

# Pesticidal effects of scent leaf (*Ocimum gratissimum* L.) on maize weevil: potency of scent leaf on *Sitophilus zeamais*

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## Abstract

Maize weevil is a major pest of stored maize grains in many regions of the world including Nigeria. A laboratory investigation was conducted to assess the potency of *Ocimum gratissimum* L. leaves for *Sitophilus zeamais* control. The *O. gratissimum* powder was used for ethanolic extract preparation by soaking 300 g of dry powdered plant material in 1.5 L of absolute ethanol for 24 hours at room temperature with continuous stirring for 10 minutes. The ethanolic extract tested for phytochemical constituents, including tannins, alkaloids, saponins, phenolic compounds, terpenoids,

steroids, glycosides, and flavonoids, showed that they were all present except steroids. Exposure of adult *S. zeamais* to different concentrations of ethanolic extract generated significant cumulative mortality, and increased as the concentration increased; 35.5%, 64.4%, 95.5%, and 100% mortalities of *S. zeamais* were recorded at 30, 150, 250, and 750 mg/mL extract, respectively. The capacity of the extract to induce 100% mortality at 750 mg/mL concentration revealed its potential as an insecticidal agent. The powder caused low weight loss and seed damage of grains treated with the highest dosage. The ethanolic extract of *O. gratissimum* displayed a high level of insecticidal activity against maize weevil suggesting a high possibility of using it as toxicant, repellent, and feeding deterrent in integrated pest management strategies of *S. zeamais*.

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## Introduction

Maize (*Zea mays* L.) is a major source of dietary carbohydrates as well as the most important cereal in sub-Saharan Africa.<sup>1</sup> While the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a major pest of stored maize grains in many regions of the world including Nigeria.<sup>2</sup> The maize weevil is one of the most destructive stored product pests of grains, cereals, and other processed and unprocessed stored products in sub-Saharan Africa.<sup>3</sup> The maize weevil causes qualitative and quantitative damage to stored products with grain loss ranging between 20% sometimes up to 90% for untreated stored maize,<sup>3</sup> and the severity of the damage depends on factors that include storage, structure, physical and chemical properties of the product. Heavy infestation of adults and larvae of maize weevil, which cause post-harvest losses, have become important constraints to storage entomology<sup>4</sup> and food insecurity in the tropics. This insect is notorious for attacking most crop food items of grain; they can breed within other types of crops as well. Although the maize weevil may not readily breed in finely processed grains, it can easily breed in products like macaroni, noodles, and milled cereals that have been exposed to excessive moisture.<sup>5</sup> The negative effects of the weevils can result in the movement of grounded particles in storage, lowering germination of seed grains,<sup>6</sup> distribution of molds and other micro-organisms through the grain mass and quality reduction caused by the occurrence of exuviae, fecal material, etc. which reduce the future of the maize produced by farmers in Africa.<sup>7</sup>

There is an urgent need to search for eco-friendly, cheap, sustainable, and safe plant extracts that could protect as well as not contaminate food products in storage systems and could be of use to small-scale holder farmers. In addition, biogenic compounds are not chemically synthesized compared to compounds that are partially or completely synthesized in the laboratory due to their bonds, and are often considered environmentally friendly<sup>8</sup> and

therefore are likely to be evaluated positively<sup>9</sup> and find wider acceptance in the long run. Plant protection products of natural origin also have the advantage of developing a valuable mode of action against insects and thus can reduce the risk of cross-resistance and at the same time provide new ideas for the design of specific target molecules.<sup>10</sup> The powders and essential oils of garlic and African curry plants have been widely used for the control of insect pests, especially storage insect pests.<sup>11</sup> Research on the potential application of biologically active compounds from garlic abounds in the literature. For instance, steam-distilled garlic oil has been investigated for toxicity against the adult maize weevil. When fresh Nigerian garlic cultivar (GUN) juice was compared to German garlic cultivar (GAG), it was discovered that fresh GUN juice caused a cumulative mortality rate of 73% and GAG had an 87% cumulative mortality rate and they were remarkably likely to induce mortality to *S. zeamais* at 28 days after treatment.<sup>11</sup> Garlic extracts have been vaporized and utilized in fumigation testing with *Callosobruchus maculatus* (Coleoptera: Bruchidae).<sup>12</sup> When compared to the control, all the exposed durations (6-96 hrs) to volatile fumes of garlic aqueous extract caused massive mortality to *C. maculatus*.

