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## Phytochemical and insecticidal properties of some botanical extracts against the lesser grain borer, *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae)

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**Abstract**

Experiments were conducted in postgraduate laboratory of the Department of Biology of Umaru Musa Yar'adua University, Katsina (UMYUK) Nigeria, to analyze phytochemical properties and assess insecticidal effects of acetic leaf extracts of *Euphorbia balsamifera*, *Lawsonia inermis* and *Mitracarpus hirtus* against *Rhyzopertha dominica* F. To analyze the phytochemicals of the test plants, preliminary tests were conducted to determine secondary metabolites while Gas Chromatography Mass Spectrum (GC-MS) was carried out to identify various active compounds present in the botanical extracts. Effects of the treatments on adult mortalities against *R. dominica*, adult emergence and grain weight losses were also determined. To determine percentage mortalities of *R. dominica* at 7 days after treatment (DAT), acetic extracts of the botanicals at the concentrations of 6.25, 12.50, 25.00, 50.00, 100.00 mgml<sup>-1</sup> and permethrin powder at 11.20 mg/20 g were applied to 20 g of sorghum grains in different plastic bottles. None of the extracts or permethrin powder was added to the control. The experiment was arranged in a completely randomized design (CRD) and replicated three times. Findings of the study revealed the presence of alkaloids, carbohydrate, phytosterols, phenolic compounds, flavonoids, saponins, tannins and cardiac glycosides in the botanical extracts. The plant extracts resulted in adult mortality of *R. dominica* which ranged from 16.67 to 63.33% within 7 days after treatment (DAT). No adult emergence was observed in all the treated grains. Also, grain weight losses of the treated sorghum varied from 3.92 to 6.63%. Acetic extracts of the botanicals could therefore be utilized to reduce *R. dominica* infestations in stored sorghum.

**Keywords:** botanicals, phytochemicals, GC-MS analysis, LC<sub>50</sub>, mortality, *R. dominica*, toxicity

**Introduction**

Sorghum is the primary food crop in virtually all parts of northern Nigeria <sup>[1]</sup>. Sorghum is also used as animal feed, bio-fuel and extensively in brewing and the industrial demand for sorghum by beer manufactures is rising steadily in step with rising demand for their products <sup>[1]</sup>.

Storage is an interim phase during transit of agricultural produce from producers to consumers. The main purpose of storage for small scale farmers in Africa is to ensure household supplies and seed for planting <sup>[2]</sup>.

*Rhyzopertha dominica* is a major cause of damage pest of wheat and rice around the world <sup>[3, 4]</sup>. Both larvae and adult produce frass and cause weight losses by feeding on grains. *R. dominica* infestation can reduce rice to dust <sup>[5]</sup>. There are three aspect of impact of *R. dominica* infestation which are loss in the quality of stored grain, loss in the quality of stored seeds <sup>[6]</sup> and the cost to prevent or control infestation <sup>[7]</sup> on wheat and rice. Larvae of *R. dominica* consume both germ and endosperm during their development in grain and thus provide more frass than *Cryptolestes ferrugineus* and *Sitophilus granarius* <sup>[8]</sup>.

In order to manage these infestations, chemical insecticides are widely used by farmers due to their rapid action. This leads to a search for alternative and environmental-friendly methods such as application of botanicals to control stored products insects pests. It is against this background that leaf extracts of *Euphorbia balsamifera* Aiton, *Lawsonia inermis* L. and *Mitracarpus hirtus* (L.) DC. were selected for their phytochemical screening as well as assessing their influence in reducing *R. dominica* infestations in stored sorghum.

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## Materials and Methods

### Collection, Identification and Preparation of Plant Materials

Leaves of *Euphorbia balsamifera* Aiton, *Lawsonia inermis* L. and *Mitracarpus hirtus* L., were collected from the bushes around Dabaibayawa village and identified at the Herbarium of the Department of Biology, Umaru Musa Yar'adua University, Katsina (UMYUK). All the plant leaves were shade dried in a well ventilated area in the Biology Laboratory 3 for 14 days before grinding and sieving them into fine powders through a laboratory sieve with 80 µm aperture size. The powders were placed in well labeled black polythene bags separately and kept in laboratory shelf at room temperature prior to use. The conventional insecticide (permethrin 0.6%) was purchased from insecticide vendor.

