





Larvicidal Activity of Ethanolic Leaf Extract of *Ocimum gratissimum* (Scent Leaf) on Larvae of *Musca domestica*

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Abstract	Article History
<p>Housefly is a domestic pest, causing serious hazard to public health, serve as mechanical vectors to pathogens causing typhoid, cholera, dysentery and others. The use of insecticides is discouraged to manage housefly therefore, alternative management strategies are inevitable. This study was aimed at determining the larvicidal activity of ethanolic leaf extracts of <i>Ocimum gratissimum</i> (scent leaf) on larvae of <i>Musca domestica</i>. Healthy leaf samples of <i>O. gratissimum</i> were collected, washed, stored, shade dried and grinded to fine powder. Whatman filter process and maceration extraction methods were used to get the extracts for the larval toxicity test. Larvae were reared according to the method described by Pavela. Qualitative and quantitative analysis of extracts were performed using standard analytical method. Phytochemical analysis of the plant extracts showed presence of saponins, flavonoid, alkaloid, tannin, steroid and protein, glycosides and anthraquinone. Four different concentrations (100, 250, 500 and 1000 ppm) of the extracts were used. Four replicates were maintained for each concentration and mortality of the larvae was recorded after 24, 48 and 72 hours. Moribund and dead larvae were recorded. The findings of this study indicated that there was 11.50 (46.00 %); 6.75 (27.00 %) and 2.75 (11.00 %) mortality after 24; 48 and 72 hrs of exposure to the extract respectively. The result showed that the mortality rate was concentration dependent as highest mortality 21 (84.00 %) was recorded in 1000 ppm ethanol after 24 hours. The Concentration-response larvicidal bioassay of the extracts have at 72 hours 2.75 (11.00%) mortality at 246.1ppm (LC50) and 6110.8ppm at (LC95). The control which contained only the larvae in distilled water and Quaker oat with no extract, showed no toxicity. This study revealed that tested botanical extracts had significant potential for controlling housefly as alternative to chemical insecticides. Putting the extracts in the form of commercially available insecticide is recommended in further investigation.</p> <p>Keywords: Mortality, larvicidal, extract, phytochemical, <i>Musca</i>, saponins</p>	<p>Received: 24 Mar 2025 Accepted: 04 Apr 2025 Published: 06 Apr 2025</p> <p>Scan QR code to view*</p>  <p>License: CC BY 4.0*</p>  <p>Open Access article.</p>
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Introduction

Musca domestica, or housefly, is a member of the Mucidae family of insects (order Diptera). Houseflies are a mechanical vector for more than 100 pathogens, including those that cause typhoid, cholera, shigellosis, bacillary dysentery, tuberculosis, anthrax, ophthalmia, poliomyelitis (Cirillo, 2016), and pyogenic cocci, making them a particular problem for hospitals and during disease outbreaks. Due to the high rate of breeding of *Musca domestica* in the environment, which act as mechanical disease vectors of diseases, constituting a public health problem, and the lack of effective chemical control, there is a need to investigate some medicinal plants that can have an activity on housefly larvae, so as to break their life cycle and serve as a means of control.

Ocimum gratissimum, sometimes known as "scent leaves," is a perennial aromatic herb that is commonly cultivated in Nigeria and is a member of the Lamiaceae family. Fungal

infections, colds, catarrh, influenza, pneumonia, and conjunctivitis are all treated with *Ocimum gratissimum*. It has inflammatory-fighting, antiseptic, antitussive, anti plasmodial, diaphoretic, and antiseptic effects (Kabir *et al.*, 2005; Ngassoum *et al.*, 2003; Ijeh *et al.*, 2005). Malaria and other diseases can be treated using the herb (Rabelo *et al.*, 2003).

Chemical insecticides are harmful to both the environment and people, some of which are very persistent and take a long time to degrade (Ekesiobi and Igbodika, (2015). Recent research by Umoh *et al.* (2020) describes the use of plants with larvicidal properties in the management of various vectors. The maximum number of eggs laid by a single female of the housefly, *Musca domestica* L (Diptera Muscidae), were found in the larval media of the control treatment, where no plant extract was applied, according to research by Sohail *et al.*, (2015) on the effects of aqueous extracts of various plants on life cycle and population build up parameters. The essential

oils of *Cinnamomum verum* and *Z. officinalis* had the least deterrent effects on *M. domestica* but had significant larvicidal and repellent effects. The effectiveness of bay essential oil against the housefly *Musca domestica* (Diptera: Muscidae) was studied by Chintalchere *et al.* (2020).