The leaves of the plant known as scent leaf (*Ocimum gratissimum* L.) contain a perfume odor. When combined with alcohol (small stout beer), it is used in Nigeria to treat certain conditions like hemorrhoids (piles) and for cooking meals. It is traditionally used to cure typhoid fever when combined with the bark of a bitter leaf tree, lemon grass, and guava leaf, and boiled for about 20 minutes. Although this plant is now not utilized historically for controlling maize weevils since farmers have not yet realized the value of using it, the necessity to control *S. zeamais* and improve the output of the stored product was what drove this research.

Although synthetic pesticides such as phosphine, ethylbromide, and DDT have long been widely used in the control of insect pests, their indiscriminate use has led to various problems including toxic residues in the treated products, environmental pollution, and growing resistance against insecticides by insects and pests.<sup>8</sup> The use of bio-pesticides is of immense importance as the crop of interest, maize, is a major staple food in the tropics, especially in Nigeria where it is eaten to lessen hunger in the population. It is therefore imperative to further broaden the list of plant products used in the control of stored insect pests of maize. This study focuses its attention on coming up with alternative measures, such as the use of plant materials that possess insignificant threats, are environmentally friendly, and require little or no skill to be used effectively. On these critical grounds, the present study intends to evaluate the use of scent leaf (*O. gratissimum*) as a pesticide to prevent damage to maize by *S. zeamais*, which is one of the major pests of stored products. This study aims to evaluate the insecticidal property of scent leaf powder and the plant extract (ethanolic and aqueous) which was made and tested against stored maize weevil (*S. zeamais*) infestation. The specific objectives of this work were to identify the potency of scent leaf to protect maize grain in storage, ascertain the minimum effective dosage against maize weevil, determine if the plant extract has a toxicity effect against the maize weevil, and ascertain the repellent effect of scent leaf against *S. zeamais*.

## Materials and Methods

### Collection and processing of plant materials

Scent leaves (*O. gratissimum*) were bought from Ogige Market, Nsukka, Enugu State, Nigeria. The leaves were air-dried in the lab-

oratory. The plant material was grounded into powder with a milling machine and the powder was sieved to obtain uniform particle size, after which it was stored in a plastic air-tight container until needed.

### Preparation of plant extract

The *O. gratissimum* powder was used for the preparation of the ethanolic extract. The extract was prepared by soaking 300 g of the dry powdered plant material in 1.5 L of absolute ethanol for 24 hours at room temperature with continuous stirring for about 10 minutes. After 24 hours, the extract was sieved with a muslin cloth. The liquid filtrate obtained was concentrated by allowing it to evaporate to dryness for 24 hours at room temperature and humidity. The aqueous extract was prepared by dissolving the same 300 g of the powdered *O. gratissimum* in 1.5 L of water and was filtered.

### Phytochemical analysis

The ethanolic extract of *O. gratissimum* was tested for different phytochemical constituents such as tannins, alkaloids, saponins, phenolic compounds, terpenoids, steroids, glycosides, and flavonoids. The presence of possible phytochemical constituents was evaluated qualitatively with the following methods:

**Test for tannins:** the extract of *O. gratissimum* was mixed with 3–4 drops of 0.1% ferric chloride solution. The formation of a brownish-green or blue-black color indicated the presence of hydrolyzable tannins.<sup>10</sup>

**Test for alkaloids:** the plant extract was mixed in 1% v/v HCl, warmed, and filtered. This filtrate was mixed with Mayer's reagent (Mercuric chloride + potassium iodide in water). The presence of alkaloids is specified by the formation of a yellow-colored precipitate.<sup>10</sup>

**Test for saponins:** about 1 mL of the extract of *O. gratissimum* was mixed with 9 mL of distilled water and shaken vigorously until the appearance of stable froth for at least 15 min which indicated the presence of saponins.<sup>10</sup>

**Test for phenolic content:** about 200  $\mu$ L aqueous extract solution was mixed with 0.75 mL 10-fold diluted folin-ciocalteu reagent. Following 5 min of incubation, 0.75 mL of 6% Na<sub>2</sub>CO<sub>3</sub> solution was added to the mixture and allowed to stand for 90 minutes at room temperature. The brown color indicated the presence of phenolic compounds.<sup>10</sup>

**Test for terpenoids:** the extract (5 mL) was mixed with chloroform (2 mL), and concentrated sulfuric acid (3 mL) was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results for the presence of terpenoids.<sup>10</sup>

**Test for steroids:** about 2 mL of the plant extract of *O. gratissimum* was mixed with 1 mL of distilled water and a few drops of concentrated sulfuric acid. The appearance of blue or green color indicated the presence of steroids.<sup>10</sup>

**Test for glycosides:** about 1 g of *O. gratissimum* was extracted with 5 mL of distilled water. Then, 2 mL of glacial acetic acid containing one drop of ferric chloride solution was added to the filtrate. This was followed by the addition of 1 mL of concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring at the interface indicated the presence of glycosides.<sup>10</sup>