### Preparation of Samples for Phytochemical Screening

Twenty grams of each of the plant powders was soaked into 100 ml of each solvent (methanol, acetone and ethanol) for 48 h and filtered using Whatman No. 1 filter paper. The filtrate was evaporated using water bath until dry extracts were obtained which were used for preliminary phytochemical screening to detect the presence of secondary metabolites such as saponins, tannins, flavonoids, carbohydrates, phenolic compounds, terpanoids and cardiac glycosides [9].

### Preliminary Phytochemical Screening of Leaf Extracts

The different botanical extracts were subjected to preliminary screening using standard procedures for the detection of alkaloids, saponins, tannins, phenolic compounds, flavonoids, carbohydrates, phytosterols, terpanoids and cardiac glycosides as described hereunder.

#### Test for alkaloids (Mayer's test)

Three ml of extract were introduced into three different test tubes and then acidified with 1 ml hydrochloric acid. Half g of the extract was diluted in 10 ml of 1% aqueous hydrochloric acid and to each of these solutions, 4 drops of mayer, wadner and dragendroff reagents were separately added. A creamy white (mayer), reddish brown (wadner) and orange brown (dragendroff) precipitates indicated the presence of alkaloids [10].

#### Test for carbohydrates (Molish's test)

Molisch's test was adopted for carbohydrates test. Two g of the extracts were dissolved in 5 ml of distilled water and filtered. Two drops of alcoholic naphthol solution were added to 2 ml of the filtrate and 1 ml of concentrated sulphuric acid was added slowly along the side of the test tube. A violent ring at the junction of two liquid confirmed the presence of carbohydrates [11].

#### Test for phytosterols (Lieberman-Bruchard's test)

Five ml of extract were treated with chloroform and the filtrate of that is treated with few drops of acetic anhydride. Then the solution was boiled and allowed to be cold, the formation of brown ring at the junction of the test tube indicated the presence of phytosterols [11].

#### Test for terpenoids (Salkowski test)

Each of the plant extracts was taken in a test tube and a few pieces of tin plus 3 drops of thionyl chloride were added to it. A violet colour indicated the presence of terpenoid [12].

#### Test for phenolic compounds (Ferric chloride test)

Two ml of acetonetic leaf extract of the test botanicals were treated with a few drops of ferric chloride solution and the formation of bluish black colour proved the presence of phenols [11].

#### Test for saponins (Foam test)

Two ml of the botanical extracts were vigorously shaken in a test tube for 2 minutes and observed for a stable persistent froth. Frothing in the test extract indicated the presence of saponins [10].

#### Test for tannins (Ferric chloride test)

Two drops of 5% ferric chloride were added to 1 ml of the test extract. A dirty green precipitate indicated the presence of tannins [10].

#### Test for flavonoids (Alkaline reagent test)

Ten mg of magnesium powder was added to 3 ml of the test extract followed by 5 drops of concentrated hydrochloric acid. A red colouration indicated the presence of flavonoids [10].

#### Test for cardiac glycosides (Keller-kilani test)

A mixture of 10 ml of 5% sulfuric acid and 1 ml of the test extract in a test tube was heated in boiling water for 15 minutes after which 10 ml of Fehling's solution was added to the mixture and boiled for another 10 minutes. A brick-red precipitate indicated the presence of glycosides in the extract [10].

### Gas Chromatography Mass Spectrum (GC-MS) Analysis of the Test Botanicals

Five ml of acetonetic extract of each of the botanicals were taken to the Ahmadu Bello University, Zaria, Nigeria for GC-MS analysis. The samples were analyzed using Thermo GC-Trace Ultra (version 5.0) Gas Chromatography Interface to Thermo MS DSQ II Mass Spectrometer Instrument employing the following conditions: capillary standard non polar column (30 x 0.25 mm x 0.25 µm) and helium was used as carrier gas at a constant flow rate of 1 mlmin<sup>-1</sup>. The oven temperature was kept at 70 °C and was programmed to reach 260 °C at a rate of 6 °C min<sup>-1</sup> and the mass range was m/z 50-650. The total running time was completed in 1 hour. The chromatogram obtained from gas chromatography was analyzed in mass spectrometry by comparing the mass spectra of unknown peaks with those stored in Willey 9 GC-MS library.

