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Ethanolic oil extract of *Ocimum gratissimum*: Potential alternative to synthetic insecticides

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Abstract
Ethanolic oil extracts of *Ocimum gratissimum* was evaluated for their contact and fumigant toxicity against the wheat grain weevil, *Rhyzopertha dominica* and damage to stored wheat under laboratory conditions. Bioassays were conducted at five dosages, 1% of plant extract at 0.2, 0.4, 0.6, 0.8, and 1.0 ml/20 g wheat grains. Adult mortality, adult emergence including inhibition rate and seed weight loss in percentages due to infestation by the weevil were parameters assessed. Results indicated that, both tests at 1.0ml achieved 98-100% weevil mortality at 24 h and not significantly ($P < 0.005$) different from other concentrations and the negative control, but significantly different from the positive control. It was evident from the results that *Ocimum gratissimum* had significant effect on the survival of the insects as low dosage is required to achieve 50 and 95% mortality in 24 h. Weevil mortality and percentage inhibition rate increases with increased plant oil dosages, while adult emergence and percentage seed weight loss reduced with increased plant oil dosage. Findings from this study shows that oil extract of *O. gratissimum* could serve as a bio-pesticide for the control of adult *R. dominica* infesting stored wheat grain.
Keywords: Bioassays, emergence, mortality, *Ocimum gratissimum, Rhyzopertha dominica* toxicity.

Introduction

A major constraint for major for survival on earth has been the conflict between man and insects because they both share the same source of feeding. Nevertheless, the ease of reproduction and multiplication of insects as well as their ability to infest both on the field and in storage has almost won the battle as reported in early study by Ogungbite *et al*., (2014). In fact, the levels of food security have been affected by the infestation of many insect pests especially in many developing countries where less attention is being given to pest management according to Martins *et al*., (2012); Oni, (2014a; 2014b).
Wheat (*Triticum aestivum L.*) is the world's most important cereal crop after maize and rice, in terms of area cultivated and amount of grain produced.
Rhyzopertha dominica (*F*) is a major pest of wheat around the world as mentioned in Flinn et al., (2004). The adult and larvae stages of this pest produce frass and cause weight loss in grains resulting from feeding. Emery and Nayak (2007) reported that infestation by *R. dominica* can reduce rice to dust which brings about high monetary loss. Bashir (2002) also mentioned that the feeding on seed germ by *R. dominica* reduces germination rates and vigour of the grains and may be followed by secondary pests and fungi.
In Nigeria today, the increasing usage of synthetic chemical insecticides are associated with many cons that is impeding their wide spread use nowadays. These problems include

pollution poisoning, residue accumulation, development of pest resistance and high cost of application and reapplication, effects on both human and environmental health according to reports by Ofuya, (1995), Oni and Ileke, (2008), Idoko and Adesina, (2012), Akinneye and Ogungbite, (2013), Oni, (2014a), Ogungbite *et al.*, (2014).This has necessitated the search for safe, ecologically sound and cheap control measures as shown in the study of Adedire and Ajayi, (1996).

A large array of botanicals have been screened for preventing post-harvest loss by insect pest infestation in the report of Glob and Wesley, (1960). However, few investigations have been carried out on insecticidal potential of oil extracts of *Ocimum gratissimum.* Several studies have been carried out that lend credence to herbalist use of this plant for treating diarrhea and other gastrointestinal infections and its widely used as a local medicinal plant for the treatment of diarrhoea, gonorrhoea infection, vaginal douches for vaginitis and in the treatment of mental illness according to the report by Vierra and Simon (2000).

It is against this background that this work evaluates the contact and fumigant potency of ethanolic extract of *Ocimum gratissimum* against *Rhyzopertha dominica* infesting stored wheat grains.

Materials and Methods

Insect Culture and Food Media

Adult *R. dominica* used in this study were obtained from infested wheat grains from Isinkan market in Akure, Nigeria. Subsequent generations were reared on clean uninfested wheat grains in Kliner jars in the laboratory under temperature at ambient room temperature 28±2^{o}C and relative humidity 75±5%.Wheat grains used as food media obtained from the Seed unit of Crop, Soil and Pest Management Department, Federal University of Technology, Akure, Nigeria was disinfested by deep-freezing for 72hours and acclimatized in the open laboratory for 6hours before subsequent assays.