**Test for flavonoids:** a portion of the extract of *O. gratissimum* was heated with 10 mL distilled water over a steam bath for 3 minutes. Then, 4 mL of the filtrate from the mixture were shaken with 1 mL of diluted ammonia solution. A yellow coloration indicated the presence of flavonoids.<sup>10</sup>

## Collection and preparation of maize grains

The maize that was used in this study was obtained from Ogige Market, Nsukka, Enugu State, Nigeria. The maize was air-dried for three days and placed in a deep freezer at  $-20^{\circ}\text{C}$  for one week to kill any storage insect pests. After disinfection, the maize grains were air-dried to prevent fungal growth. The grains were packaged in polythene bags and kept pending use.

## Culturing of *S. zeamais*

The initial stock of *S. zeamais* used for the experiment was obtained from weevil-infested grains bought from Ogige Market, Nsukka, Enugu State, and cultured in separate plastic containers under the ambient laboratory temperature. The weevils fed on the infested maize. The infested maize grains were left in the culture vial and kept in the laboratory cupboard to allow mating and oviposition of insects. This was left undisturbed till the emergence of adults. The newly emerged adults were used for the experiment.

## Experimental design

### Fumigant toxicity assay

The toxicity test was conducted according to the method described by Saghere *et al.*<sup>13</sup> Whatman filter papers were cut according to the size of the plastic container and treated with different concentrations of the plant extract ranging between 50 mg/mL and 400 mg/mL and allowed to dry for 10 minutes. These treated filter papers were gently placed at the bottom of the disposable plastic cups. At the bottom of the container, 10 g of maize were placed on the treated filtered papers, and 15 newly emerged adult insects (2–4 days old) were introduced into each cup. The cups were covered with muslin cloth and held in place with rubber bands. The experiment was done in four treatment groups (A-D) with three replicates. A, B, C, and D treatment groups received ethanolic extract concentrations of 30, 150, 250, and 750 mg/mL, respectively. For the control, only the solvent (absolute ethanol) was applied to the filter papers. The experimental setup was examined for 3 days and the number of dead insects (those that failed to respond to pen probes) was recorded every 24 hours. Probit analysis was done to ascertain the  $\text{LC}_{50}$  of the plant extract and subsequent tests were carried out below this concentration.

### Adult mortality test

According to Koomson and Oppong,<sup>14</sup> three concentrations (100, 250, and 300 mg/mL) of the plant extract were prepared. Each concentration of plant extracts was mixed for about 10 minutes with maize grain. Day-old adults of *S. zeamais* (15 in number) were released into the bottles containing plant extract-treated maize and covered with a muslin cloth. The muslin cloths were held in place with rubber bands to prevent insect exit out of the containers. Three replicates were maintained for the different concentrations of the plant extracts. The controls were left untreated. Insect mortality count was recorded every 24 hours for 21 days after treatment. Percentage adult mortality was determined using the formula:

$$\% \text{ Mortality} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

### Repellency test

The repellency test was carried out using the method of Talukder and Howse.<sup>15</sup> Three different concentrations (150, 250,

and 750 mg/mL) of the plant extract were prepared. Whatman filter papers were cut into two halves, and one half of each filter paper was treated with various concentrations of the extract as uniformly as possible by using a micro-pipette. The other halves of the filter paper were treated with only the solvent as the control. The plant-extract-treated and solvent-treated filter paper halves were air-dried to evaporate the solvent completely. Plant extract-treated and solvent-treated filter paper halves were then attached lengthwise, edge-to-edge with adhesive tape, and placed at the bottom of glass Petri dishes. Subsequently, 20 adult weevils were released at the center of the Petri dishes and the Petri dishes were covered and kept in the dark; 3 replicates were set for each concentration of plant extracts. The number of insects present on both treated and untreated halves was recorded after 1 hour and 3 hours in mild light.

Percentage repellency was computed as follows:

$$\text{PR} = \frac{\text{Nc} - \text{Nt}}{\text{Nc} + \text{Nt}} \times 100$$

where: PR, percentage repellency; Nt, insect number present on the treated strip; Nc, insect number present on control strip; Negative PR values were treated as zero.

