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Assessment of the damage caused by *Eobania vermiculata* and its control by metaldehyde on *Beta vulgaris* subsp. *cicla*

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Abstract \

The research was conducted in the Jabal Akhdar region between September 2018 and January 2019. Samples of the land snail *Eobania vermiculata* (O.F.Muller, 1774), (Helicidae) were randomly collected, cleaned, and cultured in a laboratory setting at a temperature of 25± 3°C and a relative humidity of 65±5%. The snails were placed in plastic containers with a top opening covered with gauz for ventilation. Paper tissue moistened with water was placed at the bottom of the containers to provide moisture, and Swiss chard (*Beta vulgaris* subsp. *cicla*) was provided as food. Water and food were replenished every three days. This experiment aimed to investigate the damage caused by different age groups *of E. vermiculata* snails on three age groups of Swiss chard plants, as well as the impact of metaldehyde on snails. Observations were made at 24, 48 and 72-hour intervals. The results indicated that plant age at four weeks showed a higher damage rate compared to plants at eight weeks, as older plants have greater resistance to infestation. Additionally, increasing snail age led to a higher degree of injury, with snails at 16 weeks showing the highest damage rate, followed by snails at eight weeks, and then four weeks. Furthermore, longer exposure times resulted in higher damage rates on Swiss chard plants, with five days showing the highest damage, followed by four days, and then one day. The interaction between plant age (2, 4and 8 weeks) and snail age (4and 8 weeks did not significantly affect plant damage at snail ages of four and eight weeks, but it was significant at 16 weeks. Similarly, the interaction between snail age, plant age, and exposure time did not significantly impact the average snail damage to Swiss chard plants in this study. The results on the effect of metaldehyde on *E. vermiculata* showed that the highest concentration of metaldehyde (8g) resulted in the highest mortality rates at three time periods (24, 48and 72 hours) (41.7%, 5.0%, 100 %). This was followed by 5g of metaldehyde, which recorded mortality rates of (0.00%, 25%, 66.7 %) after 24, 48, and 72h, respectively. In comparison, 3g of metaldehyde resulted in lower mortality rates (0.00%, 8.33%, 66.7%) a after the same time periods.

Keywords: *Beta vulgaris E .vermiculata*, Damage, Metaldehyde, snail age

Introduction \

Beta vulgaris L., the red beetroot, belongs to the Chenopodiaceae family of plants. It is most famous for its many cultivated variations, the most well-known of which is beetroot, often called table garden beetroot, which is a purple root vegetable. Beetroot can be boiled, baked, or used to extract juice. It can also be eaten fresh. You can enjoy red beets in salads, soups, pickles, and roasting. Unlike fruits, sucrose is the primary sugar found in beets [4]. For hundreds of years, red beets have been utilized in traditional medicine to cure dandruff, constipation, and pain in the intestines and joints [16]. *E .vermiculata*, Muller also known as the brown garden snail, is a species of land snails that is considered a global problem as a pest of agricultural, ornamental, and horticultural crops, it is common throughout many nations with varying environmental circumstances [11, 33]. Among mollusks, land gastropods are highly diversified category. Due to agriculture and human activity, a number of species of terrestrial snails and slugs have expanded into areas beyond of their original ecological range. These species damage important, profitable field crops and horticultural plants, turning them into pests in these new locations. In humid and temperate settings worldwide, terrestrial gastropods such as slugs and snails are considered to be major pests. Additionally, they spread parasitic illnesses that harm both agricultural animals and people [29]. Moreover, many of land snails are known to serve as intermediate hosts to human and livestock parasites of Platyhelminthes (15; 29) as well as the nematode parasite *Angiostrongylus cantonensis* in Egypt (33). Moreover, they act as vectors of many plant pathogens (4). Because each species has different environmental needs and behaviours, managing the population of land mollusks is not an easy undertaking [14]. Chemical management remains the most popular approach, particularly for protecting broad areas of plants from land snails, given there are only few specialist molluscicides available. It was therefore essential to discover novel, potent chemicals. According to [27]. Snails treated with the herbicide Grass ate moved more slowly after treatment than they did prior to the herbicide's application. Few chemical substances have been made available for sale with the intention of suppressing snail populations; instead, molluscicides and other insecticides, such as a few carbamates, have been employed as poisonous bai ts or contact poisons [32]. One of the most widely used molluscicides, metaldehyde, was first employed in snail baits in the early 1940s after being introduced in the late 1930s [8]. According to reports in the 1990s, 55% of crop areas treated with land snail pesticides also employed metaldehyde [12]. While compressed pellets are the most common application method, it can also be sprayed in an emulsified form or mixed into an edible matrix and used to cover an inert granular core [18]. According to [21]. metaldehyde causes irreversible, severe damage of the mucus cells required for gastropods to live on land, leading to dehydration and final death. Farmers who cultivate arable land use metaldehyde, which is non-toxic to plants, to protect crops such as potatoes, oilseed rape, and grains. Effective mollusk control is a major concern because without such a pesticide, there would be significant losses of valuable crop goods and resulting economic ramifications. It has been projected that the UK could lose up to £100 million in productivity annually due to a lack of effective slug control products [25]. When slug pellets are applied, metaldehyde concentrations in UK water bodies have often exceeded the EU's legal drinking water guideline for a single pesticide (0.1 µg/ L and 0.5 µg/L for all pesticides present) [23]. In 80% of slug pellets used worldwide, metaldehyde is the active component, usually at a weight percentage of 1.5, 3.0, or 4.0%. Its use as a molluscicide dates back to the early 1940s. Lonza produces metaldehyde under the brand name Meta® metaldehyde.

