FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 5 No. 1, March, 2021, pp 106 – 111
DOI: https://doi.org/10.33003/fjs-2021-0501-542

INSECTICIDAL AND REPELLENT ACTIVITIES OF *CITRUSSINENSIS* (L.) LEAF EXTRACT AGAINST *TROGODERMA GRANARIUM* EVERTS (COLEOPTERA: DERMASTIDAE IN STORED GROUNDNUT

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ABSTRACT
Groundnut production has been reported to be threatened by storage insect pests which caused significant damage in storage. The repellent and efficacy activities of *Citrus sinensis* leaf extract were assessed on *Trogoderma granarium* in stored groundnut. The treatment was laid in a Completely Randomized Design with 3, 5, and 7% concentration level of methanolic and petroleum ether Extract for insecticidal activities. The results of *C. sinensis* leaf extract toxicity obtained was significant at 7% concentration at 24, 48 and 72 hours after treatment with 100% and 80% mortality in methanolic and Pet. ether extracts respectively in adults. Half disc experiment was carried out to determine the repellency activities with 1, 2, and 3% concentration of methanolic and pet. ether extract. It was observed that at 2HAT the insects moved towards the extract with the Index of Repellency of 1.13 at 3 % concentration for methanolic and pet ether extract, as the hours increase from 4 to 6HAT it was observed that the extract acted as repellant towards the insect with methanol and petroleum ether extract of 3 % concentration having the highest repellency index of 0.47. Petroleum ether at 1 % concentration with the value of 1.47, 1.40 and 1.20 repellency index across the 2, 4 and 6HAT showed that the extract remains an attractant towards *T. granarium*. The results obtained from this study is an indication of the potential of methanolic and petroleum ether crude extract of *C. sinensis* in groundnut storage

Keywords: Repellency, *T. granarium*, *C. sinensis*, Petroleum ether, Repellency index

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) which is grown in most parts of Nigeria, although the northern part has the most suitable soil and climate for its production and despite the economic importance of groundnut as a source of plant protein for humans and livestock in developing countries, such as Nigeria, profitable protection of this crop is seriously constrained by the attack of *Trogoderma granarium* with the infestation in stored groundnut it has resulted into significant loss in groundnut (Musa, 2013). Insect infestation is a major contributor to the quality deterioration of durables (cereals, pulses, roots and tubers) stored in warm and humid climates and the use of chemical agents to prevent or control insect infestations has been the main method of grain protection, since it is the simplest and most cost-effective means of dealing with stored product pests (Hidalgo *et al*., 1998., Narong, 2003) However, insecticides have serious drawbacks such as pest resurgence and resistance, lethal effects on nontarget organisms, the risk of users contamination, food residues, and environmental pollution (Tapondjou *et al*., 2002). Besides the safeguards needed when working with traditional chemical insecticides (Fields et al., 2001), as well as traditional farmers' inadequate storage facilities in developing countries, which are unsuitable for efficient conventional chemical protection (Tapondjou et al., 2002), highlight the need for new and appropriate methods for insect pest control of stored goods. While fumigant has always the best source of pesticide formulation in warehouses and methyl bromide is one of the synthetic fumigant that as being used but as a result of the U.S. Environmental Protection Agency (EPA) has classified methyl bromide as a "Restricted Use Pesticide". Methyl bromide use is currently being reduced because of its ability to destroy the ozone layer more so it is highly toxic likewise some other synthetic pesticides (EPA, 2020).

The increased public concerns about the potential adverse environmental effects associated with the use of synthetic plant protection and production agrochemicals with the continued incidence of *T. granarium* on produce transported from countries where it is indigenous, as well as the potential for spread due to increased use of dry cargo containers and roll-on/roll-off road transport, make it a potential threat to global food security prompted the search for the technologies and products based on biological processes to control the pests

(Suresh, 2012, Singh et al., 2017). Serious infestations of khapra beetle on groundnut grain can render it unfit or marketable for consumption due to depletion of specific nutrients, quality can decrease (Singh, 2017). Not only do insects reduce the quantity and quality of stored food crops, but they also affect the nutritional value, viability and marketability of those crops (Oboh et al., 2017).

