Response of Some Biomarker Enzymes to Terpinolene Used as Repellent against *Rhyzopertha dominica* (Fab.) Infestation in Stored Food Grains

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Abstract: The repellent activity of terpinolene and its effects on antioxidant, neurotransmitter and detoxifying enzymes of *Rhyzopertha dominica* was evaluated under laboratory condition. The terpinolene was made into 2% concentration and 0.2, 0.4, 0.6, 0.8 and 1.0ml dosages were made from it and replicated five times. The repellent activity of the compound was carried out by placing two a filter paper that have been divided into two equal parts into a Petri-dishes with one side been treated with the compound. The activities of SOD, CAT, GPx, AChE, CarEST and GST was determined in the larvae of the insect. The result showed that, only 1.0 ml dosage and the positive control achieved 100% repellency at 0-1 h and were significantly different from other treatments. The activity of SOD decreases with increase in dosage of the compound. The lowest SOD (0.29 µmol/min/ml) was recorded by the positive control but was not significantly different from the 1.0 ml dosage of the compound. CAT activity significantly reduced at higher dosage, while the positive control recorded the lowest CAT activity. GPx activity increased increased dosage. AChE activity decreased with increase in the compound dosage. DDVP recorded the lowest AChE activity 0.25 µmol/min/ml. CarEST and GST activities increased with increase dosage of 0.6 ml and drastically reduced at 0.8 and 1.0 ml of the compound. Terpinolene compound proven insecticidal and could be formulated to target SOD and AChE enzymes having shown significant effects on them. Thus, this could be the mode of action of the compound.

Keywords: Terpinolene, active compound, neurotransmitter, antioxidant, detoxifying, mortality.

INTRODUCTION

Stored product insect pests play a fundamental role in food insecurity among the countries of the world, especially in the developing countries where insect pest management is minimal and improved or modern storage techniques is lacking or grossly inadequate. These insects are usually field to store where they multiply their destructive activities in the storage where they have more favourable conditions (Isman, M.B. 2006; Ashamo, M.O et al., 2013; Liu, X.C et al., 2013; Oni, M. O. 2014; Tedela, P. O et al., 2017). *Rhyzopertha dominica* (Fabricius) (Bostrichidae: Coleoptera) is a diverse important insect pest of many stored cereal grains and had been reported of being capable of lowering the grains quality and rendering the stored grains unfitting for human consumption if left unchecked; which ultimately leads to economic loss (Ogungbite, O. C et al., 2014; Adesina J. M & Mobolade-Adesina, T. E. 2020). Thus, this calls for an effective means of controlling or managing the insect.

The origination of synthetic insecticides in the early 1930s was a massive feat to insect control practitioners and entomologists until many unanticipated complications linked with these insecticides began to uncover in later years (Isman, M.B 2006; Pavela, R 2008 & Zibaee, A 2011). Replacing these dangerous synthetic insecticides with natural products that may have little or no adverse effects on human and environmental health therefore becomes inevitable.
Many medicinal and aromatic plant species have proven insecticidal action against wide range of insect pests and they are being advocated as potential substitute to the use of synthetic insecticides. (Zibae A, 2011) reported that many botanicals contain myriads of potential bioactive compounds that could be insecticidal in nature. Terpinolene is one of the secondary metabolites which plants used for their defence against insects. This secondary metabolite had been reported of being present in several botanicals such as Melaleuca alternifolia, M. trichostachya, Manilla elemi, Nectandra elaiophora and Dacrydium colensoi (Aydin, et al., 2013). However, the insecticidal potential of this secondary metabolite has not been well established. In view of this, the present research evaluated the repellent toxicity of terpinolene to R. dominica and also the response of some biomarker enzymes to the compound.

**MATERIALS AND METHODS**

**Insect culture**

*R. dominica* used for this work was obtained from the insect colony maintained in the Department of Crop, Soil and Pest Management laboratory, Federal University of Technology Akure, Nigeria. These insects were sub-cultured on clean, uninfested wheat grains that have been disinfested inside freezer at -10°C for 5 weeks. The insects were cultured inside a net-covered plastic container, secured with rubber band and the culture was maintained throughout the period of the experiment by replacing the devoured wheat grains with new ones.

