Efficiency of Two Phytochemical Compounds and a Biopreparation against the Common Prevailing Land Snail Species in Alexandria Governorate, Egypt.

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DOI: 10.21608/JALEXU.2022.170768.1093

ABSTRACT: Phyto-molluscicides are becoming interesting due to its environmental friendliness, accessibility and ease of application. The efficient activity of Azadirachta indica and Mentha piperita essential oils, and a commercial formulation of Bacillus thuringiensis as biopesticide, were tested for their molluscicidal activity against two terrestrial snails’ E. vermiculata and T. pisana. The bioassay technique was performed using the Dipping leaves method to estimate LC50 and LC90 values against both terrestrial snails; E. vermiculata and T. pisana, under laboratory conditions. The obtained results indicated that the tested essential oils of A. indica and M. piperita were more toxic against T. pisana than E. vermiculata snails. Where, the deduced LC50 values of A. indica were 0.87% and 2.37% for M. piperita against T. pisana. Likewise, against E. vermiculata were 2.26 and 9.523%, respectively after 72 h post treatment. On the other hand, the toxicity of B. thuringiensis was more effective against E. vermiculata than T. pisana snails, where the LC50 values were 2.16% and 8.81%, respectively, after 72hrs post treatment. This study elucidated the promising efficient use of essential oils and or biopesticides in controlling the harmful gastropod pests.

Keywords: Terrestrial snails, Land snails, essential oils, Bacillus thuringiensis, Azadirachta indica, Mentha piperita

INTRODUCTION

Mollusks are the second biggest phylum in the animal kingdom, accounting for a significant portion of the world’s fauna. Only the Gastropoda have successfully invaded land along all classes of mollusks (Sandeep et al., 2012). Mollusks are a varied category of invertebrate animals having soft, unsegmented bodies. (O’Connor and Crowe, 2005). Terrestrial gastropods are classified as agricultural pests because they damage crops and cause financial loss by eating the leaves, roots and fruits of plants, as well as numerous horticultural plants ornamental plants, wood trees, and newly sown lawn grasses. They also decrease crop quality and contaminate agricultural products with their bodies, feces, or slime (Barker, 2002; Eshra, 2004; Awad et al., 2012; Puizina et al., 2013; Awad, 2014; Ali, 2017; Hussein and Sabry, 2019). As a following result, an unpleasant odor develops, preventing both humans and animals from eating these contaminated plants (Shetaia et al., 2009).

Today’s gastropods in Egypt are observed attacking various plantations (Kassab and Daoud, 1964; El-Okda, 1979; El-Deeb et al., 1996; Abu-Bakr, 1997; Eshra, 2004). Both E. vermiculata and T. pisana are significant agricultural pests that inflict significant damage in agriculture and horticulture. They are particularly destructive in locations where they may find the right circumstances for fast growth (Puizina et al., 2013).

Methomyl and other oxime-carbamates insecticides, which are used to control snails, are moderately poisonous to land snails but extremely hazardous to mammals and beneficial insects (Hussein et al., 1994; Radwan and El-Zemity, 2007; Salama et al., 2005; Abdelgaleil, 2010). Because of the aforementioned drawbacks of conventional pesticides, the request for safe and environmentally friendly alternative molluscicides is critical in order to protect human health and the environment.

Essential oils are green insecticides that are non-toxic to mammals and other species (Ferreira et al., 2009; Stroh et al., 1998). No residue-related problems exist (Misra and Pavlostatth, 1997). Essential oils and their constituents are gaining popularity as safe
alternatives to pesticides for controlling a wide range of pests, including gastropods, acting as insecticides (Isman et al., 2001; Hussein, 2005; Radwan et al., 2008; Pavela, 2015; Klein et al., 2020; Owolabi et al., 2020).

The biological management of land snails or slugs utilizing microbial agents, i.e., bacteria, is an alternative strategy that gained more attention a few years ago and offers efficient control against land snails. *Bacillus thuringiensis* (Bt) is a soil gram-negative bacteria that generates pest-toxic compounds. Recently, it has emerged as one of the biological control agents for a number of insect pests (Dean, 1984). Numerous studies have examined this bacterium's toxicity toward a some land snails in Egypt (Zedan et al., 1999; Azzam and Belal, 2003; Kramarz et al., 2007).

The main aim of this work is to evaluate the molluscicidal effect of *A. indica*, *M. piperita*, essential oils and a Biopesticide formulation Biotect® 9.4%W.P, a commercial formulation of *B. thuringiensis*, by dipping technique application and different concentrations under laboratory conditions against the most common land snails infesting various fruit orchards: *E. vermiculata*, and *T. pisana*.

