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Insecticidal activity and repellent potential of *ocimum gratissimum* on *tribolium castaneum*

Chiazom Onyedikachi Ezenwafor-Onyeocha [1, *], Edith Nonye Nwankwo [1], Izunna Somadina Okwelogu [2], Ifeanyi Onyema Oshim [3] and Immaculata Ogochukwu Uduchi [2]

[1] *Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Nigeria.*

[2] *Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Nnewi, Anambra State Nigeria.*

[3] *Department of Medical Laboratory Science, Faculty of Basic Medicine and Health Sciences, Benson Idahosa University, Benin City, Nigeria.*

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Abstract

Background: Using botanicals for pest control has become very popular in recent years in sub-Saharan Africa and other parts of the world. These botanicals are recognized to offer protection against insect pests thanks to the natural components inherent in them. Some of these chemicals may work independently or in concert to create a desired result that lead to health challenge.

Objectives: This study was aimed at evaluating the toxicity and repellent potential of *Ocimum gratissimum* on *Tribolium castaneum*.

Methodology: The different concentrations 20% (200 mg/mL), 10% (100 mg/mL), 5% (50 mg/mL), 2.5% (25 mg/mL), and 1.25% (12.5mg/mL) of the oil extracts including control with acetone only were selected and tested to determine the LD_{50} value and LD_{90} value. Repellent activity contained in the essential oil was done using standard method. Result: Mortality increased in accordance with increase in concentration of *O. gratissimum*. The highest and lowest doses of *O. gratissimum*, 200 mg/ml and 12.5 mg/ml ($P<0.05$, $P=0.009$), caused mortalities of 90% and 43.3% respectively. The log-probit regression analysis showed that the LD_{50} and LD_{90} for this plant extract were 18.60 mg/mL and 175.99 mg/mL respectively.

Conclusion: The present study has evidently shown that *O.gratissimum* oil have insecticidal activity. *O.gratissimum* oil extract also serve as good toxicant and repellent agents in the treatment of *Tribolium castaneum* during storage.

Keywords: *Tribolium castaneum; Ocimum gratissimum*; Lethal dose; Mortalitiy

1. Introduction

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is worldwide and most destructive pest of stored products and is cosmopolitan in distribution [1]. It is the common pest of wheat flour and it also causes serious damage upon dried fruits, pulses and prepared cereal foods, such cornflakes, Quaker oat, pasta, biscuit, beans, mits and other processed cereals, [1].

* Corresponding author: Chiazom Onyedikachi Ezenwafor-Onyeocha

It is often the most common species in the pest complex attacking several wheat although its pest status is considered to be secondary, requiring prior infestation by an internal feeder. It can readily infest other grains damaged in the harvesting operation, [1]. Adult and larvae of both species are economic pests that cause quantitative and qualitative losses in tropical and subtropical regions in the world but it is of Indo-Australian origin[2].

Synthetic pesticide such as Malathion, Pirtmiphos-methyl, Chlorpyrifos-methyl, Deltamethrin and the fumigant Phosphrine are presently the main products used to protect stored grain from insect, [2]. Management of these insects pest is severely dependent upon the use of synthetic insecticide. However, application of these synthetic commercial insecticides has led to several serious problems such as environmental deterioration due to chemical residues, insect resistance against these repeatedly used chemical, deterioration of food grains due to residues and harmful effect of synthetic chemicals to the non-target organisms in the surrounding [3].

Some of the botanical extracts used in place of synthetic insecticides include *annona, azadiradita, moringa, ocimum, pergularia, Zingiber* and pepper fruits among others have proven to be effective in the control of stored products pest like *Sitophilus zeamais* and *Dermestes maculatus* [4]. Furthermore, the study was aimed at evaluating the toxicity and repellent potential of *Ocimum gratissimum* on *Tribolium castaneum*.

2. Material and methods

2.1. Study Area

The experiment was carried out in the research laboratory of Parasitology and Entomology Department, Nnamdi Azikiwe University, Awka.