**Collection of Materials**

The wheat grains used were collected from National Seed Service, Ibadan, Nigeria and the grains were disinfested inside freezer for two weeks before being used in order to eliminate any hidden infestation (if any). The Terpinolene used was received as donation from the Laboratory of Professor Murray B. Isman, University of British Columbia, Canada. The active compound was kept inside refrigerator until use. The terpinolene was made into 2% concentration and 0.2, 0.4, 0.6, 0.8 and 1.0 ml dosages were prepared from it. The Dichlorvos (DDVP) used as standard check was bought from a local agrochemical store in Akure, Nigeria and was made into 0.005% from which 0.1 dosage was made.

**Repellent Activity**

Terpinolene repellent activity was accessed by using assays on Petri-dishes to confine insects during the experiment as described by (Yang, Z. C. F et al., 2014) with little modification. Filter papers were cut into half and one halves were treated uniformly with 0.2, 0.4, 0.6, 0.8 and 1.0 ml dosages of terpinolene. The other half (negative control) was treated with 1 ml of absolute ethanol while half treated with DDVP was used as positive control. The treated and control halves were then air-dried to completely evaporate the solvent. The Petri dishes were covered after ten adult (10) unsexed insects were released into the center of each filter paper disk. Five replicates were used per treatments. Counts of the insects present on each strip were made after 1, 4 and 8 hrs. The percentage repellency (PR) of each treatment was calculated using the formula:

\[PR(\%) = \frac{N_c - N_t}{N_c} \times 100\]

Where the number of insects in the negative control half is \(N_c\), while the number of insects in the treated half is \(N_t\).

**Enzyme assays**

**Larva supernatants preparation**

The effect of terpinolene on the activities of the antioxidant, neurotransmitter and detoxifying enzymes present in adult *R. dominica* was determined at different dosages of the compound. The larvae were put inside Petri dishes that contained filter papers that have been treated with different dosages of the compound for 4 h. The supernatants of the larvae were prepared as described by Bamidele, O. J et al., 2013 and the supernatant was used for all the enzyme assays, with exception of those meant for glutathione S-transferase activities that prepared according to the procedure of Vatan, T et al., (2007). Each assay was replicated five times.

**Determination of Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) activities**

SOD activity was assayed as described by Beau-champ, C & Fridovich I. 1971 and modified by Bamidele, O. J et al., (2013). Three (3) ml of the reaction medium which contained 1.17 μM riboflavin, 0.1 M methione, 0.2 μM potassium cyanide (KCN) and 0.56 μM nitroblue tetrazolium salt (NBT) dissolved in 3 ml of 50 mM sodium phosphate buffer (pH 7.8) was added to 1 ml supernatant. The reaction was initiated at 30°C for 1 h under 40 W fluorescent tubes. Duplicate solutions that serves as blank were kept under dark. The absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity was calculated with the formula below:

\[\text{SOD} = \frac{A}{R_4 / A}\]

\[A = R_1 \left(\frac{50}{100}\right)\]

\[R_4 = R_3 - R_2\]

\[R_3 = \text{OD of sample}\]

\[R_2 = \text{OD of blank}\]

\[R_1 = \text{OD of reference}\]

Where R1 is the absorbance of the reference solution, R2 is the absorbance of the blank and R3 is the absorbance of sample after the addition of enzyme at a particular level.
Aebi, H. 1984 provided a method for determining the activity of CAT. The activity was started by adding 100μl of insect homogenates containing 3% H2O2 to 0.05M phosphate buffer (pH 7.0), recording the absorbance change at 240 nm for 3 minutes, and expressing the activity as n mole H2O /min/mg protein. This formula was used to calculate the activity:

\[
\text{CAT} = \frac{R_1 - R_2}{T}
\]

Where R1 represents the initial reading at 0 minute, R2 represents the final value at 3 minutes, and T represents the time intervals. The glutathione peroxidase (GPx) activity was determined with the method of Paglia, D. E & Valentine, W. N 1967 as modified by Bamidele, O. J et al., 2013 for 3 minutes, the reaction as monitored at 340 nm absorbance indirectly as the oxidation rate of NADPH. The non-enzymatic oxidation of NADPH with the addition of H2O2 in 0.1 M Tris buffer, pH 8.0 was tested using a blank without insect homogenate as a control. The formula below was used to calculate the activity of enzyme:

\[
\text{GPx} = \frac{2(m\text{Rate}_s - m\text{Rate}_b) \times V_{\text{Roxm}} \times df}{6.22 \times V_s} \times \frac{df}{T}
\]