### Preparation of Extract for Toxicity Test

One hundred grams of each of the plant powders were dissolved in 400 ml of acetone in conical flasks in which the mouth were properly covered with cotton and kept in the laboratory at room temperature for 48 hrs. The mixture was first separated using muslin cloth and then filtered with Whatman No. 1 filter paper using vacuum pump (Dymax 14). The filtrates were placed in a water bath for the solvent to evaporate leaving the solid crude extracts.

### Rearing of *Rhyzopertha dominica*

Whole grains of sorghum local variety called "Kaura" from Katsina central market were disinfected in an oven at 60 °C for 1 hour [13] before using them as substrate for insect rearing. Twenty five pairs of adults of *R. dominica* obtained from Institute of Agricultural Research, Zaria (IAR), Nigeria

were introduced into each of rearing bottles (500 ml capacity) containing 400 g of the disinfested sorghum. The bottles were covered with muslin cloth, secured with rubber bands and placed in an incubator at  $30 \pm 2$  °C and  $70 \pm 5\%$  r.h for oviposition. The insects were removed after seven days leaving the grains only for adult emergence. The emerged  $F_1$  individuals were sieved from the grains and used for the experiments [14].

#### Determination of Adult Mortality of *Rhyzopertha dominica*

Twenty grams of disinfested sorghum grains were weighed into 15 plastic bottles (250 ml capacity). The plant extracts were applied at the rate of 6.25, 12.50, 25.00, 50.00 and 100.00 mgml<sup>-1</sup> to 20 g of sorghum grains in each of the bottles. Another bottle contained grains treated with 11.20 g of permethrin, while no extract was added to the control. The treatments containing extracts and permethrin were thoroughly mixed with the sorghum grains using glass rod to ensure thorough admixture and allowed the extracts to evaporate for 2 hrs. Thereafter, ten newly emerged adults of *R. dominica* were introduced into each of the treated and untreated grains. Mouths of the bottles were covered with muslin cloth, secured with rubber bands and then kept in an incubator at  $30 \pm 2$ °C and  $70 \pm 5\%$  R.H. and arranged in a completely randomized design (CRD) with three replications. Dead insects were removed, counted and recorded daily for 7 days. This was followed by removing all the remaining insects (dead and alive) and leaving the grains only. The plastic bottles containing the grains were then kept under the aforementioned environmental conditions until emergence of new individuals of the insect [15].

#### Examination of Adult Emergence of *Rhyzopertha dominica*

Adult emergence of *R. dominica* from treated and untreated grains was examined immediately after determination of adult mortality. This was done by sieving all the remaining insects from the treated and untreated sorghum grains and the containers were left undisturbed in the laboratory until the emergence of new individuals. The number of emerged insects was recorded daily for 14 days.

#### Assessment of Weight Losses in Treated Sorghum Grains Infested by *Rhyzopertha dominica*

The level of damage caused by *R. dominica* on the sorghum grains were studied from the same set up. Sorghum grains from each of the containers were sieved, weighed and recorded at 49 days after introduction of the insects. Grain weight loss was determined by using the following formula [16].

$$\text{Grain Weight Loss (\%)} = \frac{\text{Initial Weight (g)} - \text{Final Weight (g)}}{\text{Initial Weight (g)}} \times 100$$

#### Statistical Analysis

Data were analyzed using GraphPad Insta 3. Normality test was done using KS test and all data were found to be non parametric. Therefore, Kruskal-Wallis test was employed to test if there was any significant difference in adult mortality among the insects and weight losses of treated sorghum grains infested by *R. dominica*. Significantly different means were separated using Dunn's multiple test for non parametric data. All analyses were performed at  $p < 0.05$ .

## Results

### Secondary Metabolites Present in Acetonic Leaf Extracts of Three Botanicals

Preliminary phytochemical screening of *E. balsamifera*, *L. inermis* and *M. hirtus* leaves revealed the presence of 9 secondary metabolites namely; alkaloids, carbohydrates, phytosterols, phenolic compounds, flavonoids, saponins, tannins and cardiac glycosides (Table 1). Alkaloids, carbohydrates, phenolic compounds and flavonoids were presence in methanolic leaf extracts of all the botanicals. Phytosterols and cardiac glycosides were absent in *E. balsamifera*. Likewise saponins and terpenoids were not found in *L. inermis*. Acetonic extracts of all the test botanicals contained carbohydrates, phytosterols, saponins, tannins flavonoids, terpenoids, and cardiac glycosides, whereas no alkaloids and phenolic compounds were found in *M. hirtus*. The results further showed that ethanolic extracts contained phytosterols, saponins, phenolic compound, flavonoids, terpenoids and cardiac glycosides in all the botanicals. However, alkaloids and carbohydrates were absent in *E. balsamifera*, while saponins, phenolic compounds and cardiac glycosides were not found in *M. hirtus* (Table 1).