### Damage assessment test with *O. gratissimum* powder

The method of Koomson and Oppong<sup>14</sup> was adopted in carrying out this test. Ten pairs (ten males and ten females) of a-day-old adult *S. zeamais* were introduced into plastic containers containing 20 g of maize grains treated with five different concentrations of the *O. gratissimum* powder (1, 2, 3, 4, and 5 g of grain). The 20 g weight of the grain served as the initial weight of the maize. The treatment groups were covered with muslin cloth and held in place with a rubber band to prevent the insect pest from escaping and to provide the needed ventilation. The setup was left to stand for 20 days. In the control, the maize was only treated with the solvent. At the end of the 20 days observation period, the weevils were sieved out and the maize grains in each container were weighed. The extent of weevil damage was assessed in terms of weight loss of the maize grains using the formula:

$$\% \text{ Weight loss} = \frac{\text{Initial weight of maize} - \text{final weight of maize}}{\text{Initial weight of maize}} \times 100$$

## Statistical analysis

Data obtained from the tests were analyzed using Statistical Packages for Social Sciences (SPSS) version 23.0 (IBM Corporation, Armonk, USA) and Microsoft Office Excel® (Microsoft Incorporated, Redmond, USA). Short- and long-term mortality of *S. zeamais* after exposure to *O. gratissimum* extract was compared using a generalized linear model with the duration of exposure and concentration acting as fixed factors. This was followed by the least significance difference (LSD) post-hoc test. Probit analysis by the Finny<sup>16</sup> method was used to estimate lethal concentrations of *O. gratissimum* extract in *S. zeamais* adults after 24, 48, and 72 h. Repellency of *S. zeamais* by *O. gratissimum* powder and loss in weight (a measure of seed damage) were compared between concentrations by one-way analysis of variance (ANOVA) and LSD. The level of significance was set at  $p < 0.05$ .

## Results

### Phytochemical analysis of *Ocimum gratissimum*

The summary of the results of the phytochemical analysis of *O. gratissimum* is presented in Table 1. Tests for tannins, terpenoids, flavonoids, resins, phenols, saponins, alkaloids, and xanthoproteics for aromatic amino acids, oils, and glycosides were positive in both ethanolic extract and aqueous extract. Steroids were not seen in the ethanolic and aqueous extract. Tannins, flavonoids, and xanthoproteics occurred in minute quantities with tannin occurring in least quantity. Alkaloids, saponins, phenols, glycosides, and resins occurred in moderate quantities. Phenols occurred more in the ethanolic extract than in the aqueous extract. Phenolics are the largest group of phytochemicals and have been touted as account-

**Table 1.** Phytochemical analysis of *Ocimum gratissimum* using ethanolic and aqueous extracts.

Parameter	Ethanolic extract	Aqueous extract
Tannins	+	+
Alkaloids	++	++
Saponins	++	++
Phenols	++	+
Terpenoids	++	++
Steroids	-	-
Glycosides	++	++
Flavonoids	+	+
Resins	++	++
Xanthoproteics	+	+
Oils	+++	++

+++ , very high; ++, moderate; +, little/traces; -, absent.

**Table 2.** Cumulative mortality of adult *Sitophilus zeamais* after 72 hours exposure to *Ocimum gratissimum* ethanolic extract.

Concentration (mg/mL)	Initial number	Number of deaths			Total death	Number survived	Mortality (%)	Survival (%)
		24 h	48 h	72 h				
0	45	00	00	00	00	45	0.00 <sup>a</sup>	100.00 <sup>d</sup>
30	45	4	5	7	16	29	35.56 <sup>b</sup>	64.44 <sup>d</sup>
150	45	7	7	15	29	16	64.44 <sup>c</sup>	35.56 <sup>c</sup>
250	45	10	14	19	43	2	95.56 <sup>d</sup>	4.44 <sup>b</sup>
750	45	45	-	-	45	00	100.00 <sup>d</sup>	0.00 <sup>a</sup>

Numbers with different letters in superscript along a column were significantly different ( $p < 0.05$ ).

**Table 3.** Cumulative mortality of adult *Sitophilus zeamais* after 72 hours exposure to *Ocimum gratissimum* powder with three replicates.

Concentration g/20 g grain	% Death in replicates			Mean mortality (%)	Survival (%)
	R1 (%)	R2 (%)	R3 (%)		
0.0	00	00	00	0.00±00 <sup>a</sup>	100±00 <sup>c</sup>
1.0	74	61	71	68.67±6.81 <sup>b</sup>	31.33±6.81 <sup>d</sup>
2.0	73	84	81	79.33±5.69 <sup>c</sup>	20.67±5.69 <sup>c</sup>
3.0	80	88	93	87.00±6.56 <sup>d</sup>	13.00±6.56 <sup>b</sup>
4.0	97	100	100	99.00±1.73 <sup>c</sup>	1.00±1.73 <sup>a</sup>
5.0	100	100	100	100±00 <sup>c</sup>	0.00±00 <sup>a</sup>

Values as % mean±SD. Numbers with different letters in superscript along a column were significantly different ( $p < 0.05$ ).

ing for most of the antioxidant activity of plants or plant products. Oil content was in the highest quantity and occurred more in the ethanolic extract than in the aqueous extract.