Then, several providers (like Certis or De Sangosse) create this active component [30]. Granular bait pellets sold under a variety of trade names such as Cekumeta®, Deadline ®, Hardy®, Metarex®, and Metason®.The main component of most slug pellets used to protect crops is metaldehyde. According to [6], this molluscicide is often found in surface water bodies above the EU statutory drinking water limit of 0.1 μg l^{-1} for a pesticide and is classified as an emerging contaminant. The stability of the microbiota in snails is crucial, just like in other animal species, as it plays a role in immune response and metabolism. However, the microbiota in snails is not stable due to noticeable dynamism resulting from its evolution over time due to various factors, including the snail's age, the type of food it eats, and environmental conditions [28; 15]. Therefore, the main objective of this research was to study the damage caused by the brown garden snail *E.vermiculata* and to determine the effect of toxic metaldehyde molluscicide used in control.

Materials and methods \

1. Sampling and Culturing of the Land Snail *E. vermiculata* Muller under Laboratory Conditions: Samples were collected randomly from the study site in the Jabal Akhdar region, Libya (32° 35′ 51.84″ N, 21° 28′ 22″ E) during the period between the months of September 2018 and January2019. They were cleaned and cultured in the laboratory under controlled conditions with a temperature of 25± 3°C and a relative humidity of 65 ± 5% inside plastic containers .The containers had a top opening covered with a piece of gauze to provide ventilate.

2. Snail Mass Rearing: The snails collected by hand from grass lawns were cleaned debris and kept in plastic container measuring 20 × 30 × 10 cm. A small window was cut in the snap cover of the container and layered with muslin cloth to facilitate aeration. Damp tissue papers were placed on the bottom of the container to maintain moisture. Fresh leaves of Swiss chard *Beta vulgaris* Subsp. *cicla* were used for feeding. Water and food were added every three days.

3. **Plant used:** Swiss chard *Beta vulgaris* subsp *cicla* was used to evaluate the damage caused by *E. vermiculata.* Young seedlings were planted in plastic cups 12 cm in depth and 9 cm in diameter. To prevent the snails from escaping grease was applied on the top of the container liners. The treatments were conducted inside a small netted farm in the Plant Protection Department laboratory.

4. Evaluating the damage by snail *E. vermiculata:* In this experiment three ages of snails, 2, 8 and 16 weeks old from one population of three snails per container and three ages of plants 2, 4, and 8 weeks old were chosen. Twelve treatments were arranged in four replicates. Small seedlings were transferred to small plastic containers. The damage was evaluated by either counting of stem and the number of holes on the leaves, or stem cutting. The observations were recorded daily for five days

5. The use of the molluscicide metaldehyde against *E. vermiculata* under laboratory conditions: This experiment was conducted to study the effect of metaldehyde on adult *E. vermiculata* snails. Three concentrations of metaldehyde (3, 5 and 8 g) were used to test the toxicity of metaldehyde on the adult snails. For each concentration, fresh lettuce leaves were dipped for three minute and then left to dry. Three snails were introduced into a box supplied with the treated lettuce disk in each treatment with four replicates for each concentration. The observations were recorded at intervals of 24, 48 and 72 hours.