2. MATERIALS AND METHODS
2.1. MATERIALS
a. Biotect 9.4%W.P a Commercial formulation of *Bacillus thuringiensis*.

b. Neem oil (Azadirachta indica) and peppermint (*Mentha piperita*) essential oil were purchased as pure oil from National Research Center, Dokki, Cairo, Egypt.

c. Acetone (pure) was purchased from Al-Nasr Pharmaceutical Chemicals Co. (ADWIC) (Egypt).

d. Homogenous disks of lettuce leaves were dipped in a series of each of *A. indica*, *M. piperita*, and *B. thuringiensis* prepared concentrations for 5 min and left for dryness. The treated lettuce disks were transferred into plastic cups, and 10 adult snails were placed in each cup. Each treatment concentration had three replicates; results were detected along three different periods; after 24, 48, and 72 h, untreated lettuce disks were used as a check treatment. Mortality percentages were recorded after 24, 48, and 72 h post-treatment.

3. Statistical analysis.

Lethal effect was evaluated as percentages of cumulative daily mortality, corrected for mortality in the control variant according to Abbott's formula (Abbott, 1925) as follows:

\[
\text{Corrected Mortality} = \frac{(\text{Mortality}\% \text{ of treated land snails} - \text{Mortality}\% \text{ of control})}{(100 - \text{Mortality}\% \text{ of control})} \times 100
\]

treatment, respectively. Noticeably, the toxicity of neem oil increased as the time of exposure increased for both snails *T. pisana* and *E. vermiculata*. The obtained results indicated that neem oil was the high toxic against *T. pisana* than *E. vermiculata* snails.

The exhibited data in Table (2) show that tested peppermint oil was more toxic upon adults of *T. pisana* than *E. vermiculata* snails, with LC₅₀ values of 6.24, 3.19, and 2.37 % after 24, 48, and 72 hours, respectively. While, its deduced LC₅₀ values against *E. vermiculata* were 15.29, 11.07, and 9.523% after 24, 48, and 72hrs respectively. These results elucidate that the extracted LC₅₀ values of peppermint oil decreased with increasing the exposure time and indicated its higher toxicity against *T. pisana* than *E. vermiculata* snails.

3. RESULTS AND DISCUSSION
3.1. Efficiency of neem; peppermint essential oils and Biotect® (commercial formulation *B. thuringiensis*) against *T. pisana* and *E. vermiculata* snails.

The toxicity of (neem oil) against *T. pisana* and *E. vermiculata* was evaluated. The calculated LC₅₀ values for *T. pisana* were 2.66, 1.29, and 0.87% after 24, 48, and 72hrs post treatment, respectively (Table, 1). Also, the extracted LC₅₀ values for *E. vermiculata* were 5.35, 4.02, and 2.26% after 24, 48, and 72 h post-treatment. The results elucidate that the extracted LC₅₀ values of neem oil decreased with increasing the exposure time and indicated its higher toxicity against *T. pisana* than *E. vermiculata* snails.
Table (1): The calculated LC50 values of neem oil on adults of *T. pisana* and *E. vermiculata* snails under laboratory conditions.

<table>
<thead>
<tr>
<th>Land snails species</th>
<th>Exposure Time (hr)</th>
<th>LC50 %</th>
<th>Confidence Limits at 50% of probability</th>
<th>LC95 %</th>
<th>Confidence Limits at 95% of probability</th>
<th>Slope ±Variance</th>
<th>X2</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td>Lower</td>
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<tr>
<td><em>T. pisana</em></td>
<td>24</td>
<td>2.66</td>
<td>2.18</td>
<td>3.80</td>
<td>11.6</td>
<td>6.80</td>
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<td>48</td>
<td>1.29</td>
<td>1.07</td>
<td>1.63</td>
<td>16.7</td>
<td>9.05</td>
<td>45.7</td>
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<td></td>
<td>72</td>
<td>0.87</td>
<td>0.73</td>
<td>1.04</td>
<td>9.69</td>
<td>5.98</td>
<td>20.7</td>
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<tr>
<td><em>E. vermiculata</em></td>
<td>24</td>
<td>5.35</td>
<td>3.25</td>
<td>6.45</td>
<td>52.2</td>
<td>49.3</td>
<td>55.1</td>
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<td>48</td>
<td>4.02</td>
<td>2.57</td>
<td>9.30</td>
<td>102.9</td>
<td>30.25</td>
<td>105.3</td>
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<td></td>
<td>72</td>
<td>2.26</td>
<td>1.60</td>
<td>4.16</td>
<td>51.5</td>
<td>17.7</td>
<td>60.4</td>
</tr>
</tbody>
</table>

Table (2): The estimated LC50 values of peppermint oil on adults of *T. pisana* and *E. vermiculata* under laboratory conditions.

