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TOXICOLOGICAL EFFECTS OF SOME SINAI'S FLORA EXTRACTS AGAINST THE GREATER WAX MOTH, *Galleria mellonella* L.

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ABSTRACT

This study was performed to assess the effectiveness of some Sinai's Flora extracted from thyme (*Origanum vulgare* L.), castor (*Ricinus communis* Linn), common horehound (*Marrubium vulgare* L.) and yellow nutsedge (*Cyperus esculentus* L.) against the larvae of greater wax moth, *Galleria mellonella* and on honeybee workers. Plants were collected from Sinai Peninsula, Egypt, during the period from November 2018 to February 2019, dried and ground. Aqueous extracts were used at 10, 15, and 20% levels and contact toxicity assay was performed in the laboratory on the larvae of greater wax moth, *Galleria mellonella*. The test was set up in a Complete Randomized Design (CRD) with 10 replications of each plant extract. The results revealed that, plant extracts tested at various levels showed variable responses against the larvae of greater wax moth, *Galleria mellonella* compared to the controls. The highest larval mortality percentage (92.15%) was recorded. The lowest LC50 values were obtained from aqueous extract of *O. vulgare* (7.07 %) against larvae of greater wax moth. The lowest toxicity effect against adults of honeybees was obtained when treated with aqueous extract of *C. esculentus* (4.24%) at 10% concentration. Hence, it be concluded that thyme extract at 20% concentration is a good natural pesticide against greater wax moth.

INTRODUCTION

Honeybees *Apis mellifera* L. are highly valued resource-insects all over the world, prized not just for producing honey, wax and other products (**Beyene and Mekonen 2019)**. Bees are vulnerable to a variety of diseases and pests **(Ritter and Akratanakul, 2006).**The greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) is a major pest of the honeybee and can cause substantial losses to combs, hive material and bees in beehives allover the world specially in a world with a warm climate **(Hachiro and Knox, 2000)** because it feeds on wax and pollen stored in the combs of active honeybee colonies **(Desalegn and Amsalu, 2001; Mohamed *et al.*, 2020).** For over a century, many intervention approaches have been tried around the world to combat the challenges of increased wax moth infestation. The most common method for controlling the larger wax moth pest is to utilise synthetic insecticides. On the other hand Synthetic insecticides have major downsides, including toxicological risks to beekeepers and bees. (**Beyene and Mekonen, 2019)**, causes the mortality of beneficial species like parasites and predators as well as mortality of pollinating insects like honey bees, risks of contaminating hive products are a global challenge **(Pirali-Kheirabadi and Silva, 2010).**The plants based insecticides being the natural plant products are safer and a great alternative to synthetic pesticides that has fewer negative effects on human health and the

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environment **(Koul *et al.*, 2008)** so their usage against pests as grown in significance everywhere around the world **(Hiremath, 1994; Lalita *et al.*, 2018).**

Natural products of plants and plant derivatives are an alternative to the insecticides currently in use bioactive **(Emara *et al.*, 2002; Tripathy and Singh, 2005**). Several Flora extracts or isolated active components have been demonstrated to be effective insecticides in both acute and chronic applications **(Moawad and Ebadah, 2007; Mohamed *et al.*, 2014)**. Sinai's Peninsula has a very wide variety of Flora and the homeland of some special and rare species. As a result, there is an increasing need for natural or alternative repellents. These products are both effective and safe for the environment. Some of this plant's extracts are frequently active against pest species. They grade into non-toxic compounds after biodegdation. Furthermore, plant-derived compounds have been discovered to be particularly efficient against insect pests that are resistant to insecticides. **(Aranson *et al.*, 1989; Kwon *et al.*, 1996; Ahn *et al.*, 1997; Koul *et al.*, 2008; Mohamed *et al.*, 2014)**. As a result, numerous studies began large-scale screening attempts for toxic effects in order to use them as insecticides **(Khambay *et al.*, 2002; El-Shazly and Hussien, 2004; Prowse *et al.*, 2006; Malarvannan and Subashini, 2007; Khalaf *et al.*, 2009; Mohamed *et al.*, 2014).** Despite the fact that plant extracts have been tested against a variety of Lepidopterous insects, no research has been done to see if plant extracts can kill wax moth larvae. It's critical to find a natural chemical with high insecticidal activity against the wax moth and low toxicity to honeybees. (**Lalita *et al.*, 2018)**.