2.2. Insect culture

The strains of *Tribolium castaneum* obtained from laboratory culture from department of Parasitology and Entomology was cultured on quaker oat purchased from an open market in Awka. The quaker oat were sterilized in the oven at 160 °C for one hour and allowed to cool for an hour. Subsequently, the adults of *Tribolium castaneum* were introduced into the sterilized oats and allowed to oviposit under temperature of 29-30°C, relative humidity of 80-87% and 12L: 12D photo regime. The F_1 adults (1-2weeks old) that eventually emerged were used for the study.

2.3. Experimental crop

The crop, white Quaker oats of 500 grams weight procured from eke Awka market was used for the study. The Quaker oat was sterilized in an oven at 40°C for 2 hours to ensure there were no prior infestation before use.

2.4. Sources of *O.gratissimum*

The *Ocimum gratissimum* leaf was purchased from a local market in Onitsha. The leaves of *O.gratissimum* were washed to remove dirts and debris. The *O. gratissimum* leaves were dried under room temperature for seven days. Thereafter, they were pulverized with an electric blender to obtain fine powder.

2.5. Hexanolic Extraction of *O. gratissimum* crude extraction

The extraction of oil from the pulverized samples was done in the laboratory using N-Hexane in Soxhlet extractor for 3hours. Fifty grams (50g) of each of the plant samples was put into a thimble and then into the Soxhlet body. N-Hexane 250mL was measured and poured into a round bottom flask system of the Soxhlet apparatus using a funnel. This was heated about 5cm above a hot electric plate while cold water was allowed to flow in and out of the apparatus to cool off the condenser compartment. After many refluxes of about of about 3hours, the N-Hexane was gradually evaporated. This process was done repeatedly until the pulverized sample for both botanicals was exhausted the solvent was distilled off at 75 °C leaving the oil extract from the samples behind.

2.6. Serial dilution of *O. gratissimum*

The pure oil extract (crude extract) was regarded as 100% concentration. This was subsequently diluted serially with acetone to obtain the graded concentration yielding, 20% (200 mg/mL), 10% (100 mg/mL), 5%(50 mg/mL), 2.5% (25 mg/mL), and 1.25%(12.5 mg/mL).

2.7. Toxicity effect of *O. gratissimum* extracts on adults of *tribolium castaneum*

2.7.1. Direct Application on Quaker oat (Contact toxicity)

15g of the Quaker oat were treated with one millilitre aliquot of the different concentrations of the extracts and left for 30 minutes to dry, this was done repeatedly for other replicates. Ten adult beetles were introduced into each of the different kilner jars and covered using a muslin cloth. Mortality record was taken after 24, 48, and 72hours

2.7.2. Residual Application on Filter Paper

The different concentrations 20%(200 mg/mL), 10% (100 mg/mL), 5%(50 mg/mL), 2.5% (25 mg/mL), and 1.25%(12.5 mg/mL) of the oil extracts including control with acetone only were selected and tested to determine the LD_{50} value and LD_{90} value. One millilitre aliquots of the different concentrations of the oil extract of each treatment was replicated three times. The oil was absorbed by the filter papers and left for 30 minutes to allow the acetone to evaporate. Ten adults of the flour beetle of mixed sexes were exposed to the treatment for 3 days. Records of mortality were taken after 24, 48, and 72 hours. Adult insects were counted dead when they refuse to move parts of their body in response to a probe with five forcep.

2.7.3. Repellency test

Repellent activity contained in the two essential oil was studied according to method adopted by Talukder and Howse [5], using Petri dishes (9cm diameter) Test solutions were prepared by dissolving different concentrations (150 mg/mL, 100 mg/mL, 60 mg/mL, 30 mg/mL) of each oil in one millilitre (1ml) acetone. Whatman No.1 filter paper (8cm diameter) was cut into two and each solution was applied to half of a filter paper as uniform as possible by using micro pipette. The other half was treated with acetone treated halves were dried for 30 minutes to evaporate the solvent completely.

Treated and untreated halves were placed in the glass Petri dish 2cm away from each other and ten adult flour beetles were released at the centre of the filter paper disc and then sealed tightly. Three replicates were set for each concentration. Observation of the number of adult insect present on both the treated and untreated halves was recorded after every 30 minutes for two hours of setting up the experiment, (30, 60, 90, and 120 minutes). The data were expressed as percentage repulsion (PR) by using the following equation [5].

$$PR = \frac{NC - NT}{NC + NT} \times \frac{100}{1}$$

Where:
PR is Percentage Repellency
NC is Number of Adult on Control Portion
NT is Number of Adult on Treated Portion.

