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

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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 LC_{50} and LC_{95} 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 LC_{50} 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 LC_{50} 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 among 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 significantly more harmful than they were in the past (**Mortada, 2002**). In many Egyptian governorates, the land snails *Eobania vermiculata, Theba pisana*, *Helicella vestalis*, *Monacha obstructa*, and *Cochlicella acuta* have been 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 Pavlostathis, 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).

2.2. Experimental snails:

Adults of the brown garden snail, *E. vermeculata* (Müller), and the white garden snail, *T. pisana* (Müller), having approximately the same age and size were collected for laboratory experiments from El-Maamoura locality, Alexandria. These snails were transferred to plastic cups covered with cloth netting and maintained under laboratory conditions of 27 °C and 65% R.H. The snails were fed on lettuce leaves daily up to the initiation of tests. The snails were allowed to acclimatize to these conditions for two weeks. Dead snails were excluded as soon as possible, whenever needed.

2.3. Laboratory bioassay (Dipping leaves method)

The essential oils of each of *A. indica* and *M. piperita*, as well as the biopesticide formulation of *B. thuringiensis*, were tested for toxicity against *E. vermiculata* and *T. pisana*. 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:

$$\textbf{Corrected Mortality} = \frac{(\textbf{Mortality\% of treated land snails} - \textbf{Mortality\% of control})}{(\textbf{100} - \textbf{Mortality\% of control})} \times 100$$

The statistical toxicity indices, LC_{50} and LC_{95}, were estimated as described by **Finney (1952),** via the LdP line program (Ehab Soft, Cairo, Egypt).

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 LC_{50} 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 LC_{50} values for *E. vermiculata* were 5.35, 4.02, and 2.26% after 24, 48, and 72 h post 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 LC_{50} values of 6.24, 3.19, and 2.37 % after 24, 48, and 72 hours, respectively. While, its deduced LC_{50} values against *E. vermiculata* were 15.29, 11.07, and 9.523% after 24, 48, and 72hrs respectively. These results elucidate that the extracted LC_{50} values of peppermint oil decreased with increasing the exposure time and indicated its higher toxicity against *T. pisana* than *E. vermiculata* snails.

Table (1): The calculated LC_{50} values of neem oil on adults of *T. pisana* and *E. vermiculata* snails under laboratory conditions.

Land snails species	Exposure Time (hr)	LC_{50} %	Confidence Limits at 50% of probability		LC_{95} %	Confidence Limits at 95% of probability		Slope ±Variance	X^2
			Lower	Upper		Lower	Upper		
T. pisana	24	2.66	2.18	3.80	11.6	6.80	32.4	2.58±0.42	1.03
	48	1.29	1.07	1.63	16.7	9.05	45.7	1.48±0.19	0.91
	72	0.87	0.73	1.04	9.69	5.98	20.7	1.57±0.19	7.41
E.vermiculata	24	5.35	3.25	6.45	52.2	49.3	55.1	1.66±0.71	0.37
	48	4.02	2.57	9.30	102.9	30.25	105.3	1.17±0.20	5.15
	72	2.26	1.60	4.16	51.5	17.7	60.4	1.21±0.20	0.63

Table (2): The estimated LC_{50} values of peppermint oil on adults of *T. pisana* and *E. vermiculata* under laboratory conditions.

Land snails species	Exposure Time (hr)	LC_{50} %	Confidence Limits at 50% of probability		LC_{95} %	Confidence Limits at 95% of probability		Slope ±Variance	X^2
			Lower	Upper		Lower	Upper		
T. pisana	24	6.24	5.62	7.8	56.23	33.33	139.9	1.76±0.25	1.38
	48	3.19	2.55	3.76	24.99	17.48	45.01	1.84±0.24	7.64
	72	2.37	1.81	2.85	15.27	11.69	23.22	2.03±0.25	4.00
E.vermiculata	24	15.29	12.01	26.27	62.91	33.29	285.1	2.68±0.56	0.73
	48	11.07	9.32	14.45	61.92	37.23	149.6	2.2±0.31	1.55
	72	9.523	7.994	12.41	75.24	41.78	215.5	1.83±0.27	2.37

The included data in **Table (3)** represents the extracted LC_{50} values for the tested commercial formulation of *B. thuringiensis* (Biotect®) against *E. vermiculata* and *T. pisana* snails; their confidence limits, slope, and X^2 values. The estimated LC_{50} 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 LC_{50} values were obtained with increasing exposure. The toxic efficiency of Biotect® was higher against *E. vermiculata* than *T. pisana* snails.