### Gas Chromatography-Mass Spectrum (GC-MS) Analysis of Acetonic Leaf Extracts of Three Botanicals

Gas chromatography-mass specterum (GC-MS) of *E. balsamifera* extracts is shown in Table 2. A total of 20 compounds were found among which Oleic acid Eicosyl ester had the highest molecular weight (562.99 g/mol) followed by Cyclopentene (4-octyl dodecyl) (350.70 g/mol) and then Hexanoic acid, 2-, hydroxide methyl ester (316.50 gmol<sup>-1</sup>), but the least (34.01 gmol<sup>-1</sup>) was found in hydrogen peroxide. In terms of area, 2-ethyl-2-hexen-1-ol was the highest (8.28%) followed by 5-methyl-2-hexanoic acid (7.17%) and heptadecanoic acid -16-methyl ester (6.90%), while the least (0.06%) was found in heptadecanal (Table 2).

Table 3 shows the GC-MS analysis of acetonic leaf extract of *L. inermis*. A total of 8 compounds were found in the botanical. Pennogenin di-acetate had the highest molecular weight (514.70 g/mol) followed by 2-butyl-6,7-dichlopentyl-1-oxo-2,3-dihydro-1h-inden-5-yl) acetic acid (399.30 g/mol) while the least was found in N,N-diethylethane-1,2-diamine (116.20 g/mol). Further, percentage area varied among the identified compounds from 1.22 to 39.63% (Table 3).

A total of 5 compounds were found when GC-MS analysis of acetonic leaf extract of *M. hirtus* was conducted (Table 4). Lanosta-7-9(11)20-triene-3-beta-18 diol, di-acetate had the highest molecular weight (524.77 g/mol), followed by pennogenin diacetate (514.70 g/mol) and cis -13-octadecanoic acid methyl ester (296.49 g/mol) but cis-vaccenic acid (282.50 g/mol) had the lowest molecular weight. The percentage area ranged from 4.96 to 44.90%.

### Adult Mortality of *Rhyzopertha dominica* Exposed to Acetonic Leaf Extracts of Three Botanicals

Results from this study show that adult mortality of *R. dominica* in treated sorghum grains differed with varying concentrations of the botanical extracts. Highest (63.33%) mortality among the botanical treatments was recorded in sorghum treated with 100.00 mgml<sup>-1</sup> *M. hirtus*, while the least (16.67%) was in treatments with 6.25 mgml<sup>-1</sup> *E. balsamifera* within 7 days after treatment (DAT). Permethrin powder resulted in 66.67% mortality while no insect was found dead in the untreated grains (Figure 1). Kruskal-Wallis test

indicated that adult mortality of *R. dominica* was significantly different ( $p < 0.05$ ) among treatments at all concentrations applied.

#### Adult Emergence of *Rhyzopertha dominica* Exposed to Acetonic Leaf Extracts

There were no adult emergence of *R. dominica* in the sorghum grains treated with acetonic leaf extracts of *E. balsamifera*, *L. inermis* and *M. hirtus*. However, the mean number of adult emergence in untreated grains was 42.

#### Weight Losses of Treated Sorghum Infested by *Rhyzopertha dominica*

Results obtained from this study showed a significant difference ( $p < 0.05$ ) in grain weight loss among the different botanicals and permethrin applied at varying concentrations. Highest weight losses were recorded in the sorghum treated with lower concentrations of the botanical extracts which decreased with higher concentrations following the order  $100.00 > 50.00 > 25.00 > 12.5 > 6.25 \text{ mgml}^{-1}$  (Figure 2). The result shows that mean weight loss in the permethrin treatment was 0.27%, while that in the control was 12.27%.