The aim of the study was to determine the larvicidal activity of aqueous and ethanolic leaf extract of *Ocimum gratissimum* (scent leaf) on larvae of *Musca domestica* (Diptera: muscidae)

The research effort and its findings were intended to add to the existing knowledge and to the baseline information about the larvicidal activities and effects of aqueous leaf extract in controlling *Musca domestica* as a mechanical vector of major parasitic intestinal diseases (Morey *et al.*, 2012).

Materials and Methods

Study Area

The Uli community is located at a latitude of 5°47'N and a longitude of 6°52'E, in Ihiala Local Government Area in the south-eastern Nigerian state of Anambra. With an average annual rainfall of 1932 millimetres and an average daily ambient temperature of 27 °C, the climate is tropical, and the vegetation has features that are primarily found in the rainforest. The wet season and the dry season are two separate climatic periods. While the dry season starts in November and lasts until March, the wet season begins in April and lasts until October.

Collection and Identification of Plant Materials

Fresh leaves of *Ocimum gratissimum* were purchased from Uli market in Ihiala local government area, Anambra State, Nigeria. They were identified by a botanist in the Department of Biological Science of Chukwuemeka Odumegwu Ojukwu University, Uli Anambra State, Nigeria.

Plant Collection and Drying

Only healthy leaves were collected and washed with sterilized water to remove surface dust and contaminants. The leaves were stored under shade and protected from light exposure in the lab for three weeks to completely dry under room temperature and humidity. After drying, they were grinded using a grinder and fully powdered samples were obtained with a greater surface area for a better extraction process.

Extraction of Plant

Cold maceration technique used by Perry *et al.*, (2019) was adopted. About 2.5 L of ethanol and 500 g of plant leaf powder were macerated for three days in a glass jar (5 L) while being stirred twice daily. The maceration was then filtered using Whatman No. 1 filter paper, and the filtrate was then dried in a rotary evaporator at 55°C while being evaporated under reduced pressure. Before being used for phytochemical screening and bioassays, the dry plant extracts were kept at -4 °C in storage. Each extract's extraction yield was calculated using the formula below:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the extract obtained (g)} \times 100}{\text{Weight of the plant powder used (g)}}$$

Phytochemical Screening in the Early Stages

Screening of qualitative phytochemicals

In order to identify the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, and reducing sugars in the chosen plant extracts, a procedure based on the earlier reports by Banso and Adeyemo (2016) and Stankovic (2011) was used.

Test for tannins

Each powdered sample was individually cooked for five minutes in a water bath with 20 cc of distilled water, then immediately filtered. A few drops (2–3) of 10% ferric chloride were added to 1 ml of cool filtrate after it had been distilled to 5 ml with distilled water, and the mixture was then checked for precipitate development and colour changes. To establish the presence of tannins, the reaction mixture was examined for a brownish green or blue-black colouring.

Test for saponins

One gram of each powdered sample was separately boiled with 10 ml of distilled water in a bottle bath for 10 mins. The mixture was filtered while hot and allowed to cool. The following tests were then carried out.

(a) **Demonstration of frothing:** 2.5 ml of filtrate was diluted to 10 ml with distilled water and shaken vigorously for 2 mins, formation of froth which is stable for some minutes indicate the presence of saponin in the filtrate.

(b) **Demonstration of emulsifying properties:** 2 drops of olive oil was added to the solution obtained from diluting 2.5 ml filtrate to 10 ml with distilled water (above), shaken vigorously for a few minutes, formation of a fairly stable emulsion indicated the presence of saponins.

Test for steroids

(a) About 0.2 g of each portion of the powdered sample was dissolved in 2 ml of chloroform. 0.2 ml of concentrated H₂SO₄ was carefully added to form a layer. A reddish-brown colour at the interface between the layer indicates the deoxy-sugar characteristics of cadenolides which indicates the presence of steroid.

(b) 2 ml of acetic anhydride was added to 0.5 g ethanolic extract of the sample with 2 ml of concentrated H₂SO₄. The colour change from violet to blue or green in some samples is an indication of the presence of steroids.