Where,
- \( m\text{Rate}_s = -1000 \times \Delta A_{340}/\text{min of sample} \)
- \( m\text{Rate}_b = -1000 \times \Delta A_{340}/\text{min of blank} \)
- \( 6.22 = \text{NADPH} 340\text{nm millimolar absorption coefficient at 1 cm path length} \)
- \( V_{\text{Roxm}} = \text{Volume of Reaction Mixture} \)
- \( V_s = \text{Volume of sample} \)
- \( 2 = \text{adjustment for 2 moles GSH oxidized to 1 mole GSSG per mole NADPH} \)
- \( \text{df} = \text{Sample dilution factor} \)

**Determination of Acetylcholinesterase (AChE) activity**

The activity of AChE was determined using the Ellman, G.L et al., 1961 method, as reported by Bocquene, G & Galgani, F 1998. The enzymes were derived from supernatants, and the substrate was acetylthiocholine iodide (ATCL) at 0.25mM. In 0.1 M phosphate buffer (pH 7.4; 600 μL), aliquots of enzyme (100 μL) and DTNB (100 μL of 0.01 M) were added, followed by 100 μL phenylpropene test solutions made in absolute ethanol. Control experiment was prepared by adding 100 μL absolute ethanol. The mixtures were incubated at 35°C for 15 min and 100 μL ACTL was added to start the reaction while the absorbance was measured at 412 nm with photo-spectrometer.

The percentage inhibition of terpinolene was calculated according to the formula: % of AChE Inhibition: 100 - [(\( V_0 \) of compound treatment/\( V_0 \) of control treatment) \( \times \) 100] (Adesina, J. M. et al., 2018).

**Determination of Carboxylesterase activity (CarEST)**

The method of Van-Aasperen, K. 1962 was used to determine the activity of CarEST. Undiluted 100μl of the homogenates and diluted 100μl of the homogenates were incubated briefly with 1ml of sodium phosphate buffer (20mM, pH 7.0) containing 250μM of naphthyl acetate for 30 minutes at 28°C. Four hundred microliter (400 μl) of freshly prepared 0.3% fast blue B in 3.3% SDS was added to stop the enzymatic reaction and the colour was allowed to develop for 15minutes at 28°C. The optical density of the sample was read at 430nm against the reagent blank in Shimadzu UV-160A spectrophotometer.

**Determination of glutathione transferase (GST) activity**

The method of Habig, W. H et al., 1974 was used to determine the GST catalytic activity. The activity was determined spectrophotometrically using Biochrome-4060 model spectrophotometer with the aromatic substrate, 1-chloro-2, 4 dinitobenzene (CDNB) by monitoring the change in absorbance, due to thioester formation at 340 nm and 25 °C. The assay mixture contained in a total volume of 1ml which comprised 0.1M potassium phosphate buffer at pH 7.4, 1 mM CDNB in ethanol,1 mM GSH and the enzyme solution. The full assay mixture's absorbance at 340 nm was compared to a control containing buffer instead of the enzyme and treated similarly. The product extinction coefficient was calculated to be 9.6 mM-1, which represents the quantity of enzyme required to catalyze the synthesis of 1mol/min/mg protein. Protein concentration was determined by the method of Bradford, M. M (1976) using bovine serum albumin (BSA) as a standard. GST activity was calculated with the formula below:

\[
\text{GST activity} = \frac{\Delta OD_{340}\text{min}/\text{min} \times 100 \mu \text{l}}{2.99 \text{mM}^{-1} \times 10\mu \text{l} \times \text{Sample dilution} \times 1000}
\]

**Evaluation of Results**

All the mean values obtained in each experimental setups and controls were subjected to one analysis of variance (ANOVA) and means were separated using Tukey’s Honestly Significant Difference Test. SPSS version 21 was used for the analysis.

**RESULTS**

**Repellent activities of terpinolene against adult R. dominica**

The repellent activity significantly (p< 0.05) varied with the treatment dosage and period of exposure (Table 1). At all dosage levels, the percentage repellent achieved by the treatment was significantly different from the negative control. Within 0-1 h of application, only the positive control and 1.0 ml of terpinolene was able to achieved up to 100% repellent against the adult beetle and were significantly different from other dosages. At 4-8 h of exposure, all the terpinolene
dosages were able to achieved above 30% repellent and were significantly (p< 0.05) different from the negative control.