**Table 1:** Preliminary Phytochemical Screening of Some Botanicals Using Different Extraction Solvents

Botanicals	Methanolic Extracts								
	AKL	CHO	PHT	PHC	SPN	TAN	FLV	TPR	CGC
<i>E. balsamifera</i>	+	+	-	+	+	+	+	+	-
<i>L. inermis</i>	+	+	+	+	+	+	+	-	+
<i>M. hirtus</i>	+	+	+	+	+	+	+	+	+
	Acetonic Extracts								
<i>E. balsamifera</i>	+	+	+	+	+	+	+	+	+
<i>L. inermis</i>	+	+	+	+	+	+	+	+	+
<i>M. hirtus</i>	-	+	+	-	+	+	+	+	+
	Ethanollic Extracts								
<i>E. balsamifera</i>	-	-	+	+	+	+	+	+	+
<i>L. inermis</i>	+	+	+	+	+	-	+	+	+
<i>M. hirtus</i>	+	+	+	-	+	+	+	+	-

AKL = Alkaloids; CHO = Carbohydrates; PHT = Phytosterols; SPN= Saponins PHC = Phenolic compounds; TAN = Tannins; FLV = Flavonoids; TPR = Terpenoids; CGC = Cardiac glycosides; + = Presence; - = Absence

**Table 2:** Gas Chromatography Mass Spectrum (GC-MS) Analysis of Acetonic Leaf Extract of *E. balsamifera*

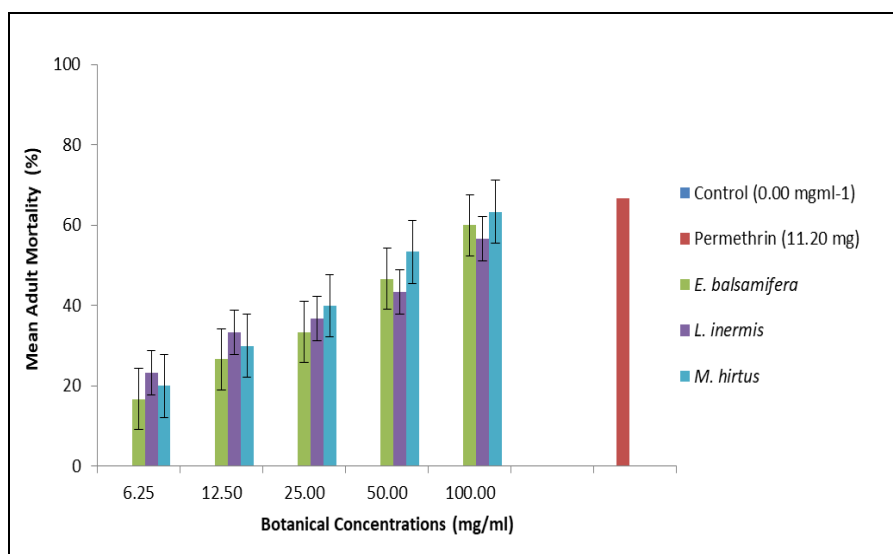
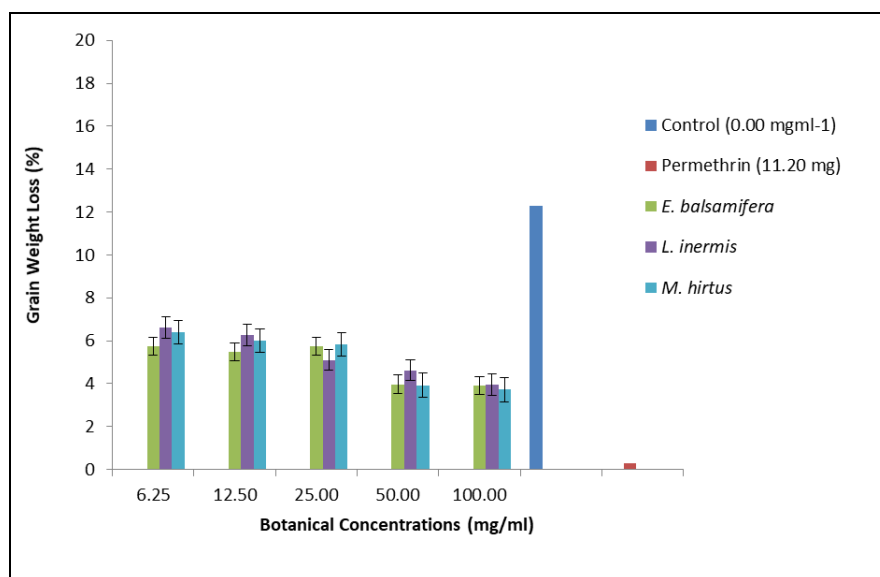
S/No.	Retention Time (min)	Name of Compound	Molecular Formula	Molecular Weight (g/mol)	Area (%)
1	1.91	Trimethylene oxide	C <sub>3</sub> H <sub>6</sub> O	58.08	1.89
2	5.24	Dodecanoic acid	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200.32	0.68
3	7.70	Tetradecanoic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228.37	0.22
4	10.04	Pentadecanoic acid 14-methyl methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.45	0.18
5	11.86	N-hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.42	1.69
6	13.92	11-octadecenoic acid, methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.49	1.15
7	14.43	Heptadecanoic acid 16-methyl methyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298.50	6.90
8	16.04	Oleic acid Eicosyl ester	C <sub>38</sub> H <sub>74</sub> O <sub>2</sub>	562.99	6.13
9	22.49	Trans -13-octadecenoic acid	C <sub>20</sub> H <sub>28</sub> O <sub>2</sub>	310.50	0.14
10	24.83	Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284.50	0.22
11	25.04	Cis-12-octadecenoic acid methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.50	0.19
12	25.08	Heptadecanal	C <sub>17</sub> H <sub>34</sub> O	254.50	0.06
13	25.97	Ethyl pentadecanoate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.50	0.14
14	26.52	Cyclopentene (4-octylidodecyl)	C <sub>25</sub> H <sub>50</sub>	350.70	1.55
15	26.76	Carbamic,-2-hydroxypropyl ester	C <sub>4</sub> H <sub>9</sub> NO <sub>3</sub>	119.12	0.62
16	27.12	Cis vaccenic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.46	0.47
17	27.48	Hydrogen peroxide	H <sub>2</sub> O <sub>2</sub>	34.02	0.59
18	28.42	5-Methyl -2-hexanoic acid	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub>	130.18	7.17
19	29.37	Hexanoic acid,2,-hydroxide methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>4</sub>	316.50	2.05
20	30.88	2-Ethyl -2-hexen -1-ol	C <sub>8</sub> H <sub>16</sub> O	128.21	8.28