Test for alkaloids

One gram of each powdered sample was separately boiled with water and acidified with 5 ml of 1 % HCl on a steam bath. The solution obtained was filtered and 2 ml of the filtrate was treated with few drops of the following reagents separately in different test tubes and observed.

(a) **Mayer's Test:** Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a creamy white precipitate indicated the presence of alkaloids in the extract.

(b) **Wagner's Test:** Filtrates were treated with Wagner's reagent (Iodine in potassium iodide). Formation of brown or

reddish- brown precipitate was regarded as evidence for the presence of alkaloids in the extract.

(c) Dragendorff's Test: Filtrates were treated with Dragendorff's reagent (solution of potassium bismuth iodide), Formation of orange-brown precipitate was regarded as evidence for the presence of alkaloids in the extract.

(d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution), Formation of yellow coloured precipitate was regarded as evidence for the presence of alkaloids in the extract.

Test for cardiac glycosides

a) Five (5) ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxy sugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

b) Ten ml of 50 % H₂SO₄ was added to 1 ml of the filtrate in separate test tubes and the mixtures heated for 15 mins followed by addition of 10 ml of Fehling's solution and boiled. A brick red precipitate indicated presence of glycosides.

Test for free anthraquinones

Five (5) ml of chloroform was added to 0.5 g of the powdered dry seeds of each sample. The resulting mixture was shaken for 5 mins after which it was filtered. The filtrate was then shaken with equal volume of 10 % ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones.

Test for combined anthraquinones

One gram of powdered sample of each sample was boiled with 2 ml of 10 % hydrochloric acid for 5 mins. The mixture was filtered while hot and filtrate was allowed to cool. The cooled filtrate was partitioned against equal volume of chloroform and the chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10 % ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for any colour change; delicate rose pink colour showed the presence of an anthraquinone.

Test for flavonoids

(a) One gram of powdered sample of each sample was separately boiled in 20 ml of water and then filtered. 5 ml of dilute ammonia solution was added to a portion of the filtrate, followed by the addition of concentrated H₂SO₄. A yellow coloration was indicative of the presence of flavonoids.

(b) One gram of the powdered dried seeds of each sample was boiled with 10 ml of distilled water for 5 minutes and filtered while hot. Few drops of 20 % sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

Test for terpenoids

Five (5) ml of each extract was mixed in 2 ml of chloroform. 3 ml of concentrated H₂SO₄ was then added to form a layer. A reddish-brown precipitate colouration at the interface formed indicated the presence of terpenoids.

Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1 % aqueous hydrochloric acid was taken as evidence for the phlobatannins.

Test for carotenoids

One gram of each sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85 % sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

Phenolics

Half gram (0.5 g) of the powdered dried seeds of each sample was boiled with 10 ml of distilled water for 5 mins and filtered while hot. Then 1ml of ferric chloride solution was added. Formation of blue-black or brown colouration indicated the presence of phenol.

Test for reducing sugars

To about 1 g of each sample in the test tube was added 10 ml distilled water and the mixture boiled for 5 mins. The mixture was filtered while hot and the cooled; 5 ml of mixture of equal volume of Fehling's solution (A and B) was added to 2 ml of the filtrate in a test tube and the resultant mixture was boiled for 2 mins. Appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugar.

Test for phenol

Ferric chloride test: 2 drops of neutral ferric chloride solution was added to 1ml of diluted aqueous solution of the test sample. A greenish purple color indicates the presence of phenolic compounds.

Quantitative Phytochemical

Determination of Alkaloids (using Harbone Method, 2010)

Five (5) grams of the sample was weighed into a 250 ml beaker and 20 ml of 20 % acetic acid in ethanol was added and covered and allowed to stand for 4 hours at room temperature. This was filtered with filter paper and the filtrate was heated to one quarter of the original volume. 5 ml of concentrated ammonium hydroxide was added drop wise until the precipitate was complete. Then, filter with the pre weighed filter paper. The residue on the filter paper is the alkaloid, which is dried in the oven at 80 °C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed. Then was calculated using the formula.

$$\% \text{ weight of alkaloid} = \frac{(\text{Weight of filter paper with residue}) - (\text{Weight of filter paper}) \times 100}{\text{weight of the sample analyzed}}$$

Determination of Tannins by Titration (according to Person method, 2004)