<table>
<thead>
<tr>
<th>Land snails species</th>
<th>Exposure Time (hr)</th>
<th>LC50 %</th>
<th>Confidence Limits at 50% of probability</th>
<th>LC95 %</th>
<th>Confidence Limits at 95% of probability</th>
<th>Slope ±Variance</th>
<th>X2</th>
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<td>Lower</td>
<td>Upper</td>
<td>Lower</td>
<td>Upper</td>
<td></td>
</tr>
<tr>
<td><em>T. pisana</em></td>
<td>24</td>
<td>6.24</td>
<td>5.62</td>
<td>7.8</td>
<td>56.23</td>
<td>33.33</td>
<td>139.9</td>
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<td></td>
<td>48</td>
<td>3.19</td>
<td>2.55</td>
<td>3.76</td>
<td>24.99</td>
<td>17.48</td>
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<tr>
<td></td>
<td>72</td>
<td>2.37</td>
<td>1.81</td>
<td>2.85</td>
<td>15.27</td>
<td>11.69</td>
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<tr>
<td><em>E. vermiculata</em></td>
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<td>15.29</td>
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<td>62.91</td>
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<td>11.07</td>
<td>9.32</td>
<td>14.45</td>
<td>61.92</td>
<td>37.23</td>
<td>149.6</td>
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<td>72</td>
<td>9.523</td>
<td>7.994</td>
<td>12.41</td>
<td>75.24</td>
<td>41.78</td>
<td>215.5</td>
</tr>
</tbody>
</table>

The included data in Table (3) represents the extracted LC50 values for the tested commercial formulation of *B. thuringiensis* (Biotect®) against *E. vermiculata* and *T. pisana* snails; their confidence limits, slope, and X2 values. The estimated LC50 values of Biotect® against *T. pisana* were 9.59, 15.7, and 8.81% after 24, 48, and 72hrs post treatment; while for *E. vermiculata* these values comprised 9.98, 16.3, and 2.16% after 24, 48, and 72hrs post treatment. The lower LC50 values were obtained with increasing exposure. The toxic efficiency of Biotect® was higher against *E. vermiculata* than *T. pisana* snails.

Table (3): The calculated LC50 values of experimental commercial formulation of *B. thuringiensis* (Biotect®) on adults of *T. pisana* and *E. vermiculata* snails under laboratory conditions.

<table>
<thead>
<tr>
<th>Land snails species</th>
<th>Exposure Time (hr)</th>
<th>LC50 %</th>
<th>Confidence Limits at 50% of probability</th>
<th>LC95 %</th>
<th>Confidence Limits at 95% of probability</th>
<th>Slope ±Variance</th>
<th>X2</th>
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<td>Lower</td>
<td>Upper</td>
<td>Lower</td>
<td>Upper</td>
<td></td>
</tr>
<tr>
<td><em>T. pisana</em></td>
<td>24</td>
<td>9.59</td>
<td>6.77</td>
<td>25.7</td>
<td>53.5</td>
<td>21.7</td>
<td>80.5</td>
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<tr>
<td></td>
<td>48</td>
<td>15.7</td>
<td>8.08</td>
<td>20.8</td>
<td>926.5</td>
<td>113.9</td>
<td>982.0</td>
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<tr>
<td></td>
<td>72</td>
<td>8.81</td>
<td>5.99</td>
<td>21.2</td>
<td>189.5</td>
<td>53.6</td>
<td>200.5</td>
</tr>
<tr>
<td><em>E. vermiculata</em></td>
<td>24</td>
<td>9.98</td>
<td>6.89</td>
<td>21.9</td>
<td>115.2</td>
<td>41.3</td>
<td>145.3</td>
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<td></td>
<td>48</td>
<td>16.3</td>
<td>10.5</td>
<td>20.8</td>
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<td>1.74</td>
<td>2.57</td>
<td>28.2</td>
<td>15.6</td>
<td>86.4</td>
</tr>
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</table>
The aforementioned results agree with Abobakr et al. (2022) who investigated the fumigant toxicity and feeding deterrent effect of extracted essential oils from Lavandula dentata, Juniperus procera, and Mentha longifolia against the land snail M. obstricta. It was found that the three tested EOs exhibited a strong feeding deterrent effect at sublethal concentrations. Also, Gabr et al. (2006) found that several neem formulations (Neemix4.5%) had molluscicidal action against M. obstricta and E. vermiculata snails in the laboratory and in the field. Also, Eshra et al. (2016) discovered that lavandula dentata oil derived from the leaves and flowers was fungitantly poisonous to T. pisana snails, with an LC50 value of 16.3 µL/L air. Boufatoum and Abu Bakri (2013) proved that the oil extract of Citrullus colocynthis with a concentration of 100 g had the greatest efficacy against T. pisana. Furthermore, the present results are in accordance with those reported by El-Zenity and Radwan (2001), who used peppermint, caraway, thyme, and chenopodium oils topically on adults of T. pisana and discovered that the oils had strong molluscicidal activity against the snails after a contact toxicity assay for 48 h.