This has resulted in the research of a new biorational pesticide, which is eco-friendly and has low mammalian toxicity without any form of residual effect and can serve as an alternative to synthetic fumigant. Due to their potential insecticidal properties, botanicals could be a viable option for insect pest control. Against insect pests, plant products had insecticidal, repellent, and antifeedant effects (Hasan et al., 2016; Ali et al., 2017).

MATERIALS AND METHODS

Study Area

The study was conducted at University of Ilorin, Crop Protection Laboratory.

Insect Culture

The experiment was performed in the Department of Crop Protection, University of Ilorin, Ilorin, Nigeria, at a temperature of 25±2^{0}C and a relative humidity of 69±3%. Fifty adults of *T. granarium* was extracted from the stock community to infest 200 g groundnut seeds in a 500 ml Kilner jar with a netted screw top.

Source of Seeds

The seed was sourced from the Institute for Agricultural Research in Samaru, Nigeria, given the groundnut variety (SAMNUT 23). The seeds were disinfected by freezing them for 72 hours at -40 degrees Celsius to eliminate any growing insects, then air-dried them for 48 hours before use.

Collection/identification and extraction of Botanical

Leaves of *C. sinensis* were collected from the tree at Parks and Garden of the University of Ilorin, Nigeria. Part of the leave was taken for authentication and identification which was done at the University of Ilorin Herbarium service room with the identification number of UILH/001/996. The leaves were air-dried for 5 days before grinding with a mortar and pestle. The leaf powder was subjected to methanol and petroleum ether extraction. One hundred and fifty grams (150 g) of the powdered leaf was weighed and soaked separately into 650 ml of the solvents in glassware and allowed to stand in the dark for 72 h, with occasional stirring after which it was filtered to get the crude extracts.

Phytochemical screening of *C. sinensis* extract

Phytochemical analysis of extract was carried out using the method described by Odebiyi and Sofowora (1978) for the detection of saponins, tannins, phenolics, alkaloids, steroids, triterpenes, phlobatannins, glycosides and flavonoids.

Table 1: Phytochemical screening of leaves of *C. sinensis*.

PHYTOCHEMICAL COMPONENTS	METHANOL EXTRACT	PET. ETHER EXTRACT
Saponin	+	_
Flavanoids	+	+
Steroids	+	+
Glycosides	_	_
Phlobatannins	_	_
Alkaloids	_	_
Tannin	_	_
Phenolics	+	_
Triterpenes	+	+

Key: + = Present, - = Absent.

Toxicity test

The test was carried out with different concentrations of extract with 3, 5 and 7% of methanol and petroleum ether extract respectively. Fifty grams of groundnut grains were weighed into 800 ml of the container and treated with the different concentrations of extract and control which has no treatment was set up. After treatments each concentration of the extract was allowed to stand for 30 minutes before introducing 10 couple of *T. granarium* adult likewise larvae for the different experiment into the container and it was covered with muslin. Readings of daily mortality were taken.

Repellency test

Nine centimeters (9 cm) Whatmann filter paper was cut in half. One (1ml) of each extract solution was applied to half filter

paper disc as uniformly as possible with a pipette and the other half treated with 1mil of acetone as a control. The extracts treated and control half-disc were air-dried for 30minutes to evaporate the solvent completely. Treated and untreated halves of same dimensions were attached with a cellotape. Each filter paper was placed in a Petri dish and 10 adult test insects (*T. granarium*) were released at the centre of each filter paper disc were covered and the Petri dishes were covered and placed in the dark at 27 ± 2 ^{0}C with relative humidity of 70 ± 5%. Each treatment was replicated 3 times for 1, 2 and 3% concentration. The number of insects present on treated (G) and control (P) areas was recorded after 2, 4 and 6 hours (Mazzonetto, 2002).

$$IR = \frac{2G}{G + P}$$

Where, IR= Index of Repellency
If IR > 1, then the extract is Attractant.
If IR = 1, then the extract is Neutral.
If IR < 1, then the extract is Repellent.