Ten (10) gram of sample was weighed in a conical flask and 100ml of n hexane or petroleum ether was added and covered

for 24 hours. The sample was then filtered and allowed to stand for 15 minutes for the solvent to evaporate. It was then re-extracted by soaking 100ml of 1% acetic acid in ethanol for 4 hours. The sample was then filtered and the filtrate collected. 25 ml of ammonium hydroxide were added to the filtrate to precipitate the alkaloids. The alkaloid was heated with electric hot plate to remove some of ammonium hydroxide still in solution. The remaining volume was measured and 5 ml of this was taken and 20 ml of ethanol was added to it. It was titrated with 0.1M NaOH using 1ml of phenolphthalein as indicator until a pink end point is reached. Tannin content was calculated in percentage ($C1V1 = C2V2$) molarity

Determination of Saponins (according to the method of AOAC Method, 2005)

10grams of the ground sample was weighed into a thimble and transferred into the soxhlet extractor chamber fitted with a condenser and flask. 250 ml of methanol was put into the flask. Extraction continued for 1hr, The saponin was exhaustively extracted by heating the flask on a heating mantle. After the thimble and its content was removed and the methanol recovered leaving the saponin and little quantity of methanol in the flask. It was then taken to an oven and kept at slanting position at a temperature of 70°C to evaporate the residual methanol. The flask and content was weighed and the difference between the flask plus saponin and flask alone was the mass of saponin extracted. The saponin content was weighed and calculated in percentage.

Calculation:

$$\% \text{ Saponin} = \frac{(\text{Weight of beaker} + \text{sample}) - (\text{Weight of empty beaker}) \times 100}{\text{weight of the sample analyzed}}$$

Determination of Flavonoids (according to the method of AOAC, 2005)

Ten (10) g of the plant sample was put in a beaker with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through what man filter paper. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Calculation:

$$\% \text{ Flavonoids} = \frac{(\text{Weight of crucible} + \text{residue}) - (\text{Weight of crucible}) \times 100}{\text{weight of the sample analyzed}}$$

Determination of phenolic (according to the method of AOAC Method, 2005)

Twenty (20) g of the sample was weighed into 250 ml conical flasks. The sample was soaked in 100 ml of 2 % concentrated HCl for 3 hours, the sample was then filtered. 50 ml of the filtrate was placed in 250 ml beaker and 100 ml distilled water added to the sample. 10ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution I.

$$\% \text{ Phenolic acid} = \frac{\text{Titre Value} \times 0.00195 \times 1.19 \times 100}{\text{sample analyzed}}$$

Gas chromatography/mass spectroscopy analysis conditions

The crude ethanolic extract was dissolved in ethanol and vortex mixed for 1 minutes and then centrifuged at 3000 rpm for 10 minutes. 1µL of the supernatant was injected into the GC and run for 30 minutes. The volatile compounds in the sample were analyzed by Gas Chromatography Mass Spectrometer (GC/MS) on an Agilent 7890B Gas Chromatography (GC) directly coupled to the Mass Spectrometer system (MS) of an Agilent 5977A. The ion source was set at 230 °C and the ionization voltage at 70 eV. The GC oven temperature was programmed from 80 °C, with an increase of 15 °C/min, to 200 °C, then 5 °C/min to 280 °C, ending with a 5 min isothermal at 28.

Rearing of Housefly Colony

The culture of housefly from ogiri (fermented castor oil seed) samples was maintained at a temperature of 28 ± 2 °C and 60–70 % relative humidity (RH) in plastic jars (35×15 cm), covered with cheese cloth. Hatched larvae were transferred to jars containing larval media (yeast, dry milk powder, wheat bran and water according to the method described by Pavela (2018) and checked daily until the pupal stage. Pupae moved into plastic jars containing wood dust for the emergence of adults. The obtained larvae were used in the larvicidal bioassays.

Larvicidal Efficacy

The larvicidal bioassays were carried out by following the methods of Busvine (2011) and Palacios *et al.*, (2019), respectively, with few modifications. The water and ethanol extracts were dissolved in 0.5 mL of Tween-80 and different concentrations of 1000, 500, 250 and 100 PPM for plant extracts in the plastic cups. Twenty five larvae were transferred into the each test solutions prepared.