Therefore, the goal of this study is to assess the impact of some Sinai's Flora extracted from thyme (*Origanum vulgare* L.), castor (*Ricinus communis* Linn), common horehound (*Marrubium vulgare* L.) and yellow nutsedge (*Cyperus esculentus* L.) against the larvae of greater wax moth, *Galleria mellonella* and on honeybee workers.

MATERIALS AND METHODS

Study Location

Experiment was performed in the apiary of the Faculty of Environmental Agricultural Sciences, Arish University, North Sinai Governorate, Egypt, during 2018/2019 seasons to evaluate the effect of some Sinai's Flora extracts *i.e.* thyme (*Origanum vulgare* L.), castor (*Ricinus communis* Linn), common horehound (*Marrubium vulgare* L.) and yellow nutsedge (*Cyperus esculentus* L.) at 10, 15 and 20% concentrations against the larvae of greater wax moth, *Galleria mellonella* and on honeybee workers.

Plants Collection and Identification

The chosen wild plants were gathered from various locations of Sinai Peninsula, Egypt, during the winter seasons (November 2018 to February 2019), based on traditional knowledge, previous study, and data from the literature. Taxonomic identification of selected plants was performed by botanists from the Faculty of Environmental Agricultural sciences, Arish University, Egypt (Table 1), Labeled and stored in plastic bags individually. The gathered plant material was then placed in a plant presser and kept in freezers similar to a cool box..

Preparation of Plant Extracts

The portions used materials from the collected wild plants were dried in the shade and mechanically ground into powder using a Wiley grinder, then passed through a fine mesh sieve to obtain fine powder (2 mm diameter. A 50-gram sample of the ground material was extracted according to the method described by **Zaitoun (2007) and Al-Ghzawi *et al.* (2008)** by cold percolation with 95% ethanol. The ethanolic extract of Thyme (*O. vulgare* L.),

Table 1. List of selected Sinai's Flora species collected and tested against the larvae of greater wax moth, *Galleria mellonella*

No.	Scientific name	Common name	Family name	Part Used	Harvested areas
1	*Cyperus esculentus* L	Yellow nutsedge	Cyperaceae	Tubers	South Sinai
2	*Marrubium vulgare* L	Common horehound	Lamiaceae	Aerial parts	El-Arish
3	*Origanum vulgare* L	Thyme	Lamiaceae	Whole plant	St. Catherine-South Sinai
4	*Ricinus communis* L	Castor	Euphorbiaceae	Seeds+leaves	Valley of El-Arish

castor (*R. communis* Linn), common horehound (*M. vulgare* L.) and yellow nutsedge (*C. esculentus* L.) were vacuum-concentrated, weighed, and the residue was applied in the testing. The extracts were then diluted in distilled water to achieve various concentration levels (10, 15 and 20%) for bioassay test on the larvae of greater wax moth, *Galleria mellonella* L. Negative and positive controls were utilized, respectively, with distilled water and ethanol solvents.

Collection of Wax Moth

Honey bee comb infested by wax moth and contain wax moth larvae were collected from the apiary of the plant production Dept., Faculty of Environmental Agricultural Sciences, Arish University, Egypt. The infested combs were transported carefully to the Environmental protection Laboratory, Faculty of Environmental Agricultural sciences, Arish University, Egypt. 4th larval instar of *Galleria mellonella* were selected to conduct the experiment. The instars were identified based on the calculated age of the larva according to its width of the head capsule and weight **(Sehnal, 1966)**. Each individual larva was placed in separate plastic Petri dish containing feeding mixture mixed with 0.1 mg of the examined plant extract, resulting in only one extract being administered to each larva. No extract was utilized as a control. Each plant extract was replicated ten times (ten replicates) for each in a complete randomized design.

Testing for Bioassay

Using distilled water, three distinct concentration levels (5, 10, and 15%) of each selected plant were created. The experimental larvae were incubated at 25°C with relative humidity 70% and 24 hours darkness. Ten larvae were immersed in petri plates with 5, 10, and 15% plant extracts to determine contact toxicity. Every day, the Petri dishes were checked for mortality or odd symptoms on the larvae by probing them with a blunt object or pupae till the adult stage emerges. If a larva did not move after being poked with a pin and turned black and mushy, it was considered dead. After the plant extract was added, the duration of the larval and pupal stages was also noted. The 50% lethal concentrations (LC_{50}) values were assessed by Probit analysis according to the method of **Finney (1971).** The percent mortality rate was determined according to **Abbott (1925)** as mentioned by equation below:

$$\text{Abbott's formula}(\%) = \frac{\text{test mortality} - \text{control mortality}}{100 - \text{control mortality}} \times 100$$