Experimental Design and Data Analysis

The experiment was carried out using Completely Randomized Design (CRD) arranged involving seven treatments with three replications each. Data were subjected to analysis of variance (ANOVA) using GENSTAT 12th Edition Statistical package and were treatments means are significant, they were separated using Tukey at P≤0.05 significance level.

RESULT

Effect of *C. sinensis* on the larvae of *T. granarium*

The mean percentage mortality of *T. granarium* larvae and adult when treated with different concentrations of methanolic and petroleum ether extract of *C. sinensis.* For larvae mortality (Table 2) at 24 hours after treatment, it was observed that none of the concentration was able to attain 50% mortality the highest been 7% concentration of methanol extract was 26.67% while the lowest was Pet. Ether at 3% concentration having no mortality while, at 48 HAT there was a significant difference in which 7% concentration of methanol extract was at 40.00% mortality while concentration of the methanol extract., for the 3 and 5% concentration was not significantly different. Pet. Ether extract concentration, the highest value of mortality was 33.33% mortality which was 7% methanol extract at 48 HAT. Finally, it was observed that there was 66.67% mortality of larvae with 7% concentration of methanolic while petroleum ether extract after 48HAT in 7% concentration was 56.67% mortality of larvae.

Table 2: Effect of methanolic and petroleum ether extract of *Citrus sinensis* on the percentage mean mortality of larvae of *Trogoderma granarium*

Treatments	Conc. (%)	HAT(MEANS±SD) 24	48	72
Methanol extract	**3**	6.67^{b} ±5.77	23.33^{ab}±5.77	33.33^{c}±11.6
	5	13.33^{ab}±11.55	36.67^{ab}±5.77	43.33^{bc}±5.77
	7	26.67^{a}±5.77	40.00^{a}±17.32	66.67^{a}±5.77
Petroleum ether extract	**3**	0.00^{b}±0.00	16.67^{bc}±11.55	36.67^{c}±0.00
	5	6.67^{b}±5.77	30.00^{ab}±0.00	46.67^{bc}±5.77
	7	16.67^{ab}±11.55	33.33^{ab}±5.77	56.67^{ab}±5.77
Control	**0**	0.00^{b}±0.00	0.00^{c}±0.00	0.00^{d}±0.00
S.E.M		3.91	4.71	2.89

Values with the same superscript(s) down the column are not significantly different at p=0.05
HAT: Hours after treatment, SD: Standard deviation, S.E.M: Standard error of mean

Effect of *C. sinensis* on the adult of *T. granarium*

Adult mortality of *T. granarium* (Table 3) at 24 hours after treatments were observed to be significantly different were 3, 5 and 7% methanol extract was 13.33, 30.00 and 40.00% mortality respectively while similar observation in pet. Ether was observed with an increase in the concentration of extract with values 16.67, 26.67 and 30.00 at 3, 5 and 7% concentration while the control had no mortality. With the same trend occurring in the concentration for the pet. ether extract while the methanol extract of 7% was having the highest value of 63.33% and the least been 3 and 5% concentration in methanol extract which were not significantly different from each other. There was 100% mortality at 7% concentration of methanolic and petroleum ether extract was 80.00% at 72HAT. Three percent (3%) and 5% concentration of methanolic and petroleum ether was observed not to be significantly different with the value of 3% concentration of methanolic extract to be 43.33% mortality while 5% of petroleum ether was found to have 53.33% mortality at 18 hours after treatments.

Table 3: Effect of methanolic and petroleum ether extract of *Citrus sinensis* on the percentage mean mortality of adult of *Trogoderma granarium*

Treatments		HAT(MEAN±SD)		
	Conc. (%)	24	48	72
Methanolic extract	**3**	13.3^{bc}±11.55	33.33^{a}±5.77	53.33^{c}±11.55
	5	30.00^{ab}±0.00	40.00^{a}±10.00	76.67^{b}±5.77
	7	40.00^{a}±10.00	56.67^{a}±15.28	100.00^{a}±0.00
Petroleum ether extract	**3**	16.67^{bc}±5.77	33.33^{a}±11.55	66.67^{bc}±10.00
	5	26.67^{ab}±11.55	36.67^{a}±5.77	66.67^{bc}±5.77
	7	30.00^{ab}±10.00	43.33^{a}±5.77	80.00^{b}±10.00
Control	**0**	0.00^{c}±0.00	0.00^{b}±0.00	0.00^{d}±0.00
S.E.M		4.56	4.86	4.08