### Mortality of *Sitophilus zeamais* adults after 72 hours of exposure to *O. gratissimum* ethanolic extract

The mortality of *S. zeamais* after 24-, 48-, and 72-hours exposure to ethanolic extract of *O. gratissimum* is summarized in Table 2. Mortality at all concentrations was significantly different from the control ( $p < 0.05$ ). The highest mortality occurred at 750 mg/mL. Mortality at 250 mg/mL and 750 mg/mL were 43 (95.56%) and 45 (100%), respectively, and were not significantly different. Mortality at other concentrations (30, 150, 250 mg/mL) was significantly different from one another. Mortality rates were dose- and duration-dependent with the highest occurring at 750 mg/mL and the least at 30 mg/mL.

Mortality after exposure to powdered *O. gratissimum* is summarized in Table 3. Mortality rate had different results with ethanolic extract in comparison to the mortality rate of powdered *O. gratissimum*. All concentrations were significantly different from the control ( $p < 0.05$ ). The highest mortality occurred at 5 g. When compared to the control, 4 and 5 g show significant differences, but the results are not significant when compared between them. Other concentrations (1 g, 2 g, and 3 g) considerably differed from one another in terms of mortality. Mortality rates were dose- and duration-dependent with the highest occurring at 5 g and the least at 1 g.

### Cumulative mortality of *S. zeamais* adult on prolonged exposure to *O. gratissimum* powder

The summary of the results of prolonged exposure to *O. gratissimum* is presented in Table 4. One death was recorded at 0 g/20 g grain on day 21. However, it was not statistically different from

day 7 and day 14 at that concentration. Exposure was dose- and duration-dependent. There were significant differences ( $p < 0.05$ ) on days 7, 14, and 21 when compared with the dose-dependent mortality at 1 g and 2 g. The concentrations appeared to be significantly different ( $p < 0.05$ ) with an increase in mortality rate as concentration increased. Mortality at 1 g and 2 g was significantly ( $p < 0.05$ ) greater than the control.

### Damage assessment test on *S. zeamais* with *O. gratissimum* powder

Table 5 summarizes the damage assessment test. The highest weight loss was seen at g/20 g grain concentration of *O. gratissimum* powder while no weight loss was seen at 5 g concentration. Concentrations 4 g and 5 g presented 0.03±0.06 and no weight loss, respectively, and were not statistically significant. Other concentra-

tions (1 g, 2 g, and 3 g) substantially differed from one another in terms of weight loss ( $p < 0.05$ ).

### Repellency effect of ethanolic extract of *O. gratissimum*

Findings from the setup to assess whether *O. gratissimum* extract repelled *S. zeamais* are summarized in Table 6. The insect showed a preference for the control stripe compared to the treatment stripes. Repulsion for the *O. gratissimum* impregnated stripes was highest at the 750 mg/mL concentration at both 1 h and 3 h observation time. The lowest repellency was observed at 150 mg/mL after both 1 and 3 h. The disparity in repellency for *S. zeamais* was significantly different between all three treatment groups after 1 h and between 150 mg/mL and other concentrations after 3 h.

**Table 4.** Cumulative mortality of adult *Sitophilus zeamais* after prolonged exposure to *Ocimum gratissimum* powder (initial number of weevils per concentration =45).

Conc. g/20 g grain	7 days Mean±SD	14 days Mean±SD	21 days Mean±SD
0	0.00±0.00 <sup>1a</sup>	0.00±0.00 <sup>1a</sup>	0.3±0.58 <sup>1a</sup>
1	9±1.54 <sup>1b</sup>	13±1.11 <sup>2b</sup>	26±1.23 <sup>3b</sup>
2	17±1.44 <sup>1c</sup>	23±1.34 <sup>2c</sup>	31±1.45 <sup>3c</sup>
3	26±1.03 <sup>1d</sup>	39±1.44 <sup>2d</sup>	42±1.10 <sup>2d</sup>
4	44±0.54 <sup>1e</sup>	45±0.00 <sup>1e</sup>	45±0.00 <sup>1d</sup>
5	45±0.00 <sup>1e</sup>	45±0.00 <sup>1e</sup>	45±0.00 <sup>1d</sup>

Values as mean±SD. Numbers with different letters in superscript along a column were significantly different, while values with different numeric superscript across a row were significantly different ( $p < 0.05$ ).