Extraction of *Ocimum gratissimum* leaf oil

Plant leaves were air dried, pulverized into fine powder using Crown Star W301U electric blender and then ethanolic soxhlet extraction was carried out. The oil extract was placed in air-tight bottle for subsequent use.

Contact Bioassay

Twenty grammes (20g) of the wheat grains was weighed into petri-dishes and 1% of the oil extract were separately applied topically to the grains at 0.2, 0.4, 0.6, 0.8 and 1.0 ml dosages. The negative control was treated with 1ml of 1% ethanol and the positive control was treated with 0.1 ml of 0.005% DDVP. Twenty (20) unsexed teneral adults of *R. dominica* was introduced separately into the treated wheat grains and the control setup. Weevil mortality was observed at 24, 48, 72 and 96 h application period. Dead and living insects were removed after seven days. Treatments were left undisturbed for one month for emergence of adults. Number of emerged adults were counted and removed. Percentage inhibition rate and weight loss were also determined.

Fumigant Bioassay

The fumigant toxicity of the oil extract were tested against *R. dominica* using the method of Zhou *et al.* (2012) as described by Yang *et al.*, (2014) with little modifications. 1% Ocimum oil extract was applied as fumigants at 0.2, 0.4, 0.6, 0.8 and 1.0 ml. Vapour of the extracts diffused into the insect inside the petri-dishes containing 20g of wheat grains *Triticum aestivum* with twenty unsexed day old *R. dominica*. The negative control was treated with 1ml of 1% ethanol while the positive control was treated with 0.1 ml of 0.005% DDVP.There were five replicates for each treatment dosages. Dead and living insects were removed after seven days, treatments were left undisturbed for one month for emergence of adults. Number of emerged adults were counted and removed. Percentage inhibition rate and weight loss were also determined.

Characterization of the oil extract of *O. gratissimum* by Gas Chromatography and Mass Spectrometry

A qualitative characterization analysis of possible alkaloids present in alkaloid extracted fraction was carried out using GC-MS using scan mode. This analysis was performed using 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies). The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% Phenyl Methyl Siloxane (30m length x 0.32mm diameter x 0.25µm film thickness) (Agilent Technologies). The carrier gas was Helium used at constant flow of 1.6 mL/min at an initial nominal pressure of 2.84 psi and average velocity of 46 cm/sec. 1µL of the samples were injected in splitless mode at an injection temperature of 260 °C. Purge flow was 21.5 mL/min at 0.50 min with a total flow of 25.8 mL/min; gas saver mode was switched on. Oven was initially programmed at 60 °C (1 min) then ramped at 4 °C/min to 110 °C (3 min) then 8 °C/min to 260 °C (5 min) and 10 °C/min to 300 °C (12 min). Run time was 56.25 min with a 3 min solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C and transfer line temperature of 280 °C. Scanning of possible alkaloid compounds was from *m/z* 30 to 550 amu at 2.62s/scan scan rate and were identified by comparing measured mass spectral data with those in NIST 14 Mass Spectral Library and literature. Prior to analysis, the MS was auto-tuned to perfluorotributylamine (PFTBA) using already established criteria to check the abundance of *m/z* 69, 219, 502 and other instrument optimal & sensitivity conditions. Analysis validation was conducted by running replicate samples in order to see the consistency of the constituent compound name, respective retention time, molecular weight (amu), Quality ion (Q-Ion) and %Total.

$$\%\text{Total} = \frac{Abundance\ of\ individual\ constituents}{Total\ Abundance\ of\ all\ consituents\ in\ extract} \times 100$$

These abundance were outputs from the *NIST 14 Library search report* of the extracts constituents. Each compound identified via the NIST 14 Library Search report has a corresponding mass spectrum showing the abundance of the possible numerous *m/z* peaks per compound

Statistical Analysis

All the mean values obtained were subjected to analysis of variance (ANOVA) and means were separated using Tukey Honestly Significant Difference Test. Mortality data and doses of *R. dominica* was further subjected to probit analysis and log transformation respectively to determine the lethal dose to 50% and 95% of the insects (LD_{50} and LD_{95}) (Finney, 1971). All analysis were carried out using SPSS 21.0 software package.