Table (3): The calculated LC_{50} values of experimental commercial formulation of *B. thuringiensis* (Biotect®) on adults of *T. pisana* and *E. vermiculata* snails under laboratory conditions.

Land snails species	Exposure Time (hr)	LC_{50} %	Confidence Limits at 50% of probability		LC_{95} %	Confidence Limits at 95% of probability		Slope ±Variance	X^2
			Lower	Upper		Lower	Upper		
T. pisana	24	9.59	6.77	25.7	53.5	21.7	80.5	2.20±0.56	0.00
	48	15.7	8.08	20.8	926.5	113.9	982.0	0.93±0.27	0.45
	72	8.81	5.99	21.2	189.5	53.6	200.5	1.23±0.27	0.50
E.vermiculata	24	9.98	6.89	21.9	115.2	41.3	145.3	1.54±0.30	0.23
	48	16.3	10.5	20.8	9584	5214	9863	0.59±0.24	8.45
	72	2.16	1.74	2.57	28.2	15.6	86.4	1.47±0.24	1.81

The aforementioned results agree with those recorded by **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. obstructa*. 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. obstructa* 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 fumigantly poisonous to *T. pisana* snails, with an LC_{50} value of 16.3 µl/L air. **Boufatoum and Abu Bakri (2013)** proved that the oil extract of *Citrullus coloeynhis* 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-Zemity 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. obstructa*. 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 bioprepation as biopesticide constituents are gaining increasing interest for use as safe alternatives to pesticides for controlling various pests, including gastropods.

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الملخص العربي

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

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

2- معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الأسكندرية – مصر

أصبحت مبيدات الرخويات النباتية مثيرة للإهتمام بسبب كونها صديقة للبيئة وسهولة الوصول إليها وسهولة استخدامها. تم تقييم النشاط الابادى لزيت النيم و زيت النعناع الفلفلى العطرى و المستحضر التجارى لبكتيريا الباسيلس ثورينجينسيس كمبيد حيوي ضد قوقع الحدائق البني و قوقع الحدائق الابيض و تم عمل التقييم الحيوي باستخدام طريقة غمس الاوراق لحساب قيم التركيز المميت لـ50 % من العشيرة و 95% للقواقع ، تحت الظروف المعملية. و أشارت النتائج المتحصل عليها أن كل من زيت النيم وزيت النعناع كان اكثر فعالية ضد قوقع الحدائق الابيض عن قوقع الحدائق البنى حيث بلغ قيم التركيز المميت لـ50 % لزيت النيم بلغ 0.87 % بينما كان لزيت النعناع 2.37 % ضد قوقع الحدائق الابيض فى حين ان، بلغت قيمة التركيز المميت لـ50 % لزيت النيم بلغ 2.26% و لزيت النعناع 9.52 % بعد المعاملة ب 72 ساعة ضد قوقع الحدائق البنى ومن ناحية أخرى ، كانت بكتيريا باسيلس ثورينجينسيس أكثر فاعلية ضد قوقع الحدائق البنى عن قوقع الحدائق الابيض فبلغت قيمة التركيز المميت النصفي للقواقع المعاملة لها هو 2.16% و 8.81 % على الترتيب بعد المعاملة بـ 72 ساعة. أظهرت هذه الدراسة أنها تدعم الاستخدام الواعد للزيوت العطرية كمبيدات حيوية ضد الرخويات الضارة.