

Toxicity of Acetonic Leaf Extracts of Three Botanicals against the Lesser Grain Borer, *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) in Stored Sorghum

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ABSTRACT

Experiments were conducted to assess insecticidal effects of leaf extracts of *Euphorbia balsamifera*, *Lawsonia inermis* and *Mitracarpus hirtus* against *Rhyzopertha dominica* F. Acetonic leaf extracts of the botanicals at varying concentrations of 6.25, 12.50, 25.00, 50.00, 100.00 mgml⁻¹ and permethrin powder at 0.056% (w/w) 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. Phytochemical analysis of the test plants was conducted. Effects of the treatments on adult mortalities as well as their LC₅₀ against *R. dominica* were also determined. 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 were found to be highly effective in killing adult *R. dominica* leading to total adult mortality within 12 days after treatment (DAT). *M. hirtus* was found to be the most effective botanical with its LC₅₀ values lower than that of the other botanicals. Acetonic leaf extracts of *E. balsamifera*, *L. inermis* and *M. hirtus* could therefore be utilized to reduce *R. dominica* infestations in stored sorghum.

Keywords: Botanicals, Phytochemicals, LC₅₀, Mortality, *Rhyzopertha dominica*, Toxicity.

INTRODUCTION

Sorghum is the primary food crop in virtually all parts of northern Nigeria (USDA, 2010). The whole grain may be grounded into flour or decorticated before grinding to produce a fine particle product used in various traditional foods (Leader, 2004; Suleiman & Rugumamu, 2017). Sorghum is also used as animal feed, bio-fuel and in brewing (USDA, 2010).

Abate et al. (2000) observed that insect pests are one of the major constraints to agricultural production in Africa. A large number of insects attack crops during all stages of growth: from seedling to storage. Some of the insect pests that attack sorghum grain in the store include the *Sitophilus spp.* (Coleopteran:

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Curculionidae), *Rhyzopertha dominica* (F) (Coleoptera: Bostrichidae), *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae), *Oryzaephilus surinamensis* (L) (Coleoptera: Silvanidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Utono, 2013, Mailafia et al., 2014).

Most of losses and damages to stored sorghum are caused by various types of insect pests. Insect infestations in stored sorghum grains lead to decrease in grains quantity, quality and seed viability (Suleiman et al., 2018). Studies on *S. zeamais* infestations on stored sorghum have been reported by others (Gofishu & Belete, 2014; Suleiman, 2014; Suleiman et al., 2018). But, little attention has been paid to the status of *R. dominica* infestations in sorghum grains.

In order to manage these infestations, chemical insecticides are widely used by farmers due to their rapid action. However, these chemicals are harmful to both non target organisms and their environment (Suleiman et al., 2018). This leads to a search for alternative and environmental-friendly methods such as application of botanicals to control stored products insects pests. Currently, there is little literature on the application of *Euphorbia balsamifera* Aiton, *Lawsonia inermis* L. and *Mitracarpus hirtus* (L.) DC. against *R. dominica* infesting sorghum grains, even though they were tested and found effective against *Callosobruchus maculatus* F. and *Sitophilus zeamais* Motsch (Chudasama et al., 2015; Suleiman et al., 2018).

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh leaves of *Euphorbia balsamifera* Aiton, *Lawsonia inermis* L. and *Mitracarpus hirtus* (L.) DC, were collected from the bushes around Dabaibayawa village and identified at Herbarium of the Department of Biology, Umaru Musa Yar'adua University, Katsina (UMYUK). All the plant leaves were shade in a well ventilated area in the Biology Laboratory 3 before grinding them into fine powders using clean pestle and mortar. 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 acetone for 48 hrs 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 (Ajuru et al., 2017).

Preliminary Phytochemical Screening of the Leaf Extracts

Acetonic extracts of the botanicals were subjected to preliminary screening using standard procedures described by Idris et al. (2014) and De Silva et al. (2017) for the detection of alkaloids, saponins, tannins, phenolic compounds, flavonoids, carbohydrates, phytosterols, terpanoids and cardiac glycosides. The procedures are outlined hereunder.