6. Statistical analysis: The data were analyzed using SAS program. The LSD test was used at a significance level of 5% for comparing means.

Results and discussion \

Effect of plant age on the average snail damage to Swiss chard plants: Results as shown in Table (1). obtained to test the damage caused by snail which depends on the ages of the snails the size of the plants they are feeding on and the number of snails introduced. In the damage assessment experiment three different ages of *B. vulgaris* subsp *cicla* (2, 4 and 8 weeks) were exposed to snails at three different ages (4, 8 and 16 weeks) with density of 3 snails/ container. The damage caused by snails was rated in Table (2). It has been observed that the older the plant, the less harmful of the snails are to the Swiss chard plant. .Results showed that the plant age at four weeks recorded a higher damage rate (2.9), compared to plant age at eight weeks which recorded a lower damage rate (1.4), This is because the older the plant, the greater its resistance to infestation.

Results presented in Table (3) showed that increasing snail age increased the degree of injury. Snails at 16 weeks of age recorded a damage rate of followed by snails at eight weeks of age with a rate of as compared to snails at four weeks of age which recorded a lower damage rate of on chard plants.

Results in Tables (4, 5) showed that the influence of time (days) on the average snails damage to

Swiss chard plants. Increasing time up to five days resulted in a higher damage rate of 2.6 followed by four days with a rate of 2.3 as compared to one day which recoded the lower damage rate of 1.4

Interaction between plant age (2, 4, 8 weeks) and snail age (4, 8 weeks) on plant damage was not significant after four and eight weeks, but was significant with snails at 16 weeks of age (Table 6). Also, interaction between snail age, plant age and time on the average snail damage to Swiss chard plant was not-significant in this study (Table 7).

Results in Tables (8 and 9), showed that the highest concentration of metaldehyde (8g) recorded the highest mortality rated at three time periods (24, 48, 72 h) (41.7, 75.0, 100%), followed by 5g metaldehyde which recorded (0.00, 25, 66.7%) after (24, 48, 72 h), compared to 3g metaldehyde which recorded the lower rates of mortality (0.00, 8.33, 66.7%) after three periods (24, 48, 72 h), respectively. On the other hand, the higher value of LC_{50} and LC_{95} were recorded after 48 hour (6.60 and 10.6), while LC_{50} and LC_{95} recorded (2.52 and 8.91) after 72 hour. Confidence Limits at 50% and 95% 48h showed higher values probability after ranging between (1.07 to 8.99) and (9.05 to 15.7), while Confidence Limits at 50% and 95% of probability after 72 h showed lower values (0.73 to 5.04) and (5.98 to 13.7), respectively.

These results are agreement with [13].who found that metaldehyde, methiocarb, thiocarb, cyanophos and monocrotophos were tested using baits against two land snails, *Monacha cartusiana* and *Eubania vermiculata*. They found that mortality after 14 days was 93, 87, 70, 45 and 36% for *M. cartusiana* and 85, 82, 63, 39 and 28% for *E. vermiculata*, respectively. Also [1] found that metaldehyde, profenofos were the most toxic to *E. vermiculata* and *M. cartusiana* with toxicity indices of 88.6, 97.3 and 100.0, 100.0, respectively. Additionally [9] studied the effect three molluscicide, Gastrotox, Mlotov, and Mesurol on tissues of the two land snails, *Monacha cantiana* and *Eobania vermiculata*. They found that the three molluscicides affected the activities of five vital enzymes, total lipid and total protein when applied against the tested land snails.In another study by [1] on toxicity methomyl, aldicarb, *Oxychilus* sp., it was oxamyl, methiocarb, du-pont-1642 and metaldehyde by using baits against *Helix* sp., *E .vermiculata*, *T. pisana*, *Rumina* sp., *Cochlicella* sp., *Helicella* sp. *Limax* sp. and found that aldicarb, methomyl, oxamyl and du-pont-1642 showed the highest toxicity, while methiocarb and metaldehyde [10] showed less toxicity. Furthermore evaluated the efficacy of the locally formulated 0.5 %, aldicarb, oxamyl, methiocarb, Lannat and metaldhyde in controlling land molluscs such as *H. aspersa*, *Eobania* sp., *Theba* sp., *Rumina* sp. and *Oxychilus* sp. The results indicated that, aldicarb, oxamyl and Lannat exihibited the highest toxicity against most snail and slug species, while methiocarb and metaldehyde were less toxic.