Contact effects of oil extract of *O. gratissimum* on adult mortality of *R . dominica*

Table 1 showed the mortality of *R. dominica* exposed to different dosages of *O. gratissimum.* Adult *R. dominica* mortality increased with increase in treatment dosages and period of application. *O. gratissimum* oil extract at 1.0 ml achieved 98% mortality of the insect within 24 h of plant extract application and was significantly ($p < 0.05$) different from other treatments, except 0.8 ml plant extract that recorded up to 90% mortality of the insect within the same hour of observation. Within 48 h period of exposure, all the treatments recorded more than 50% mortality of the insect except 0.2 ml of the oil extract. At 96 hour of application, all the treatments achieved up to 70% mortality of the insect. However, it was noted, that the treatments were not significantly different from the positive control but significantly different from the negative control.

Fumigant effects of oil extract of *O. gratissimum* against adult *R. dominica* mortality

Mortality of *R. dominica* exposed to fumes of different dosages of *O. gratissimum* was presented in Table 2. From the result, adult mortality increased with increase in dosage of the treatments and period of application. *O. gratissimum* oil extract at 0.8 and 1.0 ml records 100% mortality of the insect within 24 h of exposure and was significantly ($p < 0.05$) different from other treatments except at 0.6 ml with 82% mortality of the insect within the same hour of observation. Within 48 h period of exposure, all tested dosages of the oil extract recorded more than 70% mortality of the insect. At 96 h of application, all the treatments achieved 100% mortality of the insect. However, it was noted that the treatments were not significantly different from the positive control but were significantly different from the negative control.

Lethal dose of *O. gratissimum* oil extract contact required to achieve 50 and 95% mortality of *R. dominica* within 24 h

The lethal dosage of *O. gratissimum* oil extract required to achieve 50 and 95% mortality adult *R. dominica* was presented in Table 3. From the results, lower amount of *O. gratissimum* oil extract was required to achieve 50 and 95% mortality of the beetle within 24 h of application. It was observed that only 1.77 ml dosage of the oil extract was required to achieve 50% mortality of the insect while 3.75 ml dosage was required to achieve 95% mortality of the insect within 24 h. Also, only 6.51 ml of the *O. gratissimum* oil extract was required to achieve 95% mortality of the *R. dominica.* Thus, oil extract of *O. gratissimum* appeared to be more effective as lower dosage of it was required to achieve 50 and 95% mortality of the insect within 24 h.

Lethal dose of *O. gratissimum* oil extract used as fumigant required to achieve 50 and 95% mortality of *R. dominica* within 24 h post treatment

The lethal dosage of *O. gratissimum* oil extract required to achieve 50 and 95% mortality of adult *O. gratissimum* was presented in Table 4. Lower amount of *O. gratissimum* oil extract used as fumigant was required to achieve 50 and 95% mortality of the beetle within 24 h of application. It was observed that only 1.29 ml dosage of the oil extract was required to achieve 50% mortality of the insect while 3.98 ml dosage was required to achieve 95% mortality of the insect within 24 h. Also, only 4.17 ml of the *O. gratissimum* oil extract was required to achieve 95% mortality of the insect. Thus, oil extract of *O. gratissimum* used as fumigant appeared to be more effective.

Table 1. Contact toxicity of *O. gratissimum* oil extract against *R. dominica* adult survival

Treatments	Dosage (ml)	% mortality in hours			
		24	48	72	96
O. gratissimum	0.2	32.00±3.74cd	48.00±3.74bc	68.00±5.83bc	80.00±4.47bc
	0.4	48.00±3.74de	60.00±5.48c	88.00±4.90def	92.00±3.74cde
	0.6	60.00±3.16e	90.00±3.16d	100.00±0.00f	100.00±0.00e
	0.8	90.00±4.47f	98.00±2.00d	100.00±0.00f	100.00±0.00e
	1.0	98.00±2.00f	100.00±0.00d	100.00±0.00f	100.00±0.00e
DDVP	0.1	58.00±5.83e	100.00±0.00d	100.00±0.00f	100.00±0.00e
Negative control	0.0	0.00±0.00a	0.00±0.00a	4.00±2.45a	14.00±2.45a

Each value is mean ± standard error of five replicates. Mean followed by the same alphabet are not significantly different (p > 0.05) using Tukey Honestly Significant Test.