Test for alkaloids: 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.

Test for saponins: 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.

Test for tannins: Two drops of 5% ferric chloride was added to 1 ml of the test extract. A dirty green precipitate indicated the presence of tannins.

Test for flavonoids: 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.

Test for terpenoids: 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.

Test for phenolic compounds: The plant extracts were treated with a few drops of ferric chloride solution and the formation of bluish black colour proved the presence of phenols.

Test for carbohydrates: 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.

Test for phytosterols: 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 showed the presence of phytosterols.

Test for cardiac glycosides: 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.

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 mouths were properly covered with cotton wool 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 escape 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 (Asmanizar & Idris, 2012) before using them as substrate for insect rearing. Adults of *R. dominica* were obtained from Institute of Agricultural Research, Zaria (IAR), Nigeria. The insects were introduced into rearing bottles (500 ml capacity) containing 250 g of the disinfested sorghum. The bottles were covered with muslin cloth, secured with rubber bands and placed in an incubator at 30 ± 2°C and 70 ± 5% relative humidity (R.H.) for oviposition. The insects were removed after a week leaving the grains only for adult emergence. The emerged F₁ individuals were sieved from the grains and used for subsequent experiments (Sani & Suleiman, 2017).

Determination of Adult Mortality of *Rhyzopertha dominica*

Twenty grams of clean disinfested sorghum grains were weighed into small 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⁻¹ to 20 g of sorghum grains in each of the bottles, while 11.20 g of permethrin was added to another bottle containing sorghum as check, and no extract was added to the control. The treatments containing extracts and permethrin were thoroughly mixed with the disinfested sorghum grains with the aid of glass rod to ensure thorough admixture and allowed the extracts to evaporate and grain dried for 2 hrs. Thereafter, ten newly emerged adults of *R. dominica* were introduced into each of the treated and untreated sorghum grains. Mouths of the bottles were covered with muslin cloth, secured with rubber bands and then kept in an incubator at 30 ± 2°C and 70 ± 5% R.H. and arranged in a completely randomized design (CRD) with three replicates. Dead insects were removed, counted and recorded daily until first total mortality was observed in any of the replicates. 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 (Suleiman, 2014).

Determination of Median Lethal Concentration (LC₅₀) of the Botanical Extracts

To evaluate the LC₅₀ values, methods of Ebadollahi & Mahboubi (2011) were adopted. The number of dead beetles in each bottle containing sorghum grains treated with the botanical extract was counted at the end of six days of exposure. The LC₅₀ was calculated by using probit analysis with SPSS (Version 16.0) software package.

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 of *R. dominica* among different botanical treatments. Significantly different means were separated using Dunn's multiple test for non parametric data. All analyses were performed at $p < 0.05$.

RESULTS AND DISCUSSION

Secondary Metabolites of 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). The results also showed that *E. balsamifera* and *L. inermis* contained all the phytochemicals, whereas no alkaloids and phenolic compounds were found in *M. hirtus*.

These chemical constituents were reported to show insecticidal and medicinal activities Sofoware (1993). This is in line with Acheuk & Doumandji-Mitiche (2013) who reported that alkaloids showed dose- dependent relationships as insecticide with antifeedent activity against larvae of the migratory locust. Similarly, Ge et al. (2015) reported that three insecticidal ingredients were isolated from the total alkaloids complex of *Cynanchum mongolicum*, which exhibited marked insecticidal activity on *Spodoptera litura* and *Lipaphis erysimi*. Furthermore, it was stated that tannins can enter haemolymph of the insect through the peritrophic envelop of the gut (Barbehenn & Martin, 1994). Peritrophic envelop of insects are observed to be capable of connecting to tannins by attaching to carbohydrate of the envelop (Henn, 1997). Moreover, Upasani et al. (2003) reported that flavonoids are major class of chemicals constituting of 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.

Saponins have been observed to pose repellent or deterrent activity against insects (Sylwia et al., 2006). They may also affect the food uptake by slowing the passage of in the insect gut (Adel et al., 2000). These can secondarily influence food uptake, and as a consequences the nutrient uptake and growth. Saponins increase the permeability of plasma membrane and they are known to cause lysis of erythrocytes in vitro (Francis et al., 2002).

