day for 7 days (an insect was taken as dead when it does not respond when probed with a camel hair brush). Mortality data obtained were converted to percentage and transformed before analysis. Another set-up was repeated with nerolidol at the same concentrations and experimental conditions as above.

2.4.3. Repellent effects of oils

Behavioural response of adult *S. oryzae* to linalool and nerolidol was evaluated on A Y-tube olfactometer (internal diameter 0.5 cm, stem length 4 cm, arm length 5.5 cm). Each of linalool and nerolidol at concentrations; 1, 5 and 25 µL/mL was applied to a filter paper strip (No. 1 Whatman, Int Ltd. Maidstone, England) and placed at one arm of the tube, while the control (ethanol only) was placed on the other arm. Compressed clean air from a pump was drawn through two flow metres (at 60 ml/min rate) and later passed through two polyester (Nalophan) bags (38 x 25) cm. The bags contained the odour sources, and each was connected to each arm of the olfactometer with Teflon tubes. With the aid of a soft hairbrush, six adult female *S. oryzae* were placed at the stem inlet of the Y-tube and were observed for 5 minutes. Each concentration of the compounds was assayed in five replications with new batch of insects in each case. The number of entries from treated and control sides of the tubes was recorded and compared using a *t*-test analysis (Akinbuluma, 2017).

3. Results

3.1. Median lethal concentration (LC50) and insect mortality

The LC_{50} of linalool and nerolidol on adult *S. oryzae* were 3.98 µL/mL and 5.01 µL/mL, respectively, as shown in Figs 1 and 2). Percentage mortality of adult *S. oryzae* treated with the concentrations of linalool and nerolidol relative to pirimiphos-methyl and ethanol-treated control are presented in Tables 1 and 2). All concentrations of linalool were significantly higher ($P < 0.05$) than the ethanol-control in causing mortality to *S. oryzae* in all the days of the trials. Although percentage mortality values were highest on jars treated with pirimiphos-methyl, they were not significantly different ($P > 0.05$) from percentage mortality on grains treated with linalool (at 25 µL/mL) under days 3, 5 and 7 of the trials (Table 1). At higher concentrations of nerolidol and in later days of trials, percentage mortality was significantly higher than those in ethanol-treated control (Table 2).

3.2. Repellent effect

Significant difference ($P < 0.05$) was observed between repellence of adult *S. oryzae* caused by the concentrations of linalool at 5 µL/ml and 25 µL/mL and the ethanol control. Linalool at 25 µL/mL exhibited the highest repellent effect on *S. oryzae* (Table 3), even though nerolidol at 25 µL/mL also exhibited repellent effect that was significantly higher than the control.

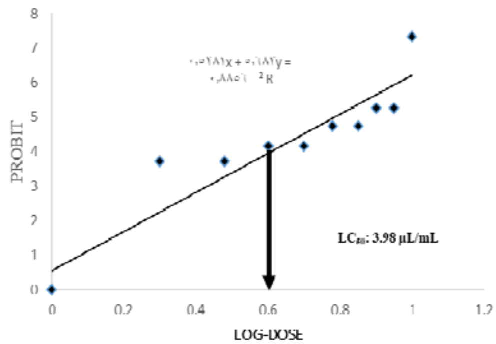

Figure 1. Median Lethal Concentration (LC_{50}) of Linalool on adult *Sitophilus oryzae*

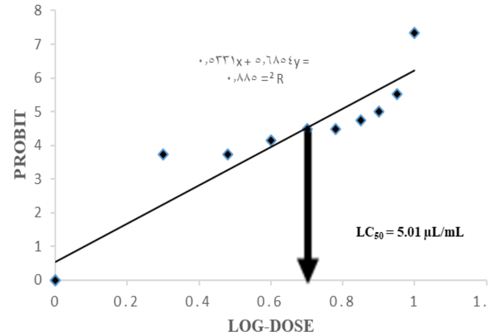

Figure 2. Median Lethal Concentration (LC_{50}) of Nerolidol on adult *Sitophilus oryzae*

Table 1. Mortality of adult *S. oryzae* treated with Linalool in the University of Ibadan, Nigeria in January, 2020

Conc. (µL/mL)	Mortality (± SE) of *Sitophilus oryzae* over 1-7 days infestation			
	1	3	5	7
0 (Ethanol)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	13.83±4.61	20.47±2.03	25.83±4.26	25.83±4.26
5	20.47±2.03	22.13±3.69	27.70±4.29	30.87±5.11
25	54.00±3.96	68.94±12.21	75.89±10.53	79.72±7.67
Pirimiphos-methyl (0.015g)	18.44±0.00	72.11±6.26	90.00±0.00	90.00±0.00
LSD (0.05)	8.63	19.35	16.37	13.68