Four (4) replicates were maintained for each concentration and mortality of the larvae was recorded after 24, 48 and 72 h for plant extracts. Dead larvae were detected when appendages did not move when probed with needle. The mortality rate of the treated larvae was calculated after 24 h of treatment by using below mentioned formula.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae} \times 100}{\text{number of larvae tested}}$$

$$\text{Corrected mortality \%} = \frac{\text{Observed mortality \%} - \text{Control mortality \%} \times 100}{100 - \text{Control mortality \%}}$$

LC₅₀ and LC₉₅ for Larvicidal Activity

The dose-response tests were performed in the range of 10–90 % mortality. Twenty five larvae were separated per dose (in triplicate), requiring a minimum of 8 doses to perform the curve. The larvae were placed in 500 ml cups containing 249 ml of dechlorinated water, along with 1 ml of the extract in the desired concentration, diluted in ethanol. For the control group, Twenty five larvae were used in 250 mL of dechlorinated water. The concentrations of extracts used for assaying were 1000, 500, 250 and 100 PPM, respectively. Mortality was recorded every 10 min during the first hour and 24 h after the start of the test, as recommended by the World Health Organization (1981). Larvae that did not spontaneously move, even if subjected to mechanical stimulation, were

considered dead. The LC calculation was performed using the Polo Plus program (see item on statistical analysis).

3.11 Statistical Analysis

The data were organized in spreadsheets using Microsoft Excel software (version, 2007). Lethal concentrations (LC) 50 %, 95 % and slope were obtained through Probit analysis with the aid of the Polo Plus software (Raymond, 1985). Significant differences in the LC50 and LC90 values were based on non-overlap of 95% confidence intervals (Hematpoor *et al.*, 2017; Wang *et al.*, 2019). The analysis of variance (Anova) and the Tukey test, at a 95 % significant level were carried out.

Results

Qualitative Phytochemical

The qualitative results are presented in Table 1. The ethanol extract contained alkaloid, glycosides, saponins, phenol, tannin, flavonoid, protein, anthraquinones and steroid compounds while terpenoids and reducing sugars were absent.

Table 1: Qualitative Phytochemical Profile of *Ocimum gratissimum* Ethanolic

Phytochemicals	Ethanol extract
Saponin	+++
Flavonoid	+
Alkaloid	+++
Tannin	++
Steroids	+
Terpenoids	-
Glycosides	+
Protein	+
Reducing sugar	-
Anthraquinones	+

Key: +++ = Present in high concentration; ++ = Present in moderate concentration; + = Slightly or sparingly present; - = Absent.

Quantitative Phytochemical

The quantitative phytochemical analysis of the scent leaf powder presented in Table 2 showed that alkaloid recorded highest concentration (26.80%), saponin (26.73mg/100g), phenol (24.36mg/100g), tannin (13.77mg/100g), steroid (5.40mg/100g) flavonoids (3.05mg/100g) and terpenoids (2.06mg/100g).

Table 2: Quantitative Phytochemical Profile of *Ocimum gratissimum* Ethanolic

Phytochemicals	Mean Value (mg/100g)
Alkaloid	26.8600±0.643 ^a
Flavonoid	3.0550±0.077 ^e
Phenol	24.3667±18.520 ^b
Saponin	26.7367±5.200 ^a
Tannin	13.7733±9.24 ^c
Steroid	5.4000±3.810 ^d
Terpenoid	2.0633±0.590 ^e

*Values are mean scores ± Standard deviation of three (3) replicates

*Data in the same column bearing different superscript differ significantly ($p < 0.05$).

Gas Chromatography-Mass Spectrometric analysis

The result from the Gas Chromatography-Mass Spectra is shown in Table 3. The result revealed eleven peaks indicating eleven different phyto compounds were present.

Table 3: Gas Chromatography-Mass Spectrometric Analysis of *Scent* leaf

S/N	Retention Time(RT)	Molecular Weight	Name of Compound
1.	14.242	206	3, 5-bis 1, 1 dimethylethyl (Phenol)
2.	17.808	228	Tetradecanoic acid
3.	18.417	240	1, 2-epoxyhexadecane(Oxirane)
4.	19.442	270	Methylhexadecanoate (Palmitic acid)
5.	20.142	242	Hexadecanoic acid (Eicosanoic acid)
6.	21.800	294	9, 12-octadecadienoic acid (Linoleic acid)
7.	22.133	296	3, 7-dimethyldodecan-1-ol (Phytol)
8.	22.850	282	6-octadecenoic acid(Oleic acid)
9.	23.217	284	octadecanoic acid(Stearic acid)
10.	28.117	386	Cholest-5, 3-ol, 5-acetate (Cholestane)
11.	28.350	390	1,2-Benzenedicarboxylic acid (Di-n-octyl phthalate)

Mortality rate of the larvae of *Musca domestica* at different concentrations of the plant extracts.