Table 2. Fumigant toxicity of *O. gratissimum* oil extract against *R. dominica* adult survival

Treatments	Dosage (ml)	% mortality in hours			
		24	48	72	96
O. gratissimum	0.2	42.00±4.90bc	72.00±3.74d	92.00±4.90bc	100.00±0.00b
	0.4	66.00±5.10de	90.00±3.16ef	100.00±0.00c	100.00±0.00b
	0.6	82.00±5.83ef	98.00±2.00f	100.00±0.00c	100.00±0.00b
	0.8	100.00±0.00f	100.00±0.00f	100.00±0.00c	100.00±0.00b
	1.0	100.00±0.00f	100.00±0.00f	100.00±0.00c	100.00±0.00b
DDVP	0.1	58.00±5.83cd	100.00±0.00f	100.00±0.00c	100.00±0.00b
Negative control	0.0	0.00±0.00a	0.00±0.00a	4.00±2.45a	14.00±2.45a

Each value is mean ± standard error of five replicates. Mean followed by the same alphabet are not significantly different ($p > 0.05$) using Tukey Honestly Significant Test.

Table 3. *O. gratissimum* oil extracts required to achieve 50 and 95% mortality of *R. dominica* at 24 h post-treatment

Treatment	Slope ± S.E	Intercept ± S.E	X^2	LD_{50} (FL 95%)	LD_{95} (FL 95%)	Sig.
O. gratissimum	2.90±0.12	-0.72±0.05	211.77	1.77(1.46-2.04)	6.51(5.09-9.65)	0.0001

X^2: Chi-square; SE: Standard error; FL: Fiducial limits; LD: Lethal dosage

Table 4. Lethal dose of fumes of *O. gratissimum* oil extracts required to achieve 50 and 95% mortality of *R. dominica* at 24 h post treatment

Treatments	Slope ± S.E	Intercept ± S.E	X^2	LD_{50} (FL 95%)	LD_{95} (FL 95%)	Sig.
O. gratissimum	3.36±0.14	-0.37±0.05	207.31	1.29 (1.04-1.51)	3.98 (3.29-5.33)	0.0001

X^2: Chi-square; SE: Standard error; FL: Fiducial limits; LD: Lethal dosage

Table 5. Adult emergence of *R. dominica*, percentage lnhibition Rate and Seed weight loss by *O. gratissimum* contact test.

Treatments	Dosage (ml)	Adult emergence	% IR	% weight loss
O. gratissimum	0.2	39.40±1.50e	42.40±2.20bc	15.28±0.34e
	0.4	34.40±0.75de	49.71±1.09c	7.80±1.16cd
	0.6	20.00±2.41bc	70.76±3.52e	4.24±0.91bc
	0.8	5.80±1.66a	91.52±2.42f	0.40±0.15a
	1.0	0.00±0.00a	100.00±0.00f	0.00±0.00a
DDVP	0.1	0.00±0.00a	100.00±0.00f	0.00±0.00a
Negative control	0.0	68.00±2.65f	0.00±0.00a	41.70±1.23f

Each value is mean ± standard error of five replicates. Mean followed by the same alphabet are not significantly different ($p > 0.05$) using Tukey Honestly Significant Test.

Effect of different dosages of *O. gratissimum* on Adult emergence, % IR of *R. dominica* and weight loss in treated wheat grains

The adult emergence and percentage inhibition rate of *R. dominica* exposed to different dosages of *O. gratissimum* as well as ability of the insect to cause weight loss of treated wheat grains were presented in Table 5. Adult emergence and percentage seed weight loss decreased with increase in treatments, while percentage IR increased with increase in treatments dosages. Only 1.0 ml of the oil extract and the positive control were able to prevent insect adult emergence and seed weight loss and their effects were significantly ($p < 0.05$) different from other treatments except 0.8 ml of the oil extract treatment. Also, only 1.0 ml of the oil extract and the positive control were able to achieve 100% inhibition of the insect. Thus, they were significantly ($p < 0.05$) different from others except 0.8 ml oil extract treatment .The treatments were significantly ($p < 0.05$) different from the negative control in terms of the adult emergence, % IR and seed weight loss.