Table 1: Preliminary phytochemical screening of acetonic leaf extracts of three botanicals

Botanicals	Secondary Metabolites								
	AKL	CHO	PHT	PHC	SPN	TAN	FLV	TPR	GGC
<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, Terpoids; CGC, Cardiac glycosides; +, Presence; -, Absence

The specific carbohydrate-binding activity of lactins enables them to recognize and attach to glycoconjugates present in insects and other organisms. Since glycoproteins are major constituents of insect digestive tract membranes, it is probable that insect gut contains specific ligand-binding molecules that are target for plant defensive lactins (Zhu-Salzman et al., 1998). Therefore, the principal active compounds detected here may be responsible for their insecticidal activities of the organism.

Effect of Acetonic Leaf Extracts of Three Botanicals on Adult Mortality of *Rhyzopertha dominica*

Results from this study show that adult mortality of *R. dominica* in treated sorghum grains varied with different botanical extracts at varying exposure periods. At 6 DAT, the adult mortality was directly proportional to varying concentrations of all the three botanical extracts (Figure 1).

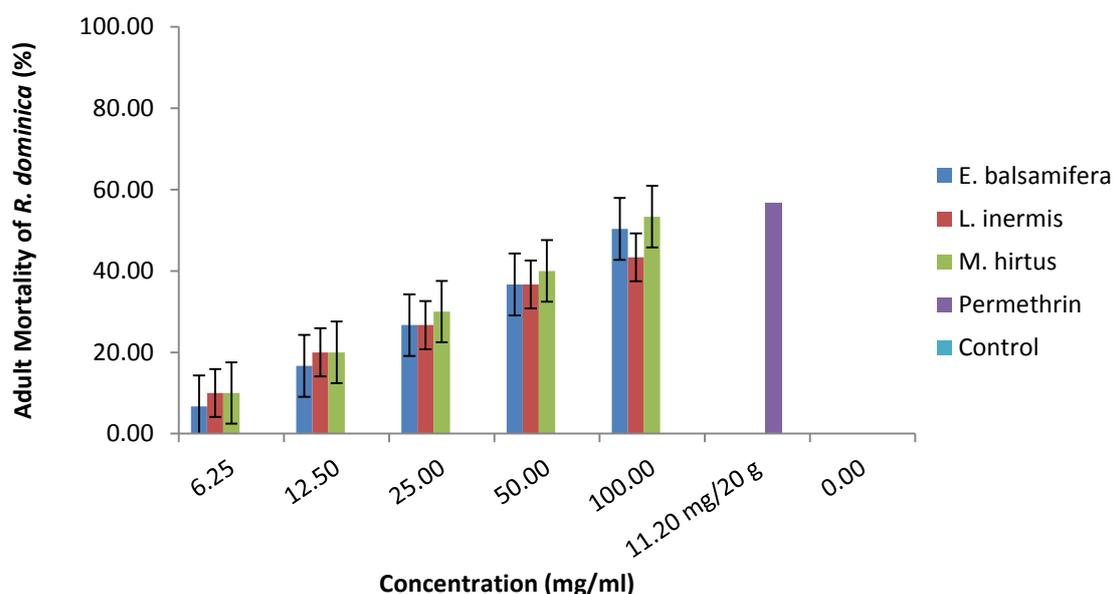


Fig. 1: Adult mortality of *Rhyzopertha dominica* exposed to acetonic leaf extracts of three botanicals at 6 days after treatment (DAT)

Highest (53.33%) mortality was recorded in sorghum treated with 100.00 mgml⁻¹ *M. hirtus*, while the least (6.67±3.33%) was in treatments with 6.25 mgml⁻¹ *E. balsamifera* within the first 6 days. Furthermore, the mortality among the botanical types at 100.00 mgml⁻¹ was in the order: *M. hirtus* > *E. balsamifera* > *L. inermis* after the aforementioned exposure period. Permethrin powder resulted in 56.67% 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 among treatments at 12.50 mgml⁻¹ ($KW = 11.283$; $p = 0.0236$) and 25.00 mgml⁻¹ ($KW = 10.998$; $p = 0.0266$). However, the mortality was not

significantly different ($p > 0.05$) at the rest of the concentrations (Figure 1).