The effects of the ethanolic scent leaf extract against the larvae of *Musca domestica* at after 24, 48, and 72, hours of exposure are shown in Table 4 below. There was 11.50 (46.00%) mortality of the larvae within 24hrs of exposure in ethanolic extract. Also, 6.75 (27.00%) mortality after 48hrs of exposure in ethanolic extract. Finally, 2.75 (11.00%) mortality after 72hrs of exposure in ethanolic extract. Result also showed that 4.00(16.00%) of the larvae were still alive after 72hrs of exposure in ethanol. The result showed that the mortality rate was dose dependents as the highest mortality 21 (84.00%) was observed in 1000ppm ethanol after 24hrs, SE (sample E) containing 50mg/ml concentration of the extracts. SD (Sample D) containing 37.5mg/ml concentration of the extract produced 100% mortality against the larvae at 48 hours of exposure and 4 (16.00%) after 48hrs indicating 100% mortality. The control which contained only the larvae in distilled water and Quaker oat with no extract, showed no toxicity. The percentage mortality rate of the larvae at different concentrations is also shown in Table 4.

Mortality rate of the larvae of *Musca domestica* at different concentrations of the plant extracts

The effects of the leaf extract against the larva of *Musca domestica* at after 24, 48, and 72, hours of exposure are shown in Table 5 below. There was 11.50 (46.00%) mortality of the larvae within 24hrs of exposure in ethanolic extract. Also, 6.75 (27.00%) mortality after 48hrs of exposure in ethanolic extract. Finally, 2.75 (11.00%) mortality after 72hrs of exposure in ethanolic extract. The result also shown that 4.00(16.00%) of the larvae were still alive after 72hrs of exposure in ethanol.

The result showed that the mortality rate is dose dependents as (16.00%) after 48hrs indicating 100% mortality. The control highest mortality 21 (84.00%) was observed in 1000ppm ethanol after 24hrs, SE (sample E) containing 50mg/ml oat with no extract, showed no toxicity. The percentage concentration of the extracts. SD (Sample D) containing 37.5mg/ml concentration of the extract produced 100% mortality against the larvae at 48 hours of exposure and 4

Table 4: Mortality rate of the larvae of *Musca domestica* at different concentrations of leaf extracts of *Ocimum grattissimum*

Scent leaf	No of larva of <i>Musca domestica</i> used	Mortality rate (%) at 24hrs	Mortality rate (%) at 48 hrs	Mortality rate (%) at 72hrs	No (%) of alive larvae
Ethanolic extract					
100ppm	25	5 (20.00%)	8 (32.00%)	5 (20.00%)	7 (28.00%)
250 ppm	25	7(28.00%)	10(40.00%)	3 (12.00%)	5 (20.00%)
500 ppm	25	13 (52.00%)	5 (20.00%)	3 (12.00%)	4 (16.00%)
1000 ppm	25	21 (84.00%)	4 (16.00%)	0 (0.00%)	00.00%)
Mean	25.00	11.50 (46.00%)	6.75 (27.00%)	2.75 (11.00%)	4.00(16.00%)
Control					
0.00mg/L	25	0	0	0	25.00 (100%)

Table 5: Concentration response larvicidal bioassay of scent leaf extracts against larva of *Musca domestica*

Scent leaf	Time	% Mortality	LC ₅₀ (ppm)	LC ₉₅ (ppm)
	24	11.50 (46.00%)	159.1	3950.1
Ethanol	48	6.75 (27.00%)	229.9	5707.6
	72	2.75 (11.00%)	246.1	6110.8