**Table 3:** Gas Chromatography Mass Spectrum (GC-MS) Analysis of Acetonic Leaf Extract of *L. inermis*

S/No.	Retention Time (min)	Name of Compound	Molecular Formula	Molecular Weight (g/mol)	Area (%)
1	13.85	trans-13-octadecanoic-acid methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.50	33.09
2	15.43	Cis -13-octadecenoic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.50	39.65
3	29.00	2-Butyl -6,7-dichlopentyl-1-oxo-2,3-dihydro-1h-inden-5-yl) acetic acid	C <sub>20</sub> H <sub>24</sub> Cl <sub>2</sub> O <sub>4</sub>	399.30	38.05
4	29.42	N,N-diethylethane-1,2-diamine	C <sub>6</sub> H <sub>16</sub> N <sub>2</sub>	116.21	22.63
5	29.76	Methacrolein diacetate	C <sub>8</sub> H <sub>12</sub> O <sub>4</sub>	172.18	34.88
6	29.08	Cycloode canol, 1-aminomethyl-	C <sub>13</sub> H <sub>27</sub> NO	213.36	4.53
7	29.19	Pennogenin di-acetate	C <sub>31</sub> H <sub>46</sub> O <sub>6</sub>	514.70	1.22
8	29.91	2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethan-1-ol	C <sub>7</sub> H <sub>14</sub> O <sub>3</sub>	146.18	1.26

**Table 4:** Gas Chromatography Mass Spectrum (GC-MS) Analysis of Acetonic Leaf Extract of *M. hirtus*

S/No.	Retention Time (min)	Name of Compound	Molecular Formula	Molecular Weight (g/mol)	Area (%)
1	13.60	9,12-octadecenoic acid methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.50	16.34
2	13.72	Cis -13-octadecenoic acid methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.49	21.77
3	15.21	Cis-vaccenic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.50	44.90
4	27.49	Pennogenin di-acetate	C <sub>31</sub> H <sub>46</sub> O <sub>6</sub>	514.70	12.02
5	27.87	Lanosta-7-9(11)20-triene-3beta-18 diol, di-acetate	C <sub>34</sub> H <sub>52</sub> O <sub>4</sub>	524.77	4.96