At 8 DAT, the adult mortality of *R. dominica* was also directly proportional to the varying concentration of all the botanicals and ranged from 20.00 to 80.00% within 8 DAT (Figure 2). The highest mortality was recorded in sorghum treated with *E. balsamifera* at 100.00 mgml⁻¹ followed by *M. hirtus* and *L. inermis*. Permethrin powder resulted in 86.67% mortality while no dead insect was found in the control (Figure 2). Kruskal-Wallis statistics indicated that the adult mortality of *R. dominica* was significantly different among the treatments at all the concentration.

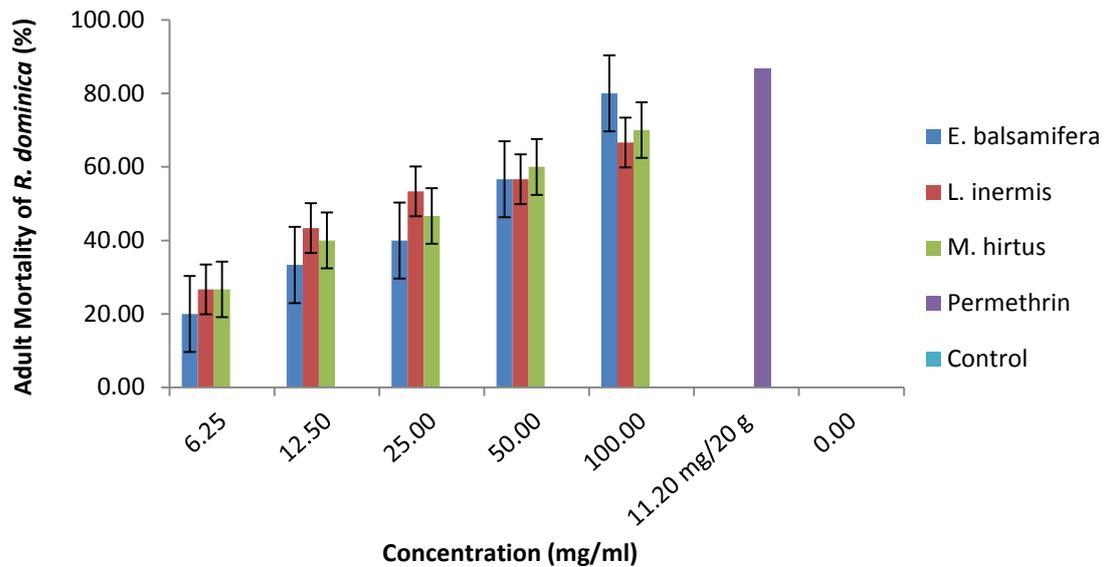


Fig. 2: Adult mortality of *Rhyzopertha dominica* exposed to acetonc leaf extracts of three botanicals at 8 days after treatment (DAT)

Figure 3 Shows that the adult mortality of *R. dominica* was highest (90.00%) in grains treated with *M. hirtus* and *L. inermis*, respectively, while *E. balsamifera* caused the least (33.33%) at 10 DAT. The result indicated that, indicate that the mortality in permethrin treated grains was 93.33% and no dead insect was found in the control. Kruskal-Wallis

statistics showed significantly different adult mortality of *R. dominica* among the treatments at 6.25 mgml⁻¹ ($KW = 12.872$; $p = 0.0119$), and 25.00 mgml⁻¹ ($KW = 10.669$; $p = 0.0101$). However, the difference in mortalities was not significant ($p > 0.05$) at 12.50, 50.00 and 100.00 mgml⁻¹ with 10 DAT (Figure 3).