Effect of different dosages of fumes of *O. gratissimum* on Adult emergence, % IR of *R dominica* and weight loss in treated wheat grains

The adult emergence of *R. dominica* exposed to fume of different dosages of *O. gratissimum* as well as percentage inhibition rate and ability of the insect to cause weight loss of treated wheat grains were presented in Table 6. Adult emergence of the insecst and percentage seed weight loss decreased with increase in the dosages, while percentage IR increased with increase in treatments dosages. Only the positive control was able to prevent adult emergence and seed weight loss and its effect was significantly ($p < 0.05$) different from other treatments. Also, only positive control was able to achieve 100% inhibition of the insect. Thus, it was significantly ($p < 0.05$) different from others. The treatments were significantly ($p < 0.05$) different from the negative control in term of the adult emergence, % IR and seed weight loss.

Active compound present in the leaves oil extract of *O. gratissimum*

The active compounds present in the leaves oil extract of *O. gratissimum* is presented in Table 7. Ninety-four active compounds were found present in the oil extract of the plant. Phenol, 2,3,5,6-tetramethyl-Naphthalene, 1,2,3,4,4a,5,6,8a- octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2. alpha.,4a.alpha., 8a.beta.)]-, 4-Hydroxy-2-methylacetophenone, Phytol and n-Hexadecanoic acid are the five major compounds with 60.21, 4.95, 4.24, 6.06 and 1.07% respectively.

Table 6. Adult emergence of *R. dominica,* percentage lnhibition Rate and Seed weight loss by *O. gratissimum* fumigant test.

Treatments	Dosage (ml)	Adult emergence	% IR	% weight loss
O. gratissimum	0.2	42.40±1.749ef	38.01±2.56bc	21.59±0.96gh
	0.4	36.00±1.00de	47.37±1.46d	17.30±0.87efg
	0.6	30.00±1.22cd	56.14±1.79e	13.19±0.92de
	0.8	24.60±1.17c	64.04±1.70f	9.28±0.70cd
	1.0	13.80±0.80b	79.82±1.17g	3.47±1.04ab
DDVP	0.1	0.00±0.00a	100.00±0.00h	0.00±0.00a
Negative control	0.0	68.00±2.65g	0.00±0.00a	41.70±1.23i

Each value is mean ± standard error of five replicates. Mean followed by the same alphabet are not significantly different ($p > 0.05$) using Tukey Honestly Significant Test.

Table 7. Active compounds present in the leaves oil extract of *O. gratissimum* using GC-MS analysis

SN	Compound Name/Hit Name	Percentage Total of all compound (% Total)	Entry Number in NIST14 Library
1	Phenol, 2,3,5,6-tetramethyl-	60.208	24433
2	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	4.948	68904
3	4-Hydroxy-2-methylacetophenone	4.238	155852