Such in accordance with those obtained by [13] who finding are tested metaldehyde, methiocarb, thiocarb, cyanophos and monocrotophos using baits against two land snails, *M. cartusiana* and *E. vermiculata*. They found that mortality after 14 days was 93, 87, 70, 45 and 36% for *M. cartusiana* and 85, 82, 63, 39 and 28% for *E. vermiculata*, respectively. Similar toxic effects were also found by [20] who reported that methomyl and metaldehyde, gave the highest reduction percentage for *M. cartusiana* compared to the other tested compounds under field tested metaldehyde against the land snail *M .cartusiana* under laboratory conditions. Additionally [7] and field conditions. They revealed that the highest concentration of metaldehyde gave 100% mortality after 7 days. [2] evaluated the molluscicidal activity of methomyl and Protecto under field conditions. Methomyl gave the highest toxic effect against, *M cartusiana* and *E. vermiculata* snails while Protecto was the least effective compound during 15 days after treatment. [1] found that metaldehyde, profenofos were the most toxic to *E. vermiculata* and *M. cartusiana* with toxicity indices of 88.6, 97.3 and 100.0, 100.0, respectively.

The principal toxic effect of metaldehyde is through stimulation of the mucous gland which causes excessive sliming, leading to death by dehydration [18]. Also, [3] studied that metaldehyde can affect mollusks either by contact, with absorption through the skin, or through the gut when eaten. The main effect is that of an irritant, causing the molluscs to produce masses of mucus, leading to dehydration and sometimes death. Loss of mucus also means that the animals can no longer move around. Another study mentioned that damage caused by terrestrial gastropods dependents not only their activity and population density but also on their feeding habit which differ from species to species [14]. The injuries affected by land snail species varied greatly from place to place depending on the abundance of the animals, the nature and extent of their food supply and weather conditions [22].

Table (1): Effect of plant age on the average snail damage to Swiss chard plant

Plant age (week)	Average of snail damage to Swiss chard plant
2	2.8
4	2.9
8	1.4
$LDS_{(0.05)}$	**0.12**

Table (2): Interaction between plant age and snails ages on plant damage.

Plant age (week)	Snail age (weeks)		
	4	**8**	**16**
2	0.88	1.06	5.00
4	1.00	1.00	5.00
8	0.80	1.26	1.93
$LSD_{(0.05)}$	**0.20**		

Table (3): Effect of shell age on the average snail damage to Swiss chard plant.

Snail age (week)	Number of snails on chard plant
4	0.9
8	1.2
16	4.0
$LDS_{(0.05)}$	**0.11**

Table (4): Effect of time (days) on the average snail damage to Swiss chard plant.

Time (days)	Number of snails on chard plant
1	1.4
2	1.8
3	2.0
4	2.3
5	2.6
$LDS_{(0.05)}$	**0.15**

Table (5): Interaction between shell ages on the average snail damage to Swiss chard plant.

Plant age (week)	Time (days)				
	1	**2**	**3**	**4**	**5**
2	1.66	2.11	2.11	2.77	2.77
4	1.66	2.33	2.33	2.33	3.00
8	0.77	0.88	1.44	1.66	1.88
LSD$_{(0.05)}$	**0.26**				

Table (6): Interaction between snail age on the average snail damage to Swiss chard plant.

Snail age (week)	Time (days)				
	1	**2**	**3**	**4**	**5**
2	0.00	0.55	0.88	1.11	1.77
4	0.22	0.77	1.00	1.66	1.88
8	0.88	4.00	4.00	4.00	4.00
LSD$_{(0.05)}$	**0.26**				

Table (7): Effect of plant age, snail age and time (days) on the average snail damage to Swiss chard plant.

Plant age (week)	Snail ages (week)	Time (days)				
		1	**2**	**3**	**4**	**5**
2	4	0.00	0.66	0.66	1.33	1.33
	8	0.00	0.66	0.66	2.00	2.00
	16	5.00	5.00	5.00	5.00	5.00
4	4	0.00	1.00	1.00	1.00	2.00
	8	0.00	1.00	1.00	1.00	2.00
	16	5.00	5.00	5.00	5.00	5.00
8	4	0.00	0.00	0.00	1.00	2.00
	8	0.66	0.66	0.66	2.00	1.66
	16	1.66	2.00	2.00	2.00	2.00
LSD$_{(0.05)}$		**0.23**				

Table (8): Effect of metaldehyde on mortality percentages of *Helix aspara*.