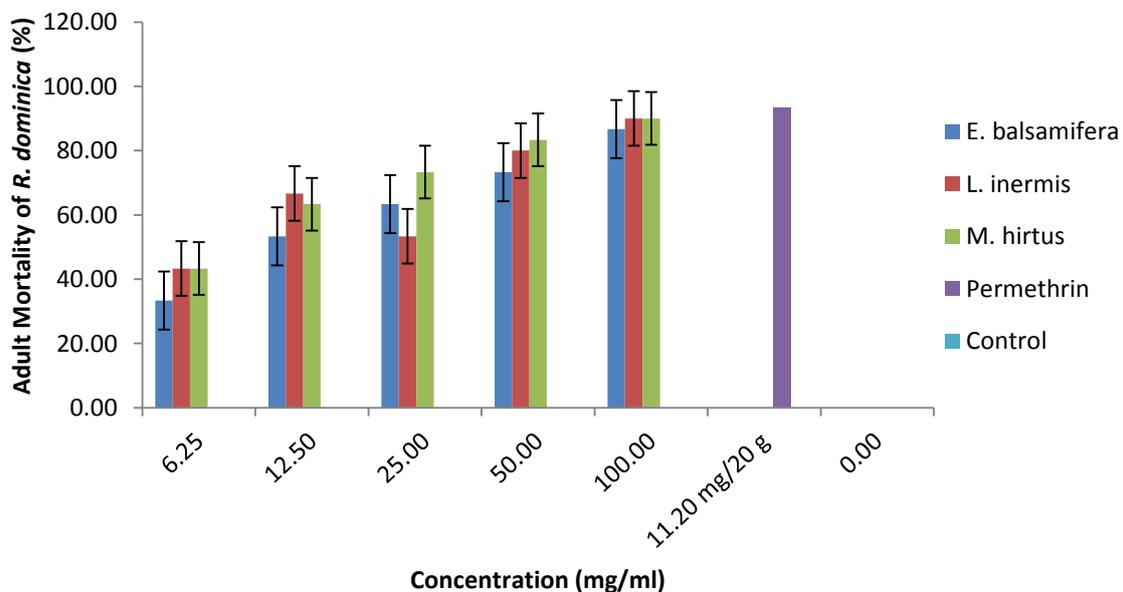


Fig. 3: Adult mortality of *Rhyzopertha dominica* exposed to acetonc leaf extracts of three botanicals at 10 days after treatment (DAT)

Similar trend to that recorded at 10 DAT was recorded at 12 DAT as shown in Figure 4. The results show that total (100.00%) mortality was recorded in sorghum treated with 100.00 mgml⁻¹ *M. hirtus* and *E. balsamifera*, respectively, while the least (53.33%) was in grains treated with 6.25 mgml⁻¹ *E. balsamifera*. Permethrin powder resulted in 100.00% mortality while no insect was found dead in the untreated grains (Figure 4).

Kruskal-Wallis statistics indicated that adult mortality of *R. dominica* was significantly different among treatments at 12.50 mgml⁻¹ ($KW = 11.543$; $p = 0.0211$), 25.00 mgml⁻¹ ($KW = 10.989$; $p = 0.0267$), 50.00 mgml⁻¹ ($KW = 10.665$; $p = 0.0306$) and 100.00 mgml⁻¹ ($KW = 10.953$; $p = 0.0271$). But it was not significantly different at 6.25 mgml⁻¹ ($KW = 9.151$; $p = 0.0574$).