**Fig 1:** Mean Adult Mortality of *R. dominica* Exposed to Acetonic Leaf Extract of Three Botanicals at 7 Days After Treatment**Fig 2:** Weight Losses in Sorghum Grins Treated with Three Acetonic Leaf Extracts and Infested by *R. dominica*

## Discussion

### Phytochemical compounds of the selected botanicals with insecticidal properties

Findings of this study revealed that *E. balsamifera*, *L. inermis* and *M. hirtus* were rich in most of the secondary metabolites such as alkaloids, carbohydrates, saponins, phytosterols, phenolic compound, tannins, flavonoids, terpenoids and cardiac glycosides. These chemical constituents were reported to show insecticidal and medicinal activities [17, 18].

It was reported that three insecticidal ingredients were isolated from the total alkaloids complex of *Cynanchum mongolicum*, these three alkaloids exhibited marked insecticidal activity on *Spodoptera litura* and *Lipaphis erysimi* Ge *et al.* [19].

Furthermore, It was stated that tannins can enter haemolymph of the insect through the peritrophic envelop of the gut [20].

Henn [21] observed that peritrophic envelop of insects are capable of connecting to tannins by attaching to carbohydrate of the envelopes. Hydrolysable tannins of oak are well known as phenolics, which can negatively influence the growth of the gypsy moth [22].

Another finding by Upasani *et al.* [23] showed that, flavonoids are major class of chemicals constituting 5-10% of known secondary metabolites involved in plant defense mechanisms by exerting toxic effects on insects. Their biogenesis is reported in response to stress conditions besides their natural presence in measured quantities the phenyl propanoid (cinnamate CoA) pathway is mainly involved in biosynthesis of all known flavonoids.

Different hypotheses on the mode of action have been drawn so far to explain the insecticidal activities of saponins,. They may pose a repellent or deterrent activity as suggested by

Sylwia *et al.* [24]. Saponins may also affect the food uptake by slowing the passage of in the insect gut, perhaps due to a reduction of the digestibility [25]. These can secondarily influence food uptake, and as a consequences the nutrient uptake and growth. Saponins were also found to increase the permeability of plasma membrane and they are known to course lysis of erythrocytes *in vitro* [26].

In the present study, GC-MS analysis confirmed the presence of different compounds in acetonic leaf extracts of *E. balsamifera*, *L. inermis* and *M. hirtus*. Some of the isolated compounds such as oleic acid Eicosyl ester, decanoic acid, hydrogen peroxides and N,N-diethyl nediamine were reported to have some insecticidal properties [27, 28]. Also, Oileic acid has the property of insectifuge, anti-inflammatory, cancer preventive and hypercholesterols [27]. Furthermore, decanoic acid can be used as insecticide (PT18). Decanoic acid kills insect upon contact with a sufficient dose with a delay of a few hours up to seven days depending on the species and the individual. It is speculated that the active substance damages the chitin cuticle of arthropods leading to desiccation [28].

#### **Effect of acetonic botanical extracts on the survival of *R. dominica***

Findings of this study revealed that acetonic extracts of *E. balsamifera*, *L. inermis* and *M. hirtus* were effective in causing adult mortality of *R. dominica* in stored sorghum. It was observed that the mortality was directly proportional to varying concentrations used for the study. This is supported by findings of Alvi *et al.* [29] who reported that adult mortality of *R. dominica* increased from 27.83 to 72.11% when the concentration of leaf extracts of *Rhazya stricta* was raised from 5 to 20%.

This study has also found that extension of exposure periods increased adult mortality of *R. dominica* in treated sorghum grains concurring with Alvi *et al.* [29] who reported that *Rhazya stricta* leaf extract caused significant mortality against *R. dominica* which increased with increase in exposure time. Similarly, Ileke and Bulus [30] reported that adult mortality of *R. dominica* in wheat grains treated with *A. indica* and *Piper guineense* increased with increase in exposure period.