**Table 5.** Weight loss (g) assessment in grains infested with *Sitophilus zeamais* and exposed to *Ocimum gratissimum* powder.

Concentration g/20 g grain	Weight loss (g) in replicates			Mean weight loss (g)
	R1 (%)	R2 (%)	R3 (%)	
0.0	3.3	2.8	3.0	3.03±0.25 <sup>c</sup>
1.0	2.7	1.9	2.2	2.27±0.40 <sup>d</sup>
2.0	1.2	1.3	1.0	1.17±0.15 <sup>c</sup>
3.0	0.6	0.4	0.3	0.43±0.15 <sup>b</sup>
4.0	0.0	0.0	0.1	0.03±0.06 <sup>a</sup>
5.0	0.0	0.0	0.0	0.00±0.00 <sup>a</sup>

Values as mean±SD. Numbers with different letters in superscript along a column were significantly different ( $p < 0.05$ ).

**Table 6.** Repellency of *Sitophilus zeamais* adults after 1 h and 3 h exposure to *Ocimum gratissimum* extract.

Concentration (mg/mL)	Nc	Nt	Repellency (%)
150	9.33±0.58 <sup>b</sup>	6.33±0.58 <sup>a</sup>	19.22±1.36 <sup>c</sup>
250	12.33±1.15 <sup>a</sup>	6.67±0.58 <sup>a</sup>	29.80±0.34 <sup>b</sup>
750	9.33±1.15 <sup>b</sup>	2.00±0.00 <sup>b</sup>	69.93±2.54 <sup>a</sup>
3 h			
150	10.00±1.00 <sup>a</sup>	6.33±1.53 <sup>a</sup>	23.12±6.60 <sup>b</sup>
250	10.67±1.16 <sup>a</sup>	5.33±0.58 <sup>ab</sup>	33.33±0.00 <sup>a</sup>
750	5.67±1.16 <sup>a</sup>	2.33±0.58 <sup>b</sup>	68.14±0.56 <sup>a</sup>

Values as mean±SD. Values with different letters in superscript along a column were significantly different ( $p < 0.05$ ). Nc, number in control; Nt, number in treatment.

## Discussion

The pitfalls of synthetic pesticides have led to increased interest in the application of botanical pesticides for crop production. Pesticidal plants are considered to be non-pollutant, less toxic, and easily biodegradable in the environment. These pesticidal plants contain non-persistent active ingredients, many of which are ultraviolet labile and easily oxidized by microorganisms, posing little to no toxicity to stored products.<sup>17</sup> In recent years, experts studying pests of stored products have become increasingly interested in these botanicals.<sup>18</sup>

In the present study, the extract of *O. gratissimum* was screened for its insecticidal action against *S. zeamais*. The study indicates that the extract can be used in the control of maize weevil. Exposure of the adult *S. zeamais* to different concentrations of the ethanolic extract generated significant cumulative mortality in all cases and increased as the extract concentration increased; 35.5%, 64.4%, 95.5%, and 100% mortalities of *S. zeamais* were recorded at 30, 150, 250 and 750 mg/mL of extract, respectively. The ability of the extract to induce 100% mortality at the concentration of 750 mg/mL revealed its potential as an insecticidal agent. The mortality of *S. zeamais* also was dependent on the concentration of the extract and the duration of the exposure. This study is in agreement with the findings of Campolo *et al.*<sup>19</sup> who reported that *O. gratissimum* essential oil was able to induce 100% adult *S. zeamais* mortality at high dosages but the lower rates of the extracts were not significant compared to control. It was discovered that the dosage and time were related to the *S. zeamais* mortality recorded in this study.<sup>19</sup> Moreover, the smallest LD<sub>50</sub> values were observed for *Aster ageratoides* (Asteraceae) (LD<sub>50</sub>=27.16 µg/cm<sup>2</sup>), and *Litsea salicifolia* (Lauraceae) (LD<sub>50</sub>=0.079 µL/insect) against the closely related species of *S. zeamais*, indicating that these essential oils could be insecticidal sources for curculionid stored product pests at very lower doses.<sup>19</sup> While in this investigation, high doses of *O. gratissimum* extract (250 and 750 mg/mL) caused a high rate of death to *S. zeamais* (95.56% and 100% respectively). Atanda *et al.*<sup>20</sup> were in accordance with the present work indicating that by using *O. gratissimum* peel powder, the mean mortality of *S. zeamais* adults increased with increasing concentration and day of exposure. The percentage mortality increased from 26.7%, 45.0%, and 78.3% for a dosage of 15 g after 24, 48, and 72 hours, respectively.