The average value was then classified according to repellency classes from 0-V where;

Class 0= < 0.1
Class I= 0.1→20
Class II= 20.1→40
Class III= 40.1→60
Class IV= 60.1→80
Class V= 80.1→100% respectively, Mcgovern *et al*., [6].

2.8. Statistical analysis

All data were subjected to analysis of variance (ANOVA). The means were separated using LSD at 5% level of significance [P=0.05], the standard errors of the means were calculated. The mortality data obtained were corrected using Abbot Formular [7].

$$PM = \frac{Po - Pc}{100 - Pc} \times \frac{100}{1}$$

Where:
Po= Observed Percentage Mortality

Pc= Control Mortality
Pm= Corrected Mortality

3. Results

3.1. Direct application and Residual application of *Ocimum gratissimum* to *T. castaneum*

The result in (Table1) which is the contact toxicity showed there was dose dependent mortality response to the botanical insecticide *O. gratissimum*. Mortality increased in accordance with increase in concentration of *O. gratissimum*. The highest and lowest doses of *O. gratissimum*, 200mg/ml and 12.5mg/ml caused mortalities of 90% and 43.3% respectively. The statistical analysis showed that the dose where significantly different ($P<0.05$, $P=0.009$) for each other. The log-probit regression analysis showed that the LD_{50} and LD_{90} for this plant extract were 18.60 mg/mL and 175.99 mg/mL respectively (Figure 1).

Similarly, the residual application on filter paper result in (Table 2) showed that the highest and lowest dose of *O. gratissimum*, 200 mg/mL and 12.5 mg/mL caused mortalities of 50.0% and 0.00%.

The result of the repellence test of *O. gratissimum* in (Table 3) showed that the overall percentage repellence of *O. gratissimum* ranges from 35 to 61% from the lowest to highest concentrations of 30mg/mL to 150mg/mL

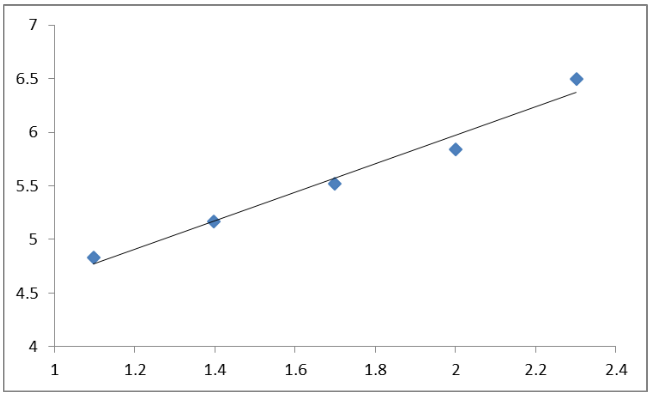

Figure 1 Probit against Log-concentration of ocimum leaf extract on tribolium

y = 1.3321x + 3.3088
$\mathbf{R^2}$ = 0.9758
5= 1.3321x + 3.3088
X=1.270 log concentration
LD_{50}=18.60mg/ml
LD_{90}=175.99mg/ml

Table 1 Mean mortality count of T.castaneum after treatment with Ocimum by Direct application on quaker oat

Concentration (Mg/ml)	**Time (hrs)**			**Mean (±S.e)**	**%Mortality**	**Probit**
	24	48	72			
200	4.67	7.67	9.33	7.22 ± 1.4	93.00	6.50
100	3.00	6.33	8.00	5.77 ± 1.5	80.70.0	5.84
50	2.67	5.00	7.00	4.89 ± 1.3	56.7	5.52
25	2.33	4.67	5.67	4.22 ± 0.9	43.3	5.71
12.5	2.00	3.00	4.33	3.11 ± 0.7		4.83
Control	0.00	0.00	0.00			
Mean (± S.e)	2.93 ± 0.5	5.33 ± 0.8	6.86 ± 0.9			
% Mortality	29.3	53.3	68.6			
Probit	4.46	5.08	5.48			

Means of three replicates (± S.e), P value for Concentration =0.009, P value for time= 0.008, L.S.D = 3.333