Findings of this study have revealed that leaves of *E. balsamifera*, *L. inermis* and *M. hirtus* were significantly toxic against adult *R. dominica* causing considerable adult mortality within 7 days. Similar results were recorded by Suleiman and Suleiman [31] that 90.00% adult mortality of *Callosobruchus maculatus* was observed in cowpea treated with leaf powder of *E. balsamifera* at 1.0/20 g (w/w) within 96 hours of exposure.

The promising effect of *L. inermis* on the survival of *R. dominica* in this study agrees with previous report that 100% mortality of *Trogoderma granarium* exposed to 1, 2, 4 and 6% of leaf powder of the botanical was achieved at 14 days after treatment [32].

There is scanty information on the efficacy of acetonic extract of *M. hirtus* against *R. dominica*. However, Suleiman *et al.* [33] reported that ethanolic extract of the plant species caused in high mortality of *S. zeamais*. The mortalities of *R. dominica* exposed to the plant extracts reported in this study could have been possible due to obstruction of spiracle of the insect body, thus impairef respiration which led to death of the insect [33].

Acetonic extract of *E. balsamifera*, *L. inermis* and *M. hirtus* used at different concentrations were found to have toxicity similar to permethrin powder against *R. dominica*, even though the beetles responded faster in permethrin than the

botanical extracts. Permethrin has been discribed as a synthetic pyrethroid that act by interfering with the electric signal passing down the axon of insect nerval cells leading to loss of coordination and ultimate death [33]

All the three botanicals in different concentrations were effective against the beetle. This was possible because plant contain secondary metabolites which are enormous storehouse of compounds such as alkaloids, flavonoids, terproids, saponins, tannins and cardiac glycosides found in the leaf extracts of *E. balsamifera*, *L. inermis* and *M. hirtus*. Presences of alkaloids, flavonoids, saponins and tannins in the acetonic extract of the plant leaf was concluded to be of insecticidal effects against *R. dominica*.

#### **Effect of acetonic leaf extracts on adult emergence of *R. dominica***

All the three botanicals tested had total inhibition rate in adult emergence of *R. dominica* in sorghum grains treated with acetonic leaf extracts as no adult had emerged. Complete suppression of adult emergence of *R. dominica* by acetonic leaf extract of the test botanicals is in accordance with other findings. For instance, Suleiman *et al.* [34] reported that complete inhibition of adult emergence of *S. zeamais* was achieved in grains treated with methanolic and ethanolic extracts of *E. balsamifera*, *L. inermis* and *M. hirtus*.

It could be possible that the complete inhibition in adult emergence of *R. dominica* by acetonic leaf extract of the test botanicals and permethrin might be due to total mortality observed some days after treatment. Also botanicals might be toxic to the few eggs deposited and as such led to elimination of the adult emergence of *R. dominica*. Concurring with Chudasama *et al.* [35] that toxic substance present in the extract may enter into the egg through chorion and suppressed further embryonic development.

Non- emergence of *R. dominica* adults in the treated sorghum grains could be as a result of high mortality of adult insect observed some days after treatment, thus disrupting mating and sexual communication as well as deterring female from laying eggs and complete suppression of the developmental stages of insect [35]. Reduced adult emergence could be due to high mortality of the insect which might consequently reduce the rate of mating and oviposition [34, 35].

#### **Effect of acetonic leaf extracts on sorghum grain damage infested by *R. dominica***

The acetonic leaf extract of *E. balsamifera*, *L. inermis* and *M. hirtus* used in this study were found to be effective in protecting the sorghum grains from damage caused by *R. dominica*. These might be attributed to the insecticidal activities of the botanicals against *S. zeamais* and *C. maculatus* as observed by others [31, 36]. Similar observations were made by Adedire and Ajayi [37] who reported 65.24% damage of maize grain caused by *S. zeamais* treated with 1.0 ml/10 g acetonic extract of *C. sinensis*.

#### **Conclusion**

Phytochemical screening of the test botanicals has shown the presence of various bioactive compounds some of which have been reported to be of insecticidal properties. Findings of this study revealed that the acetonic leaf extracts of *E. balsamifera*, *L. inermis*, and *M. hirtus* had a great effect in adult mortality of *R. dominica*.

The acetonic leaf extract of *E. balsamifera*, *L. inermis* and *M. hirtus* tested in this study were found to be effective in

protecting the sorghum grains from damage caused by *R. dominica* due to observed high mortality of the insect, non-emergence of F<sub>1</sub> generation and few perforations to the grains.

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