Table 1: Repellent activity of terpinolene against adult R. dominica

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dosage (ml)</th>
<th>% repellent in hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-1</td>
<td>1-4</td>
</tr>
<tr>
<td>Terpinolene</td>
<td>0.2</td>
<td>0.00±0.00a</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.00±0.00a</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>22.00±4.90b</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>60.00±8.94c</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>100.00±0.00d</td>
</tr>
<tr>
<td>DDVP</td>
<td>0.1</td>
<td>100.00±0.00d</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.0</td>
<td>0.00±0.00a</td>
</tr>
</tbody>
</table>

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

Effect of terpinolene on the antioxidant enzymes activities in R. dominica

The effect of terpinolene on the activities of SOD, CAT and GPx was directly proportional to its dosages (Figure 1, 2 & 3). Statistically significant differences existed between the treatments at $F_{6, 28} = 29.940$, $p < 0.0001$ (SOD), $F_{6, 28} = 13.939$, $p < 0.0001$ (CAT) and $F_{6, 28} = 54.988$, $p < 0.0001$ (GPx). Tukey multiple comparison showed that for the SOD, significant difference did not exist between 0.2 and 0.4 ml terpinolene at $p = 0.519$. Significant difference existed between positive control and 0.8 ml (p= 0.082) of terpinolene. While for CAT, no significant difference existed between 0.8 ml and 1.0 ml (p= 0.832) of the compound. Tukey multiple comparison showed that GPx exhibited non-significant difference between 0.2 ml and 0.4 ml of terpinolene at $p= 0.556$ and between 0.4 ml and 0.6 ml of terpinolene at $p= 0.973$. The highest SOD activity was recorded by the negative control (5.77 µmol/min/ml) while the lowest activity of 0.29 µmol/min/ml was recorded by the positive control. None of the dosages was able to inhibit the activity of SOD completely. The lowest CAT activity (0.86 µmol/min/ml) was recorded by the positive control while the highest activity (7.38 µmol/min/ml) was recorded at 0.4ml of terpinolene and was significantly different from others. At 1.0 ml dosage of terpinolene, the highest activity of GPx (11.05 µmol/min/ml) was recorded while the lowest activity (0.84 µmol/min/ml) of the enzyme was recorded in the positive control.

Figure 1: Effect of terpinolene on the activity of SOD in R. dominica larvae
Figure 2: Effect of terpinolene on CAT activity in *R. dominica* larvae

Figure 3: Effect of Terpinolene on GPx activity of *R. dominica* larvae

**Effect of terpinolene on AChE and CarEST activities**

The activities of AChE and CarEST varied with the dosages of the compound (Figure 4 and 5). Statistically significant variations existed between the treatments at $F_{6, 28} = 55.646, p < 0.0001$ (AChE) and $F_{6, 28} = 38.480, p < 0.0001$ (CarEST). For AChE, the Tukey post hoc test showed that there were no statistically significant differences existed between 0.2 ml and 0.4 ml terpinolene ($p=0.808$) as well as between 0.2 ml and the negative control ($p=0.998$). For CarEST, the Tukey post hoc test showed that there were no statistically significant differences existed between 0.2 ml of the compound and the negative control at $p=0.291$ as well as between 0.2 ml and 0.4 ml of the compound ($p=1.000$). The highest AChE activity was recorded by the 0.4 ml of compound (6.31 µmol/min/ml) while the activity of the enzyme is almost inhibited as only 0.0112 µmol/min/ml activity of the enzyme was recorded by the positive control and was significantly ($p<0.05$) different. The highest CarEST activity was recorded by the 0.6 ml dose of compound (7.90 µmol/min/ml) while the lowest activity of the enzyme was recorded by the positive control (0.0231 µmol/min/ml) and was significantly ($p<0.05$) different from other except 1.0 ml compound.
Effect of terpinolene on the activities of GST

The activity of the enzyme dependent on the dosages of the compound (Figure 6). Significant statistical difference existed between the dosages at \( F_{6, 28} = 22.587, p < 0.0001 \). Tukey multiple comparison showed that significant difference was existed between the 0.2 ml and 0.6 ml of the compound at \( p = 1.000 \). Also, significant difference existed between 0.2 ml of the compound and 0.8 and 1.0 ml of terpinolene at \( p = 1.000 \) and \( p = 0.280 \) respectively. The highest GST activity of 36.52 µmol/min/ml was recorded at 0.6 ml of terpinolene while the lowest activity of the enzyme (7.43 µmol/min/ml) was recorded by the positive control but its effect was not significantly different from 1.0 ml dosage of the compound.