4	Phytol	6.063	157065
5	n-Hexadecanoic acid	1.073	117418
6	4-Amino-6-hydroxypyrazolo[3,4-d]pyrimidine	1.073	24513
7	19-Norpregn-4-ene-3,20-dione	1.018	124916
8	N-(4-(9-Acridin-9-yl)-3-methoxyphenyl)-N,N-dimethylamine	1.016	195589
9	Camphene	0.881	16040
10	9-Oxabicyclo[6.1.0]nonan-4-one	0.850	1751
11	2,5-Di(trifluoroacetyloxy)acetophenone	0.746	200280
12	Caryophyllene oxide	0.677	83535
13	Benzenepropanal, .beta.-methyl-	0.662	5461
14	Hexadecanoic acid, ethyl ester	0.631	144309
15	3,5-Nonadien-7-yn-2-ol, (E,E)-	0.617	15140
16	Supraene	0.595	243217
17	.gamma.-Sitosterol	0.581	245060
18	Geranyl acetate, 2,3-epoxy-	0.566	70781
19	2-Isopropylidene-3-methylhexa-3,5-dienal	0.554	36179
20	Cyclooctene, 3-ethenyl-	0.525	255695
21	1,7-Octadiene, 3,6-dimethylene-	0.478	6265
22	Benzene, 2-ethyl-1,3-dimethyl-	0.464	15215
23	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	0.430	10150
24	Phenol, 2-methyl-5-(1-methylethyl)-	0.428	24389
25	p-Cymen-7-ol	0.361	25185
26	4-Fluorophenylhydrazine	0.330	11376
27	3-Penten-2-ol	0.324	18113
28	Benzene, 1-ethenyl-4-nitro-	0.309	68698
29	3,5-Octadienoic acid, 7-hydroxy-2-methyl-, [R*,R*-(E,E)]-	0.307	17427
30	3-Buten-1-ol	0.249	667
31	Benzene, 1-methyl-4-(1-methylethenyl)-	0.244	2279
32	Acetic acid, 3-methylenebicyclo[3.2.1]oct-6-en-8-yl ester	0.236	61615
33	Decane, 2-methyl-	0.224	47759
34	p-Cymene-2,5-diol	0.220	24185
35	Vitamin E	0.219	250946
36	Phytol, acetate	0.211	157776
37	5-Phenyl-3-(2-thienyl)-3a,6a-dihydropyrrolo(3,4-d)isoxazole-4,6(5H)-dione	0.208	186691
38	2-Hexyn-1-ol	0.206	3190
39	Caryophyllene-(I1)	0.206	18073
40	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-	0.203	51414

41	Spiro (6,6-dimethyl-2,3-diazobicyclo [3.1.0] hex-2-ene-4,1'-cyclopropane)	0.202	16648
42	Bicyclo[4.1.0]heptane,-3-cyclopropyl,-7-hydroxymethyl, trans	0.201	36538
43	7,10,13-Hexadecatrienoic acid, methyl ester	0.190	171414
44	Octadecanoic acid, ethyl ester	0.188	186704
45	(Z,Z)-.alpha.-Farnesene	0.188	68625
46	Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	0.182	18899
47	Phenol, p-tert-butyl-	0.178	24358
48	Cyclopropane, 1,1'-ethenylidenebis-	0.171	14415
49	Glyceraldehyde	0.163	16874
50	16-Epi-estriol-3-TMS-phenylboronate	0.163	255904
51	6-Benzylaminopurine, N-acetyl-	0.161	127279
52	.alfa.-Copaene	0.159	68522
53	Pyridine-3-carbonitrile, 1,2-dihydro-4-[4-(1,1-dimethylethyl)phenyl]-6-phenyl-2-oxo-	0.157	157927
54	4,4-Dimethylpent-2-enal	0.155	6630
55	Biphenyl, 4,4'-bis(trimethylsilyl)-	0.151	16103
56	Heptadecane, 2-methyl-	0.151	10524
57	3-Acetylamino-6-methoxy-2H-naphtol[1,2-b]pyran-2-one	0.149	142823
58	Bis[4-[4-hydroxy]piperidino-3-aminophenyl]sulfone	0.148	255538
59	p-Mentha-1,5,8-triene	0.147	5592
60	Spiro[2.2]pentane-1-carboxylic acid, 2-cyclopropyl-2-methyl-	0.146	34989
61	Dodecane, 2-methyl-	0.143	83629
62	1-Iodo-2-methylundecane	0.139	115554
63	3,9-Epoxy-p-mentha-1,8(10)-diene	0.136	24458
64	6-Azabicyclo[3.2.1]octane	0.132	15172
65	[1,1'-Biphenyl]-4-ol, 3,5-bis(1,1-dimethylethyl)-	0.125	142236
66	Butane, 2-(2,2-dichloro-1,3-dimethylcyclopropyl)-	0.123	60365
67	2-(5-Methyl-furan-2-yl)-propionaldehyde	0.122	17812
68	Pyridine, 2-ethoxy-	0.116	16152
69	trans-Isoeugenol	0.208	34539
70	Benzenemethanol, 4-methyl-	0.110	29364
71	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one	0.104	119458
72	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.103	155865
73	o-Cymene	0.100	15254
74	Oxalic acid, allyl nonyl ester	0.098	116960
75	Benzene, 1,1',1'',1''',1''''-(1,3-cyclopentadiene-1,2,3,4,5-pentayl)pentakis-	0.097	255904
76	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-	0.096	83533