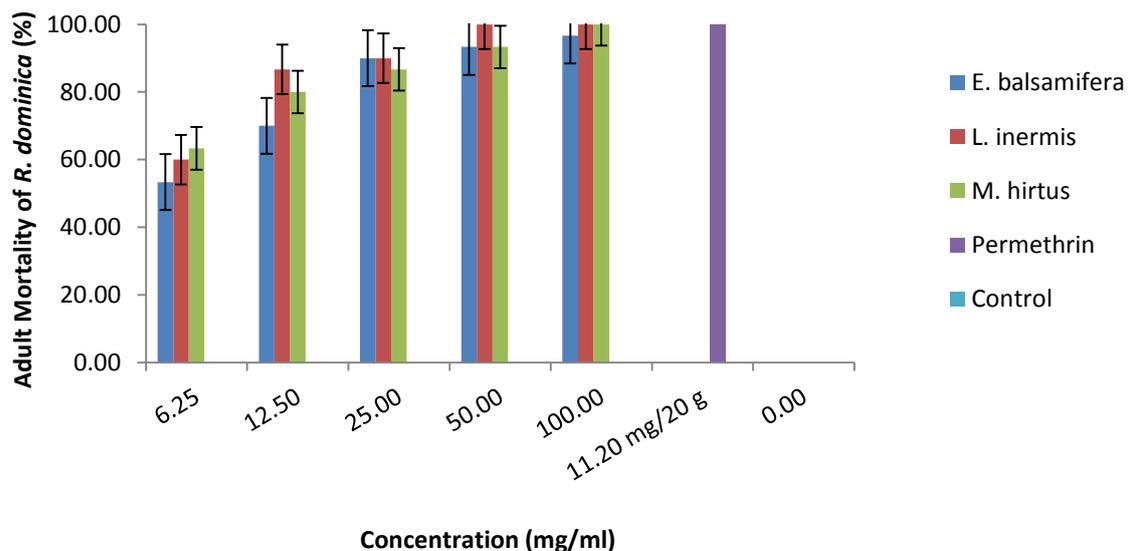


Fig. 4: Adult mortality of *Rhyzopertha dominica* exposed to acetonc leaf extract of three botanicals at 12 days after treatment (DAT)

Findings of this study revealed that acetonc extracts of *E. balsamifera*, *L. inermis* and *M. hirtus* were effective in causing adult mortality of *R. dominica* infesting stored sorghum. It was observed that the mortality was directly proportional to varying concentrations used for the study. Similar results were reported by others (Aziz et al., 2013; Popovic et al., 2017; Alvi et al., 2018; Asemave & Anure, 2019). For instance, Ileke & Bulus (2012) reported 10.00% adult mortality of *C. maculatus* when exposed to 0.1 g *Azadirachta indica* A. Juss powder for 24 hours which increased to 50.00% at the concentration of 0.8 g of the same powder within the same period.

This study has also found that extension of exposure periods increased adult mortality of *R. dominica* in treated sorghum grains. This was similarly observed by Alvi et

al. (2018) who reported that *Rhazya stricta* leaf and seed extract caused significant mortality against *R. dominica* and *Trogoderma granarium* which increased with increase in exposure time. Similarly, Asamave & Anure (2019) also reported that *A. indica* and *Piper guineense* extracts caused significant adult mortality of *R. dominica* in wheat grains which increased with increase in exposure time.

Findings of this study have revealed that leaves of *E. balsamifera*, *L. inermis* and *M. hirtus* were significantly toxic against adult *R. dominica*. The plant species resulted in total adult mortality of *R. dominica* within 12 DAT even at lower concentration. This is supported by a report that a high (90.00%) adult mortality of *Callosobruchus maculatus* was recorded in cowpea seeds treated with leaf

powder of *E. balsamifera* at 1.0/20 g (w/w) within 96 hours of exposure (Suleiman et al., 2018). *E. balsamifera* caused higher mortality rate of *C. maculatus* and recorded 100% mortality at all doses (Suleiman & Suleiman, 2014). The total mortality of *R. dominica* caused by *L. inermis* leaf powder confirms the previous report that 100% mortality of *Trogoderma granarium* exposed to 1, 2, 4 and 6% of the leaf powder was achieved within 14 days after treatment (Al-Moajel, 2004).

Although there is limited report on the efficacy of acetonic extract of *M. hirtus*, Suleiman et al. (2018) reported that both ethanolic and methanolic extract of the plant species resulted in high mortality of *S. zeamais*. The high mortalities of *R. dominica* exposed to the plant extracts reported in this study could have been possible due to blockage of spiracles of the insect body, thus impairing respiration leading to death.