In addition, the foregoing results of the biocide, B. thuringiensis, are in agreement with the findings of Zedan et al. (1999) who investigated the effectiveness of B. thuringiensis var israelensis against the land snail M. obstricta. They determined that the bacterial formulation was superior to methomyl. On the contrary, Genena et al. (2008) found that when E. vermiculata and Monacha cantiana snails were treated with eight strains of B. thuringiensis, none of the strains had any detrimental effect or caused death in each snail species. Also, Kramarz et al. (2007) conducted study on the Bt toxin (Cry1Ab's) impact upon the land snail Helix aspersa under laboratory environment, the toxin had no detrimental effects on the snail throughout the following stages of life.

CONCLUSION

The aforementioned study of the molluscicidal activity using neem and peppermint essential oils and the commercial formulation of Bacillus thuringiensis as biopesticide, were tested for their molluscicidal activity against two terrestrial snails: E. vermiculata and T. pisana. The bioassay technique was performed using the dipping leaves method against both terrestrial snails under laboratory conditions. The obtained results indicated that neem and peppermint oils were more toxic against T. pisana than E. vermiculata snails. On the other hand, the toxicity of B. thuringiensis was more effective against E. vermiculata than T. pisana snails. Essential oils and Bt biopreparation as biopesticide constituents are gaining increasing interest for use as safe alternatives to pesticides for controlling various pests, including gastropods.

REFERENCES


الملخص العربي

فعالية مركبين كيميائيين نباتيين ومستحضر حيوي على القواقع الأرضية السائدة في محافظة الإسكندرية، مصر.

حسن على عبدالحميد مصباح 1 أحمد كمال خليل مراد 1 السيد حسن عشري 2 ومسعودة رمضان الزروق 1

1- قسم وقاية النبات – كلية الزراعة (سابا باشا) – جامعة الإسكندرية – مصر.

أصبحت مبيدات الرخويات النباتية مثيرة للإهتمام بسبب كونها صديقة للبيئة وسهولة الوصول إليها وسهولة استخدامها. تم تقييم النشاط الابادي لزيت النيم وزيت النعناع الفلفلي العطري ومستحضر التجاري لكثيريا الباسيلس ثورينجنسيس كمبيد حيوي ضد قوقعة الحدائق الأبيض وقوقعة الحدائق البني. تم قياس النشاط الابادي لبكتيريا الباسيلس ثورينجنسيس كمبيد حيوي ضد قوقعة الحدائق الأبيض وقوقعة الحدائق البني. وتم استخدام طريقة غمس الأوراق لحساب تركيز المميت لـ 50% من العشيرة و 95% للقواقعة تحت الظروف المعملية. وأظهرت النتائج المعالمة أن كل من زيت النيم وزيت النعناع كان أكثر فعالية ضد قوقعة الحدائق الأبيض عن قوقعة الحدائق البني حيث بلغ تركيز المميت لـ 50% لزيت النيم 0.87% بينما كان تركيز النعناع 2.37% ضد قوقعة الحدائق الأبيض في حين أن تركيز النعناع 0.26% لـ 50% لزيت النيم. و 9.52% بعد المعالمة بـ 72 ساعة ضد قوقعة الحدائق البني ونسبة أخرى كانت بكتيريا باسيلس ثورينجنسيس أكثر فعالية ضد قوقعة الحدائق البني عن قوقعة الحدائق الأبيض فينها. وبلغ تركيز المميت النصفي للقواقعة المعالمة لها هو 2.16% و 8.81% على الترتيب بعد المعالمة بـ 72 ساعة. أظهرت هذه الدراسة أنها تدعم استخدام الزيوت العطرية كمبيدات حيوية ضد الرخويات الضارة.