Figure 4: Effect of terpinolene on AChE activity in R. dominica larvae

Figure 5: Effect of terpinolene on activity of CarEST in R. dominica larvae

Figure 6: Effect terpinolene on GST activity in R. dominica larvae
**DISCUSSION**

Due to the various adverse effects associated with synthetic chemical insecticides, many researches have been conducted on other means of managing and controlling insect infestations. Botanical powders and extracts have been reported to possess high rate of insecticidal potentials. In spite of a lot of works that have been done on plant products, only few of myriad active compounds present in this botanicals have been tested against different species of stored product insect pests (Isman, M. B 2006; Forim, M. R et al., 2012; Forim, M.R et al., 2012; Martins, C. H. Z et al., 2012 & El-Wakeil, N. E., 2013) opined that immediately a botanical extract or powder has been proven insecticidal, and the potential active compounds present in it have been identified, the next thing is to test the compounds singly for their insecticidal potential.

The result of this work revealed that terpinolene had a significant effect on adult *R. dominica* as it was able to repel the insect. The ability of the compound to repel the insect could be due to the fact that active compounds from plant has repulsive odours that discourage insect from feeding on protected commodities (Maia, M.F & Moore, S. J 2011). Insects detect odours of botanical active compounds when the odour bind to odorant receptor neurons of the insect and these compounds often block the olfactory organ of the insect (Lee, S. E et al., 2001; Gershenzon, J & Dudareva, N. 2007 and Ditzen, M. M et al., 2008) reported that many botanical volatile active compounds have high vapour toxicity against insects. Thus, the ability of the terpinolene used in this work could be that the active compound had a toxic vapour against the adult *R. dominica*. The result of this work agreed with the findings of Yang et al., (2014) in which five different isolated active compounds *Litsea cubeba* repelled adult *Lasioderma serricorne* and *Liposcelis bostrychophila*. The result also supports the findings of Nerio, L. S. J et al., (2010) and Caballero-Gallardo, K. J et al., (2011) in which several botanical active compounds were found to have significant repellent activities against wide range of insect pests.

The result obtained on the effects on terpinolene on the antioxidant, neurotransmitter and detoxifying enzymes in the adult *R. dominica* revealed that the compound had a significant effect on most of the enzymes. SOD being the first line of defence against toxic substance, preventing the accumulation of oxygen free radicals by removing superoxide radicals (O$_2^-$) to oxygen and hydrogen peroxide (H$_2$O$_2$) Kolawole, A.O et al., 2014 : Oni, M.O et al.,2019 was significantly inhibited by terpinolene at different dosages. Thus, this showed that the compound had some levels of toxicity against the enzyme. The decrease in the SOD activity with increase in the dosage of the compound indicates that Reactive Oxygen Species (ROS) may have accumulated in the cell of the insect larvae, probably the SOD was unable to scavenge them. The ability of the compound to significantly reduce the activity of SOD when compared to the DDVP effect on the enzyme showed that O$_2^-$ and H$_2$O$_2$ may have accumulated in the cell of the insect, causing some levels of oxidative damages to the terpinolene-stressed *R. dominica* (Caballero-Gallardo K. J et al., 2011). The findings of this work acquiesced with the work report of Kolawole, A.O et al., 2014 in which SOD activity in adult *C. maculatus* was drastically reduced at high dosage of a biopesticide. Similar results were also reported in the works of Wu, H.J et al., 2011: Aslanturk A.S et al., 2011 in which methidathion was found to decrease the activity of SOD in the mid-gut tissue of *Lymantria dispar*.