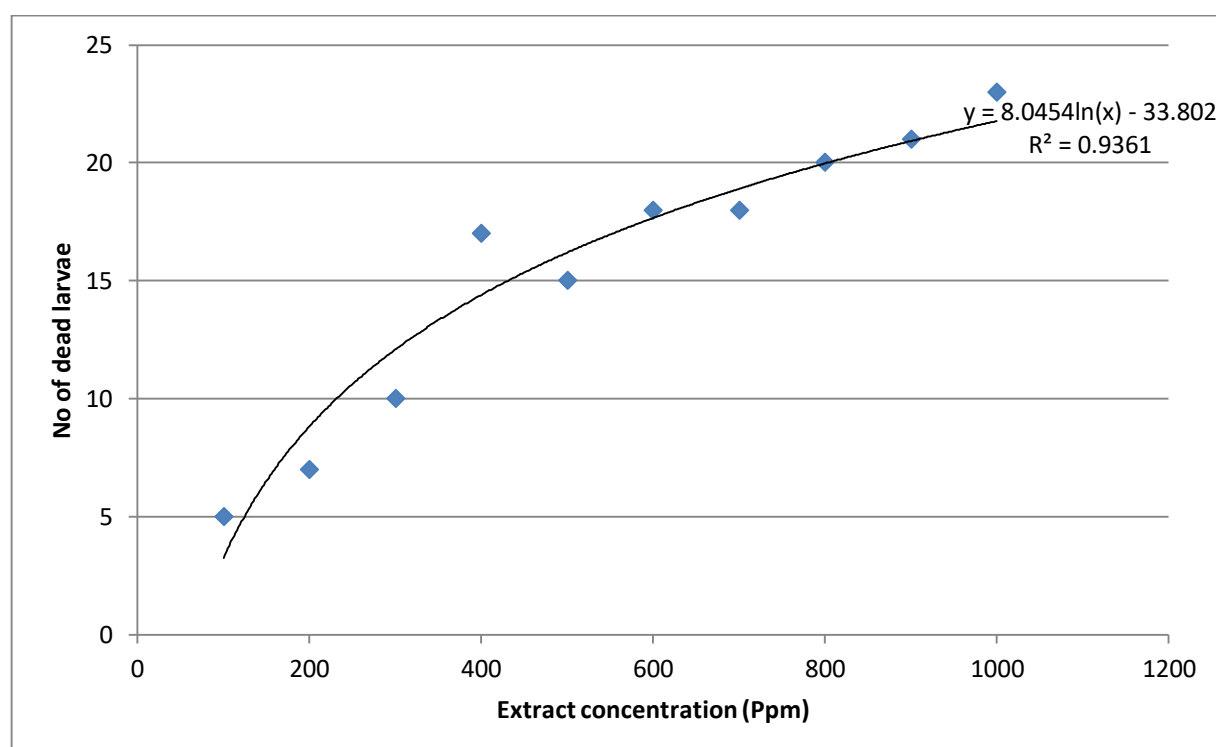


Figure 1: Mortality rate of the larva of ethanolic scent leaf extracts against larvae of *Musca domestica*

The analysis of the LC₅₀ and LC₉₀ of all the concentrations against larva of *Musca domestica* (Table 5) indicated agree with previous studies of plant extracts and highlights their potential of acting as efficient larvicides on housefly that are resistant to different types of insecticides, whose use has already led to the development of resistant populations in

Nigeria (for e.g.: Temephos (Valle *et al.*, 2019) . pyrethroids (Costa *et al.* 2020).

Discussion

The study has revealed that ethanol extracts of scent leaf contained phenols, glycosides, alkaloid, flavonoid, steroids, and saponins. This is in line with the study by Ifemeje *et al.* (2014). The larvicidal properties of this plant could be attributed to the presence of these bioactive compounds. The result showed that the ethanolic extract of scent leaf contained higher quantity of the five (5) out of the seven (7) parameters determined in the study. Several available literature reported the presence of tannins, saponin, flavonoids, steroids, phenols and cardiac glycosides both ethanol extracts of scent leaf (Usman and Osuji, 2007; Azando *et al.*, 2011; Ifemeje *et al.*, 2014).

In this research, the GC-MS analysis revealed the presence of eleven compounds from the ethanol leaf extract fraction of scent leaf while the quantitative phytochemical screening showed that alkaloid have highest concentration (26.80%), saponin (26.73mg/100g), phenol (24.36mg/100g), tannin (13.77mg/100g), steroid (5.40mg/100g) flavonoids (3.05mg/100g) and terpenoids (2.06mg/100g). Alkaloids are beneficial chemicals to plants serving as repellent to predators and parasites (Ojo *et al.*, 2013). This probably endows these group of agents its antimicrobial activity. Several alkaloid containing medicinal plants are reported to have been used by the early man as pain relievers, as recreational stimulants or in religious ceremonies to enter a psychological state to achieve communication with ancestors or God (Oliver, 1980).

Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Okwu, 2011; Abiodun *et al.*, 2025; Ekesiobi *et al.*, 2024). Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation (Okwu, 2011). According to Ezech (2015), saponin have anti-hypercholesterol, anti-inflammatory, cardiac depressant property and also appears to kill or inhibit cancer cells without killing the normal cells in the process (Okwu, 2011).

The presence of phenol in the scent leaf showed they possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Egba *et al.*, 2012).

Presence of tannins as shown in the result suggests the ability of this plant to play a major role as antidiarrhoeic and antihemorrhagic agent (Asquith and Butler, 1986; Ekesiobi *et al.*, 2017). Steroidal compounds are of importance in pharmaceuticals because of their relationship with compounds used as sex hormones (Okwu, 2011). The presence of tannins in coconut oil and water might have accounted for its sharp

taste and have been reported to hasten the healing of wounds and inflamed mucous membranes.

Cardiac glycosides are known to lower the blood pressure according to many reports and functional for heart diseases. The results obtained in this study thus suggest that spices are an increasingly valuable reservoir of bioactive compounds of potential substantial socioeconomic importance (Egba *et al.*, 2012).

The results in Table 4 showed that extracts exhibited good larvicidal activities on the larva of *Musca domestica* with varying susceptibility. The varying susceptibility observed here was in line with reports from previous findings that various housefly species showed differential susceptibility to different plant extracts Pathak *et al.*, (2020). Sample with 1000ppm ethanol concentration exhibited the highest mortality rate after 24hr of exposure by eliminating all the larvae at once. Average mortality indicated that the extracts exhibited significant mortality rate on the targeted larva of *Musca domestica*. The bioassay showed that the toxic effect of the extracts were proportional to the concentrations with the highest concentration being the most effective. This is in support of the findings of Okigbo *et al.* (2020).

Studies focusing on the investigation of extracts from plants from the perspective of discovering new ovicides, larvicides, adulticides and repellents have been an important strategy for controlling agricultural pests, vectors of medical-veterinary importance or urban viruses. Housefly [*Musca domestica* (Diptera: Muscidae)] is a major medical and veterinary insect that causes irritation, spoil foods and act as a vector for >100 species of pathogens. It causes serious threat to human health and livestock (Morey and Khandagle, 2012). Housefly is considered as cosmopolitan pest and distributed all over the world. However, as the World Health Organization does not establish criteria to recognize larvicidal activity, in the present study, we choose the level of 90–100% of larvicidal activity for 5 different concentrations as previously suggested. The analysis of the LC₅₀ and LC₉₀ of all the concentrations against larva of *Musca domestica* (Table 4.5) indicated comparable LCs values among them, indicating activity of scent leaf regardless of insect resistance. The results agree with previous studies of plant extracts and highlights their potential of acting as efficient larvicides on housefly that are resistant to different types of insecticides, whose use has already led to the development of resistant populations in Nigeria (for e.g.: Temephos Valle *et al.*, 2019) pyrethroids Costa *et al.*, 2020).

Conclusion

The phytochemical analysis of the plant extracts revealed the presence of several bioactive secondary metabolites that singly or in combinations may be responsible for the larval toxicity. The findings of the present investigation indicated that there was 11.50 (46.00%) mortality of the larva of *Musca domestica* within 24hrs of exposure in ethanolic extract 6.75 (27.00%) 48hrs in ethanolic extract and 2.75 (11.00%) mortality after

72hrs in ethanolic extract. The result showed that the mortality rate is concentration dependents as highest mortality 21(84.00%) was observed in 1000ppm ethanol after 24hrs, 100% mortality against the larvae at 48 hours of exposure and 4 (16.00%) after 48hrs indicating 100% mortality. The control which contained only the larvae in distilled water and Quaker oat with no extract, showed no toxicity. The results suggest the promising role of the scent leaf ethanol and aqueous extracts as an alternative in controlling larva of *Musca domestica* of susceptible and insecticide resistant strains. This study has proven that the extracts of *Ocimum gratissimum* possess chemical compounds that are active against larva of *Musca domestica* and is believed to be a promising strategy in controlling insect population in our society. Therefore these plant extracts could be formulated into larvicides to prevent insect borne diseases.

Conflict of interest: The authors declare no conflict of interest.

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