Findings from the present investigation show that *O. gratissimum* is rich in phytochemicals such as alkaloids, saponin, phenol, glycoside, terpenoid, resin, and oil. Specific biologically important compounds have been identified in extracts from the plant by previous works.<sup>21,22,23</sup> The extract from the leaves of *O. gratissimum* possesses good antioxidant potential presumably due to its phytochemical constituents.<sup>24,25</sup> There is no difference regarding the phytochemical constituents between the extract used in this study and other extracts made from the same plant from other studies.<sup>8,23</sup>

The results of the repellency test showed that the plant extract repelled the weevils at a high level with the maximum percentage repellency of 69.93% occurring at the highest concentration of 750 mg/mL. Similarly, Campolo *et al.*<sup>19</sup> in their work on the potential of *O. gratissimum* peel oil as a fumigant and repellent to control maize weevil, demonstrated that the powder and essential oil of this plant have a moderate repellency effect against adult *S. zeamais*. This repellent action increases the potential practical value of *Citrus sinensis* (Sapindales: Rutaceae) in protecting grain from attack by *S. zeamais* insect pest.<sup>26</sup>

The results of this study also proved that the powdered form of *O. gratissimum* was able to protect the maize grains against direct-

feeding damage and weight loss of *S. zeamais*. This was evident in the lower percentage of weight loss recorded in the treated groups. This could be due to the mortality and antifeedant effects of the extract.<sup>19</sup>

In all the tests, the effectiveness of the ethanolic plant extract was dose-dependent with the severity of their effects increasing with an increase in concentration. Different botanical effectiveness at higher dosages to various storage pests has been reported by several authors.<sup>27,28,29</sup> In the present study, both plant extract and powdered form of *O. gratissimum* are effective in controlling *S. zeamais* at high dosages when compared with the existing studies. There is, however, little research on the synergistic pesticidal properties of *O. gratissimum* extract when mixed with other botanical or synthetic insecticides.

The exact mechanism by which *O. gratissimum* extract worked to kill *S. zeamais* was unclear, although its high levels of insecticidal phytochemicals may have caused toxicity, repellency, and gastrointestinal poisoning effects. The insects then consume the poisoned seeds and are physically abraded by their cuticles, losing bodily fluid or developing spiracles that are blocked as a result.<sup>30</sup> *O. gratissimum* extract is effective against *S. zeamais* because it disrupts the metabolic, biochemical, and physiological features of insect pests.<sup>19</sup> When exposed to different doses of *O. gratissimum*, adult insects lost significant weight because their energy content was reduced.<sup>19</sup>

## Conclusions

Botanical pesticides provide additional lines of defense against insect infestations due to the synergistic potency of these metabolites and the gradual evolution of insect pest resistance to a variety of bio-active substances that can be safe to use, distinctive in their mode of action, and simple to produce. Contrarily, there is only one active component in conventional synthetic pesticides. In the present study, both powdered and ethanolic extracts of *O. gratissimum* displayed a high level of insecticidal activity against the maize weevil, *S. zeamais*. This suggests the high possibility of using it as a toxicant, repellent and feeding deterrent in integrated pest management strategies of *S. zeamais*. Therefore, these results would add to the database of botanical products available for use as non-synthetic biopesticides.

## References

- IITA. Functional cereal in sub-Saharan Africa. Intl Institute of Tropical Agric Annual Report and Res Highlights, 2019. pp 77-8.
- Adedire CO, Akinkulore RO, Obembe OM. Efficacy of cashew kernel extracts in the control of the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae). Archives Phytopathol Plant Protect 2012;45:831-9.
- Baributsa D, Baoua IB, Baoye ON, et al. PICS bags safely store unshelled and shelled groundnuts in Niger. J Stored Prod Res 2017;72:54-8.
- Cosmas P, John CT, Agathar K, et al. Use of botanical pesticides in controlling *Sitophilus zeamais* (Maize Weevil) on stored *Zea mays* (Maize) grain. Modern Concepts & Devpt in Agron 2018;1:1-4.
- Fox CW, Stillwell RC, Wallin WG, Hitchcock LJ. Temperature and host species affect nuptial gift size in a seed-feeding beetle. Funct Ecol 2006;20:1003-11.
- Rani PU, Devanand P. Efficiency of different plant foliar