At low dosages, the activity of CAT increased significantly but was reduced significantly with further increase in the dosage of the compound. The increase in CAT activity revealed that there might have been some increase in the SOD activity, which could have in-turn caused decrease in H$_2$O$_2$ as opined by Aslanturk, A.S et al., 2011. Hence, it lowers the risk of hydroxyl radical accumulation (Fridovich I. 1997). This supported the report of Orr W.C & Sohal R.S 1994, which opined that CAT protects cells against oxidative stress and extends lifespan of insects. The decrease in the activity of CAT at high dosages of terpinolene infer that there could be some accumulation of H$_2$O$_2$ in the insect due to inability of the enzyme to catalyze it. This was in support of the Kaur, A et al., 2014 findings, in which CAT activity was find to reduced significantly at high dosage of a biopesticide. It was noted in this work that GPx activity increased regardless of the dosage of terpinolene. GPx, which is the second line of defence against oxidative stress in insect, is usually being activated when CAT fails to catalyze H$_2$O$_2$(Duntas, L. H 2012; Oni, M. O et al., 2019). The increase in the activity of this enzyme showed that the enzyme must have caused the glutathione-dependent reduction of lipid peroxides and hydrogen peroxide for detoxification at the membrane level into less reactive species by using GSH as substrate (Alli, Z.Y 2012; Sankar, P et al., 2012). The increase in the GPx activity showed that the enzyme was able to prevents the progressive accumulation of free radicals and in-turn protects the cell of the *R. dominica* against oxidative stress and lipid peroxidation (Sankar P et al., 2012). This result disagreed with the findings of Aslanturk, A.S et al., 2011 in which methidathion caused increase in oxidative stress of *L. dispar* larvae.

The activity of AChE significantly reduced when compared to the negative control and almost got inhibited at 1.0 ml of the compound. The reduction in the activity of AChE implies that there may have been some increase in the ACh concentration, which could have led to buildup of the neurotransmitter at nerve synapse and neuromuscular junctions (Rajashekar, Y et al., 2014). The inhibitory effect of terpinolene on the
activity of this enzyme could also be attributed to fact that botanical active compounds have broad impact across the nervous system of insects, which is attenuated by modified acetylcholine and acetate function as reported by Rajashkekar, Y et al., (2014). The result of this study was in acquiesced with the findings of (Kim, D. L et al., 2008: Begum, N et al., 2010; Olmedo, R et al., 2015; Prakash, K.S.B 2015 & Adesina, J.M et al., 2018) botanical-based insecticides were found to decrease AChE activity in insects.

In insects, CarEST is involved in many important physiological processes and plays an important role in the detoxification of insecticides to less toxic metabolites (Wheelock, C. E et al., 2005; Tarigan, S et al., 2016). In this work, it was noted that the activity of the enzyme increased at low dosages but its catalytic activity reduced significantly at high dosages. This work agreed with the findings of Smirle, M. J et al., (1996) that the activity of the enzyme was significantly reduced in neem oil stressed Choristoneura rosacea larvae. Ortego, F et al., 1999 reported the reduction of CarEST activity in the limnoid-stressed Leptinotarsa decemlineata larvae. This result also supports the findings of Nathan, S.S et al., 2008: Caballero, C et al., 2008 & Malahat, M M et al., 2015.

GST is a multifunctional enzyme responsible for the detoxification of many toxic substances in insects was greatly reduced in the larvae of R. dominica exposed to high dosages of terpinolene. This reduction in the activity of GST implies that the protein content of the enzyme may have been affected, as GST is an enzyme with 85% protein content Terrie, L.C 1984. Thus, this support the reports of War et al., (2013) and Tarigan, S et al., 2016 that botanical insecticides have ability to reduce total protein in insects. Ebadolahi, A et al. 2013 & Adesina, J.M et al., 2018 reported a similar result in which botanical extract was found to cause low protein content in the body of Tribolium castaneum Sitophilus oryzae and in turn cause inhibition of GST.

CONCLUSION
In respect of the findings from this research, it is derived that terpinolene compound effects more repellent activity against the adult insects and displayed some level of inhibitory effects against the enzymes at higher dosages. The compound had more effects on the SOD and the AChE activities compared to other enzymes, this implies that the mode of action of the compound could be the inhibition of these two enzymes. Inclusion of terpinolene compound in integrated pest-management strategies for the control of R. dominica is hereby suggested.

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Conflicts of Interest: Authors claim no conflict of interest.

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