Table 2 Mean mortality count of tribolium castaneum after treatment with ocimum by residual application on filter paper

Concentration (Mg/ml)	**Time (hrs)**			**Mean (±S.e)**	**%Mortality**
	24	48	72		
200	2.33	3.33	5.00	3.55 ± 0.8	50.0
100	1.67	3.00	3.67	2.78 ± o.6	36.7
50	1.33	2.33	2.67	2.11 ± 0.4	26.7
25	0.67	1.33	2.33	1.44 ± 0.5	23.3
12.5	0.00	0.00	0.00	0.00	
Control	0.00	0.00	0.00		
Mean (± S.e)	1.20±0.4	1.99±0.6	2.73±0.8		
% Mortality	12.0	19.9	27.3		

Means of three replicates (±S.e), P value for concentration = 0.001, P value for time = 0.271, L.S.D =1.460

Table 3 Percentage Repellence of *Tribolium castaneum* when exposed to *Ocimum gratissimum* after 120 minutes

Concentration (Mg/mL)	**30mins**	**60mins**	**90mins**	**120mins**	**Mean ±S.e**
150	42.80	43.40	65.46	71.40	61.56±7.3
100	45.40	53.80	69.28	77.77	55.77± 7.4
60	42.80	41.75	37.95	90.99	53.37±12.6
30	20.00	10.35	53.86	56.45	35.17±11.7