Means whose differences are greater than LSD $_{(0.05)}$ within a column are significantly different

Table 2. Mortality of adult *S. oryzae* treated with Nerolidol in the University of Ibadan, Nigeria in January, 2020

Conc. (µl/ml)	Mortality (± SE) of *Sitophilus oryzae* over 1-7 days infestation			
	1	3	5	7
0 (Ethanol)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	0.00±0.00	4.61±4.61	11.25±6.70	15.86±5.62
5	4.61±4.61	14.61±4.61	23.83±4.61	40.47±2.03
25	9.22±5.83	19.22±5.32	42.50±2.34	58.22±1.66
Pirimiphos-methyl (0.015g)	20.47±2.03	78.75±6.70	90.00±0.00	90.00±0.00
LSD (0.05)	9.88	14.50	11.41	8.36

Means whose differences are greater than LSD $_{(0.05)}$ within a column are significantly different

Table 3. Repellent effect of linalool and nerolidol on adult *S. oryzae* in the University of Ibadan, Nigeria in January, 2020

Essential oil constituents	Conc (µL/mL)	Control	Test	t stat	t critical
Linalool	1	3.25	2.75	1.00	3.18
	5	5.00	1.00	4.90	3.18
	25	5.25	0.75	9.00	3.18
Nerolidol	1	3.25	2.75	0.52	3.18
	5	3.75	2.25	1.00	3.18
	25	4.75	1.25	9.00	3.18
	-	-	-	-	(df: 3, at 5%)

Mean values are significant where *t*-stat is greater than *t*-critical within the same row

4. Discussions

Botanical essential oils and their constituents do not only exhibit activities including fumigant and contact toxicity, antifeedant and repellence on stored product insect pests, but they also possess some advantages like low mammalian toxicity, biodegradation and availability in developing countries (Isman 2000; El-Bakry, 2016).

The low LC_{50} values of linalool and nerolidol shows that both oil constituents possess contact toxicity against adult *Sitophilus oryzae.* Ayvaz *et al.* (2010) also reported LC_{50} value as low as 12.74 µL/L from the essential oils of oregano, *Origanum onites* L. on *Acanthoscelides obtectus* on stored beans. A more recent study revealed that the essential oils from *Syzygium aromaticum* and *Aegle marmelos* gave an LC_{50} of 15.34 and 16.133 µL, respectively, on *S. oryzae* adults at 48 h exposure (Mishra *et al.,* 2012).

Although the mortality of *S. oryzae* increased with increasing concentration of the essential oil components and with exposure period, linalool and nerolidol were generally toxic to the insects. This might be due to the presence of terpenes in them, agreeing with the reports of Garcia *et al.* (2005) that the several monoterpenes and some sesquiterpenes were toxic to insects of stored produce. Earlier reports have shown that crude essential oils and their components are effective against insect pests of stored produce. Akinbuluma (2020) reported that the essential oil constituents from stem bark of *Cedrela odorata* caused high mortality and reduced egg-laying capacity of maize weevil. Similarly, oil from flowers of *Hyptis spicigera* elicited high insecticidal activity against *S. oryzae* (L.) and its toxicity was attributed to the presence of 1,8 cineole, carvacrol, α-pinene and β-pinene, in the crude oil (Ngamo *et al.,* 2007c). Specifically, linalool and nerolidol have been effective against some insect pests. Insecticidal properties of linalool are implicated as being a reversible inhibitor of acetylcholinesterase (Weaver *et al*., 1991). Chang *et al.* (2009) have shown that linalool is a major component in basil oil active against tephritid fruit flies, *Ceratitis capitata*, *Bactrocera dorsalis* and *Bactrocera cucurbitae*, while Lapczynski *et al.* (2008) and Ferreira *et al.* (2012) reported that nerolidol is a naturally occurring compound present in the oil of many plants with a floral odour. A recent study also revealed the presence of linalool and nerolidol and other compounds as constituents of essential oils in *Piper guineense* fruits (Akinbuluma, 2017).

In this study, linalool and nerolidol were highly repellent to *S. oryzae*, indicating that these compounds can actually exhibit a 'sniff and run' property on the insect. Olfactometry test, however, showed that linalool was more repellent than nerolidol to the insects. Muller *et al.* (2009) reported that linalool, among other compounds tested, significantly repelled more mosquitoes than untreated control, and Kim *et al.* (2010) also reported that linalool from origanum, produced 85% repellence to *Tribolium castaneum.*

5. Conclusions

This study concludes that linalool at 25 µL/mL was very effective in causing mortality and was highly repellent to adult *Sitophilus oryzae*. Therefore, the use of essential oils, especially their main components could be applicable to the management of *Sitophilus oryzae* and as a valid alternative to synthetic insecticides.

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