Values with the same superscript(s) down the column are not significantly different at p=0.05
HAT: Hours after treatment, SD: Standard deviation, S.E.M: Standard error of mean

Repellency

Repellency test was carried out on adult of *T. granarium* with concentration of 1, 2 and 3% of methanol and petroleum ether extract with the repellency index (IR) which shows if the extract was an attractant or a repellant at this level of concentration with the values of IR: <1 repellency; 1 neutral; >1 attractant. At 2 hours after treatment, the extract acted as an attractant but as the hours increasefrom 2 hours after treatment to 6 hours after treatment it was observed that the extract acted like a repellant towards the insect with methanol and petroleum extract of 3% concentration having the highest repellency index of 0.47 while petroleum ether of 1% concentration the value of 1.47, 1.40 and 1.20 repellency index across the 2, 4 and 6 hours after infestation which shows that at that level of concentration the treatment acted like an attractant towards the insect.

Table 4: Effect of methanolic and petroleum Ether extract of *C. sinensis* on repellency of *T. granarium*

Treatments		Index of repellency(IR)*		
		2H	4H	6H
	Conc. (%)			
Methanolic	**1**	1.33	0.96	0.80
	2	1.20	1.13	0.67
	3	1.13	0.93	0.47
Pet. Ether	**1**	1.47	1.40	1.20
	2	1.53	0.87	0.53
	3	1.13	0.87	0.47

*IR: <1 repellency; 1 neutral; >1 attractant, H=hour

DISCUSSION

The insecticidal potentials of methanol and petroleum ether leaf extracts were compared in this analysis which shows that increase in the concentration of the plant extract causes an increase in the mortality of the larvae and adult of *T. granarium* with 7% concentration been the most effective of all the concentration. At 24 to 72 hours after release, the peak concentrations of both methanol and petroleum ether extracts were the most toxic to larvae and adults, respectively. At the highest concentration (7%) of methanol and petroleum ether leaf extracts, adult and larval mortality were greater than 50% at 72 HAT while methanolic extract attained 100% mortality in adults. This study corroborates the conclusions of the previous report of Ojebode et al., 2016 were the fixed oil of orange peel had the highest mortality in the group of fixed oils, followed by *Azadirachta indica* and finally lemongrass which was due to the high toxicity effect of the oils against adult of *Callosobruchus maculatus*.

Sagheer et al., (2013) reported that the essential oils of *C. sinensis*, *C. aurantium*, *C. paradisi*, and *C. reticulata* have been used at 2, 4, and 8% concentrations against *T. granarium* larvae over three exposure intervals. The findings showed that *C. aurantium* oil had the highest mortality effect, followed by *C. reticulate* oil, and *C. sinensis* oil had the lowest mortality effect. This corroborates with the lower mortality observed in the larvae compared to the adult of *T. granarium*.

Bilal et al., (2015) reported that rough lemon (*C. jambhiri*) was the most effective cultivar against adults of *Tribolium castaneum*, with an RD50 value of 4.40 %, followed by freutrall early (*C. raticulata* var freutrall) 4.41 %, and kinnow

had the highest RD50 value (8.14 %), followed by red blood orange (*C. sinensis*) 4.84 % this confirms the repellency of *C. sinensis* leave extract on *T. granarium.*

Zewde and Jembere 2010 reported the repellency effect of *C. sinensis* peel oil among other citrus oils. The study results indicate that *C. sinensi*-derived materials may be used as a pulse protectant against *Z. subfasciatus* by small-scale farmers this corroborates with the findings of the repellence activities of the *C. sinensis* extract as a repellant against *T. granarium.* This implies that the active compounds that acted as repellents and fumigants may be chemically distinct.

CONCLUSION AND RECOMMENDATION

The current study's findings indicated that *C. sinensis* extract could be used as a groundnut protectant in the form of a repellant and toxicant against *T. granarium* by small-scale farmers. As a result, research into integrating, optimizing, and adjusting for the control of stored product insects is needed.

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