Means of three replicates (±S.e), P value for concentration = 0.324

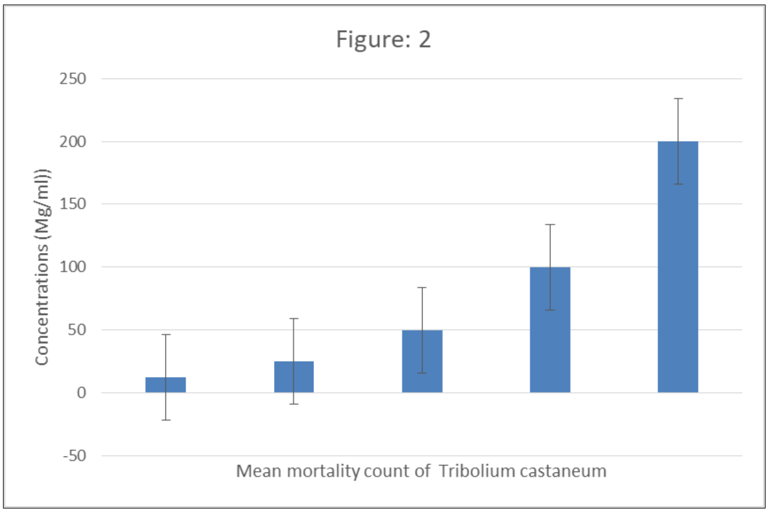

Figure 2 Mean mortality count of *Tribolium castaneum*

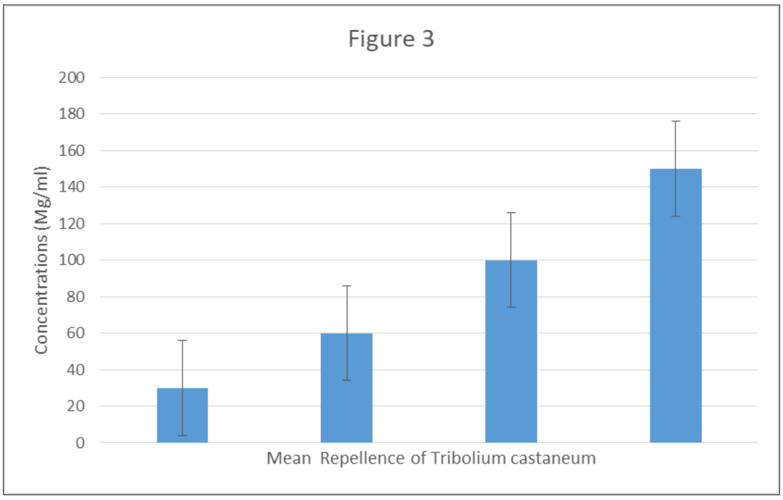

Figure 3 Mean Repellence of *Tribolium castaneum*

4. Discussion

It has been suggested that botanical insecticide may improve the effectiveness of conventional insecticides and make it possible to lessen side effects [8]. The present study has demonstrated the great potential of botanical insecticides *O. gratissimum* in the control of *Tribolium castaneum*. High mortalities of adult *Tribolium* were observed in the direct method application and residual application on the filter paper method, this indicated high susceptibility of this insect to these botanical insecticides. This is in line with Isman [8] in plant essential oils for pest and disease management [9], He reported that *O. gratissimum* having 90% of Eugenol as its active ingredient which makes it possess insecticidal properties. Also, Obeng-ofori and Reichmuth [10], demonstrated Eugenol the major constituent of the oil of *O. gratissimum* to be effective against four different storage pests.

The result further showed that the LD_{50} and LD_{90} values of *O. gratissimum* were 18.60mg/mL and 175.99mg/mL. Findings also showed that the lethal time required to kill 50% of the insects were 43.30hours for *O. gratissimum*[10].

In the Repellency test conducted, *O. gratissimum* showed good potential as repellent and toxicant agents to adults of *T.castaneum* as *O.gratissimum* showed percentage repellent range of 35%-61%. This confirms the findings of several studies which demonstrated the highly toxicant and repellent effect of some of these species against stored product beetles [11; 12].

5. Conclusion

The present study has evidently shown that *O.gratissimum* oil have insecticidal activity. *O.gratissimum* oil extract also serve as good toxicant and repellent agents in the treatment of *Tribolium castaneum* during storage.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Karunakara, C., Jayas, D.S., White, N.D.G., (2004). Identification of wheat kernels damaged by the red flour beetle using X-ray image. Biosystems Engineering, 87(3): 267-274.

[2] Jeyasankar, A., Chennaiya V. and Chinnamani T., (2016). Evaluation of five essential plant oils as a source of Repellent and Larvacidal Activities against Larva of Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). Journal of Entomology, 13: 98-103.

[3] Grunwald S., Fast, A., Mulle, K., Boll, M., Kler, A., Bonn Lander, B., (2014). Feed a Grape seed extract extends the survival of the red flour beetle, Tribolium castaneum under heat stress. Nutrition and Medicine, 2(1): 415-422.

[4] Marie, G.M., Martin, B., Pascale chalier, Richard kamga, Leonard, S.T., Marc cretin, (2013). Ocimum gratissimum essential oil and modified montmorillonite clay, a means of controlling insect pests in stored products. Journal of Stored Product Research, 52:57-62.

[5] Talukder, F.A., and Howse, P.E., (1994). Laboratory Evaluation of Toxic and Repellent Properties of the pithray tree, Aphanamixus polystachya wall and parker, against Sitophilus oryzae(L). International Journal of Pest Management, 40:274-279.

[6] Mcgovern, T.P., Gillenwater, H.B., and Mcdonald, L.L., (1977). Repellents for adult tribolium castaneum mandelates. Journal of Georgia Entomology Society, 12: 79-84.

[7] Abbott, W.S (1925). Abbott's formular. A method of computing the effectiveness of an insecticides. Journal of the American Mosquito Control Association.3(2):302

[8] Isman Murray B. (2000). Plant essential oils for pest and disease management. Crop and Protection, 19: 603-608

[9] Oshim, I.O., Urama, E.U., Olise, A.N and and Odeyemi, S (2019). In-vitro Screening of Antimicrobial Activities of Ocimum gratissimum on Clinical Isolates. South Asian Journal of Research in Microbiology. 4(1): 1-7, 2019

[10] Obeng-ofori D., and Reichmuth C.H., (1997). Bio activity of Eugenol, a major component of essential oil of Ocimum suave (wild) against four species of stored product, Coleoptera. International journal of Pest Management, 43:89-94.

[11] Alok-Krishna, Veena-Prajapati, Bhasney S., Tripathi A.K., (2005). Potential toxicity of new genotypes of Tagetes (Asteraceae) species against stored grain insect pests. International Journal of Tropical Insect Science, 25: 122-128.

[12] Zia A., Islan M., Naz F., Iuyas M., (2011). Bio efficacy of some plant extracts against chickpea beetle, Callosobruchus chinensis Linnaeus (Coleoptera: Bruchidae) attacking chickpea. Pakinstan journal of zoology, 43:733-737.