Acetonic leaf extracts 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 described as a synthetic pyrethroid that acts by interfering with the electric signal passing down the axon of insect nerve cells leading loss of coordination and ultimate death. Shade drying of the botanicals could also have an impact on their mortality effects against the beetles. This was possible because the botanicals might contain all the active

ingredients as there might not be photo and thermo-degradation due to exposure to sunlight (Suleiman et al., 2018).

All the selected botanicals in different concentrations were effective against the beetle even at lower concentration. There was possible because plant contain secondary metabolites which are vast repository of compound such as phenolic compound and steroids reported to have a wide range of biological activities and great impact on insecticidal activities. Other bioactive compounds such as alkaloids, flavonoids, terpenoids, saponins, tannins and cardiac glycosides were found in the leaf of *E. balsamifera*, *L. inermis* and *M. hirtus* are believed to be in nature. Presence of alkaloids, flavonoids, saponins and tannins in the acetonic extract of the plant leaf was concluded to be of insecticidal effects against *R. dominica*. The characteristic smell of *E. balsamifera*, *L. inermis* and *M. hirtus* might have contributed greatly in their insecticidal activity by attacking the insect from the grains resulting into an adult mortality (Suleiman et al., 2018).

Median Lethal Concentrations of Three Acetonic Leaf Extracts against *Rhyzopertha dominica*

Table 5 shows that *E. balsamifera* had the highest values of median lethal concentration (LC_{50}) 88.39 $mgml^{-1}$ followed by *L. inermis* (59.15 $mgml^{-1}$) and *M. hirtus* as (53.70 $mgml^{-1}$). Chi-square test indicated that there was no significant difference ($p > 0.05$) in LC_{50} and LC_{99} of each of the test botanicals (Table 2).

Table 2: LC_{50} of acetonic leaf extracts of three botanicals against *Rhyzopertha dominica*

Botanicals	Slope \pm S.E.	LC_{50} ($mgml^{-1}$)	95% CL		X^2	p value
			Lower	Upper		
<i>E. balsamifera</i>	2.642 \pm 0.01	88.39	58.24	238.29	2.519	0.641
<i>L. inermis</i>	2.007 \pm 0.02	59.15	36.86	1544.59	1.567	0.667
<i>M. hirtus</i>	2.351 \pm 0.01	53.70	35.67	205.63	1.479	0.687

CL, Confidence limit

LC₅₀ and LC₉₉ of acetonic leaf extract of *E. balsamifera*, *L. inermis* and *M. hirtus* showed their efficiency in causing 50 % adult mortality of *R. dominica* within 6 DAT. Recent findings reported LC₅₀ of methanolic and ethanolic extracts of the study botanicals against *S. zeamais* (Suleiman et al., 2018). Their findings revealed that all the botanicals were effective in causing 50% mortality at a very low concentration within short periods. Results from this study have shown that *M. hirtus* had the lowest values of LC₅₀ inferring it to be the most effective botanical extracts against the study insect. Although all the botanicals showed considerable effects in killing 50% of *R. dominica*, their lethal concentrations were higher than the recommended dose for permethrin powder against the insect. The effectiveness of the selected plants in killing adult beetle at lower concentration at short period of time is in consistent with Suleiman et al. (2018) that ethanolic and methanolic extracts of *E. balsamifera*, *L. inermis* and *M. hirtus* were highly effective against *S. zeamais*. However, the LC₅₀ values were lower than what was obtained by Suleiman et al. (2018) against *S. zeamais* which could probably be due to species variation.

CONCLUSION

It was found that application of acetonic leaf of *E. balsamifera*, *L. inermis* and *M. hirtus* the selected botanical resulted in high adult mortality of *R. dominica* even at the lower concentration of 6.25 mgml⁻¹. The LC₅₀ of the botanicals against the insect within six days after application were found to be higher than the recommended dose of permethrin powder, but below the higher concentration of the extracts used. The toxicity of the test botanicals against adult *R. dominica* infers that they could be utilized to reduce the weevils' infestations in stored sorghum. However, further investigations on their toxicity on mammals are hereby recommended.

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Conflict of Interest

The authors declare that no competing of interest.

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Availability of Data and Materials

The data sets generated and analyzed during this study are available from the corresponding author on reasonable request.

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