Conc. (g)	Mortality % ±SD		
	24h	**48h**	**72h**
3	0.00±0.00	8.33±0.5	66.7±0.82
5	0.00±0.00	25.0±0.96	66.7±1.15
8	41.7±1.23	75.0±0.5	100±0.00

Table (9): LC_{50} and LC_{95} and Confidence Limits at 50and 95% of probability of *E.vermiculata*.

Exposure Time (hr)	LC_{50} Gm	Confidence Limits at 50% of probability		LC_{95}%	Confidence Limits at 95% of probability		Slope	X^2
		Lower	Upper		Lower	Upper		
48	6.60	1.07	8.99	10.6	9.05	15.7	8.04±0.84	10.03
72	2.52	0.73	5.04	8.91	5.98	13.7	2.99±0.52	23.2

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الملخص العربي/

تقدير الضرر الناتج عن قوقع الحدائق *E.vermiculata* ومكافحته بالميتالدهيد علي نبات السلق *Beta vulgaris* subsp. *cicla*

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أجري هذا البحث في منطقة الجبل الأخضر خلال الفترة ما بين شهري سبتمبر2018 ويناير 2019 حيث تم جمع عينات القوقع عشوائياً و تنظيفها وتربيتها تحت ظروف المعمل وكانت درجة الحرارة 25± 3 درجة مئوية والرطوبة النسبية 65±5% داخل اوعية بلاستيكية ذات فتحة علوية مغلقة بقطعة من الشاش لتوفير التهوية، تم وضع القواقع في حاويات بلاستيكية، مع وضع قطع من المناديل الورقية المبللة بالماء في الأسفل وذلك لتوفير الرطوبة داخل الوسط الذي يتم تربية القواقع فيه، مع إضافة اقراص من اوراق نبات السلق للتغذية والماء كل ثلاثة أيام.، تم استخدام مبيد الميتالدهيد فى صورة طعم جاهز لدراسة تأثيره على القوقع *E. vermiculata* تم وضع ثلاث قواقع في كل مكرره ، وتم تسجيل الملاحظات على فترات 1 ، 6 ، 12 ساعة. تم تحليل البيانات بواسطة برنامج SAS أظهرت النتائج المتحصل عليها أن عمر النبات عند أربعة أسابيع سجل أعلى معدل ضرر، مقارنة بعمر النبات عند ثمانية أسابيع الذي سجل أقل معدل تلف، وذلك لأنه كلما زاد عمر النبات زادت مقاومته للإصابة،كما أن زيادة عمر القوقع, أدى إلى زيادة درجة الضرر، حيث سجل عمر القوقع عند 16 أسبوع أعلى معدل ضرر ، يليه عمر القوقع عند ثمانية أسابيع، مقارنة بعمر القواقع عند أربعة أسابيع الذي سجل معدل ضرر أقل في نبات السلق. ومع ذلك، فإن زيادة الوقت حتى خمسة أيام سجلت معدل الضرر الأعلى على نبات السلق، تليها أربعة أيام، مقارنة بالوقت بعد يوم واحد الذي سجل معدل الضرر الأقل، على التوالي. أما التداخل بين عمر النبات (2،4، 8 أسابيع) وعمر القواقع (4،8 أسبوع) في تلف النبات فلم يكن معنوياً بعد أربعة وثمانية أسابيع، في حين كان معنوياً مع عمر القواقع (16 أسبوع). كما أن التداخل بين عمر القواقع وعمر النبات والوقت علي متوسط ضرر القواقع لنبات السلق لم يكن معنوياً تحت هذه الدراسة. وقد اوضحت الدراسة انة عند تعريض قوقع الحدائق *E. vermiculata* الي ثلاث تركيزات من مبيد الميتالهيد 8 جرام , 5 جرام , 3جرام علي ثلاث فترات (24, 48, 72 ساعة) كانت أعلي نسبة موت عند تركيز 8جرام متبوعا بتركيز 5جرام وأقل نسبة سجلت عند تركيز 3 جرام.

الكلمات الدالة: Metaldehyde, , *Beta vulgaris E . vermiculata*, عمر القوقع، الضرر