- extracts on grain protection and seed germination in maize. Res J Seed Sci 2019;4:1-14.
7. Ibrahim YJ, Abugri AD, Afun JVK. Efficacy of ethanolic leaf extract of *Chromolaena odorata* in controlling *Sitophilus zeamais* in stored maize. J Expermtl Agr Intl 2016;14:1-10.
  8. Ileke KD, Adesina JM, Obajulaye EO. Synergistic effects of two botanical entomocides as pest-protectants in maize grains. J Biol Res 2016;89:34-9.
  9. Laothawornkitkul J, Taylor JE, Paul ND, Hewitt CN. Biogenic volatile compounds in the earth system. New Phytologist 2009;183:27-51.
  10. Khaliq A, Nawas A, Ahmad MH, et al. Assessment of insecticidal potential of medicinal plant extracts for control of maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Basic Res J Agric Sci Rev 2018;3:100-4.
  11. Nwachukwu ID, Asawalam EF. Evaluation of freshly prepared juice from garlic (*Allium sativum* L.) as a biopesticide against the maize weevil, *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae). J Plant Protect Res 2014;54:132-8.
  12. Ahmed AES, Abdelbagi AO. Evaluation of the fumigant action of garlic (*Allium sativum*) aqueous extract against the cowpea seed weevil *Callosobruchus maculatus* (F.). Univ J Agr Res 2014; 2:71-82.
  13. Saghere M, Mansoor-ul-Hasan, Latif MA, Iqbal J. Evaluation of some indigenous medicinal plants as a source of toxicant, repellent and growth inhibitors against *Tribolium castaneum* (Coleoptera: Tenebrionidae). Pakistan Entomol 2011;33:87-1.
  14. Koomson CK, Oppong EK. Entomotoxicant potential of Christmas bush, *Alchornea cordifolia* (Schum. & Thonn.) leaves powder in the control of maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) infesting stored maize. J Entom Zool Stud 2018;6:2649-54.
  15. Talukder FA, Howse PE. Evaluation of *Aphanamixis polystachya* as a source of repellents, antifeedants, toxicants and protectants in storage against *Tribolium castaneum* (Herbst). J Stored Prod Res 1995;31:55-61.
  16. Finny DJ. Probit Analysis. 3rd Ed, Cambridge University Press, Cambridge, 1971.
  17. El-Wakeil N. Botanical pesticides and their mode of action. Gesunde Pflanzen 2013;65.
  18. Suleiman M, Rugumamu CP, Ibrahim ND. Insecticidal toxicity of some botanicals against *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) in stored sorghum grains in Nigeria. J Entomol Zool Studies 2018;6:1280-7.
  19. Campolo O, Giunti G, Russo A, et al. Essential oils in stored product insect pest control. J Food Quality 2018;6906105.
  20. Atanda SA, Pessu PO, Agoda S, et al. The concepts and problems of post-harvest food losses in perishable crops. African J Food Sci 2011;5:603-13.
  21. Sulistiarini D, Oyen LPA, Nguyen XD. *Ocimum gratissimum* L. In: Plant resources of South-East Asia. No. 19: Essential oils Plants. Prosea Foundation, Bogor, Indonesia 2014; pp. 140-2.
  22. Dubey NK, Tiwari TN, Mandin D, et al. Antifungal properties of *Ocimum gratissimum* essential oil (ethyl cinnamate chemotype). Fitoterapia 2000;7:567-9.
  23. Holecz FB, Ueda-Nakamura T, Filho BPD, et al. Effect of essential oil of *Ocimum gratissimum* on the trypanosomatid *Herpetomonas samuelpessoai*. Acta Protozool 2003;42:269-76.
  24. Thabrew MI, Hughes RD, McFarlane IG. Antioxidant activity of *Osbeckia aspera*. Phytotherapy Res 2015;12:288-90.
  25. Halliwell B, Gutteridge JMC. Free radicals, antioxidants and human diseases: where are we now? J Lab Clin Medicine 2016; 119:598-20.
  26. Ubi GM, Obua P. Potential use of orange (*Citrus sinensis*) and grape (*Citrus paradisi*) peels powder as bioinsecticides in the management of maize weevil (*Sitophilus zeamais*). J Environ Sci Computer Sci Engineer Tech 2019;8:265-72.
  27. Yankanchi SR, Gadache AH. Grain protectant efficacy of certain plant extracts against rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae). J Biopesticides 2010;3:511-13.
  28. Talukder D, Muslima-Khanam LA. Toxicity of four plant based products against three stored product pests. J Bio-Sci 2009;17: 149-53.
  29. Abdelgaleil SAM, Mohamed MIE, Shawir MS, Abou-Taleb HK. Chemical composition, insecticidal and biochemical effects of essential oils of different plant species from Northern Egypt on the rice weevil, *Sitophilus oryzae* L. J Pest Sci 2016; 89:219-29.
  30. Akhtar S, Hasan MUH, Sagheer M, Javed N. Anti-feedant effect of essential oils of five indigenous medicinal plants against stored grain insect pests. Pakistan J Zool 2015;47:1045-50.