77	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1.alpha.,2.beta.,5.alpha.)]-	0.094	154846
78	2-Methyl-2-vinyloxirane	0.091	23313
79	Naphthalene, 6-chloro-1-nitro-	0.084	71200
80	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	0.083	15254
81	4-Hydroxy-4-(4,6-dimethylcyclohex-3-enyl)butan-2-one	0.080	76047
82	p-Cresol	0.079	15230
83	3-[N-Acetyl-4-acetylanilino]propionic acid	0.077	110337
84	6-Octen-1-ol, 3,7-dimethyl-, formate	0.073	51058
85	26-Nor-5-cholesten-3.beta.-ol-25-one	0.073	231233
86	3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	0.072	25390
87	3-Aminopyrazine-2-carboxylic acid	0.071	15253
88	Cyclopropane, 1-cyclopropylethynyl-2-methoxy-3,3-dimethyl-	0.069	35022
89	1,8-Cyclopentadecadiyne	0.066	66843
90	2,4-Diaminophenol	0.066	46018
91	2,5-Methano-1H-indene, octahydro-	0.065	68590
92	(E)-2-((8R,8aS)-8,8a-Dimethyl-3,4,6,7,8,8a-hexahydronaphthalen-2(1H)-ylidene)propanal	0.062	81725
93	Hexadecanoic acid, methyl ester	0.061	130819
94	2-Cyclopenten-1-one, 2,3,5-trimethyl-4-methylene-	0.061	16949

Discussion

Plant extracts have been considered as possible alternative to synthetic chemical insecticides and have been in practice since ancient time for the management of storage insect pests. Many medicinal plants and spices have been used as pest control agents as stated by Ofuya and Dawodu (2002), Ashamo (2007), Ogungbite and Oyeniyi (2014). However, despite the efficacy of many of these botanical insecticides, the mode of action of many of them have not been fully investigated as reported by Isman (2006), Dayan (2009), Begum et al, (2013). Therefore, before any botanical extract could be recommended for commercial use, its mode of action should be determined.

The results of this study showed that *O. gratissimum* leaves oil extract have insecticidal potential against infestation of *R. dominica.* The result revealed that the effectiveness of the oil extract was dependent of the dosages of the plant oil extract. The probit analysis done on the mortality of the insect showed that few amount of the oil extract of *O. gratissimum* was required to cause high mortality of the insect. Adult *R. dominica* are known for their characteristic feeding at the larvae and adult stage, therefore, the oil extract of the plant may contain some anti-nutrient properties that might have caused loss of appetite to the insect. Thus, led to high mortality of the insect due to starvation. Fumigant activity of the Ocimum oil extract may have resulted from fumes blocking the respiratory openings thereby promote suffocation and death similar to report of Oni et al. (2011). The plant oil extract may have also disrupted the normal respiratory activities of these insects leading to asphyxiation and subsequent death of the insects. This result was in agreement with previous studies in which oils of *A. indica, Z. zanthoxyloides, A. occidentales, M. oleifera* and many other botanical extracts were used as protectant as described by Oni (2014b), Ogungbite and Oyeniyi (2014), Oni and Ogungbite (2015). Also, the result of high toxicity of *O. gratissimum* against the *C. maculatus* may be due to some of the potential active compound found present in the leaves oil extract of the plant. These phytochemicals have been reported to have considerable adverse effects on normal life cycle of insects by Yang *et al*., (2014). Thus, the effect of the oil extract of the plant could be due to these potential active compounds according to Yang et al. (2006).

The oil of *O. gratissimum* used in this work has proven insecticidal against wheat beetle, *R. dominica*. Nevertheless, the insecticidal potential of this plant oil depended on the dosage of the treatment and the period of application. The result showed that the oil had more fumigant toxicity effects on the survival of the insects, while it had more contact toxicity effect on adult emergence of the insect, as well as the ability of the insects to cause seed weight loss.

Therefore, oil of *O. gratissimum* has shown insecticidal efficacy against *R. dominica* infestation and since the characterization of the oil extract revealed different active compounds present in the oil, the compounds can be synthesized for commercialization for its incorporation into pest management tactics against stored products insects.

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