The effect of the wild plant extracts on honeybees' morality

The effect of the wild plant extracts on morality honeybees (*Apis mellifera*) was performed by collecting honeybee workers from colonies headed with open mated local ligustica queens and kept in special designed small cages (10 cm × 20 cm × 20 cm). Each cage was closed on all sides with a net (meshes 2 mm × 2 mm). Each cage

was supplied with fifty worker bees, moreover adequate sugar syrup and sprayed with different wild plant extracts at different concentrations, (seven replicates/each). The cages were incubated at 25°C. For five days, the number of dead bees was record every day. The experiment was carried out in a completely randomized way.

Statistical Analysis

The experiment was set up in a Complete Randomized Design (CRD). The information was calculated according to COSTAT computer program **(Anonymous, 2005),** The graphs were created in Excel and evaluated using the Analysis of Variance (ANOVA) approach, with Duncan's multiple range test (P>0.05) used to separate the means **(Steel and Torrie, 1980)**.

RESULTS AND DISCUSSION

Extracts of four Sinai's Flora extracts including yellow nutsedge (*Cyperus esculentus* L.), common horehound (*Marrubium vulgare* L.), thyme (*Origanum vulgare* L.) and castor (*Ricinus communis* Linn) at different levels of 10, 15 and 20% were used in this experiment to determine their effectiveness against the larvae of greater wax moth, *Galleria mellonella* larvae in laboratory.

Effect of Sinai's Flora Extracts on Mortality of Wax Moth *Galleria mellonella*

Concerning the mortality percent of greater wax moth *Galleria mellonella* treated with some Sinai's Flora extracts is depicted on Table 2 and illustrated in Fig. 1. The results showed that, the extract of *O. vulgare* caused the highest toxicity rate (92.15%) for larvae of *G. mellonella* after the exposure, followed by extract of *R. communis* (81.47%). While, extract of *M. vulgare* (9.67%) showed lowest toxicity action against larvae of greater wax moth, *Galleria mellonella* after exposure compared to control.

Table 3 and Fig. 2 show the results of comparative action of aqueous extracts of Sinai's Flora at medium lethal concentration (LC_{50} level) based on co-toxic factor. The results indicated that the lowest LC_{50} values were obtained from aqueous extract of *O. vulgare* (7.07%) and *R. communis* (8.45%). While, the highest LC_{50} values were achieved by extract of *M. vulgare* (43.19%), followed by *C. esculentus* (26.66%) after exposure.

Studies showed that different thyme species have larvicidal, adulticidal, and oviposition deterrent activities against insects **(Damtie and Mekonnen (2021).** The essential oils of Thymus vulgaris had larvicidal activities against greater wax moth, *Galleria mellonella* L. larvae **(Owayss and Abd-Elgayed (2007)**. Also, **Almadani and Hiware (2020)** showed that the total mortality of greater wax moth larvae increased with increasing thyme concentration.

Effect of Sinai's Flora Extracts on Mortality of Bees

The effect of the different plant extracts on the mortality of honeybees is indicated in Table 4 and Fig. 3. The results revealed the highest toxicity effect was achieved when adults of honeybees *Apis mellifera* treated with aqueous extract of *Ricinus communis* (25.75%) at high concentration (20%), followed by aqueous extract of *O. vulgare* (14.62%) against adults of honeybees after exposure based on co-toxic factor. On the contrary, the lowest toxicity effect was obtained when adults of honeybees treated with aqueous extract of *C. esculentus* (4.24%) at 10% concentration. **Rahimi *et al*. (2017)** found that the percentage of honeybees' mortality in control hives and the honey bees treated by thyme extract did not have a significant difference.

Table 2. Effect of different Sinai's Flora extracts on the mortality (%) of wax moth (mean ± SD)

Plant extract	Conc. (%)	Mortality of wax moth (%)
	10	29.21±1.17g
Cyperus esculentus	15	34.90±0.82f
	20	41.25±1.88e
	10	9.67±0.72i
Marrubium vulgare	15	12.85±0.11i
	20	27.20±3.18h
	10	62.55±1.62d
Origanum vulgare	15	71.38±2.23c
	20	92.15±2.59a
	10	63.09±2.21d
Ricinus communis	15	70.11±1.83c
	20	81.47±2.75b
Control		4.52±0.01j

The same letter is not significantly different (P≤0.05) as determined by the least significant difference.

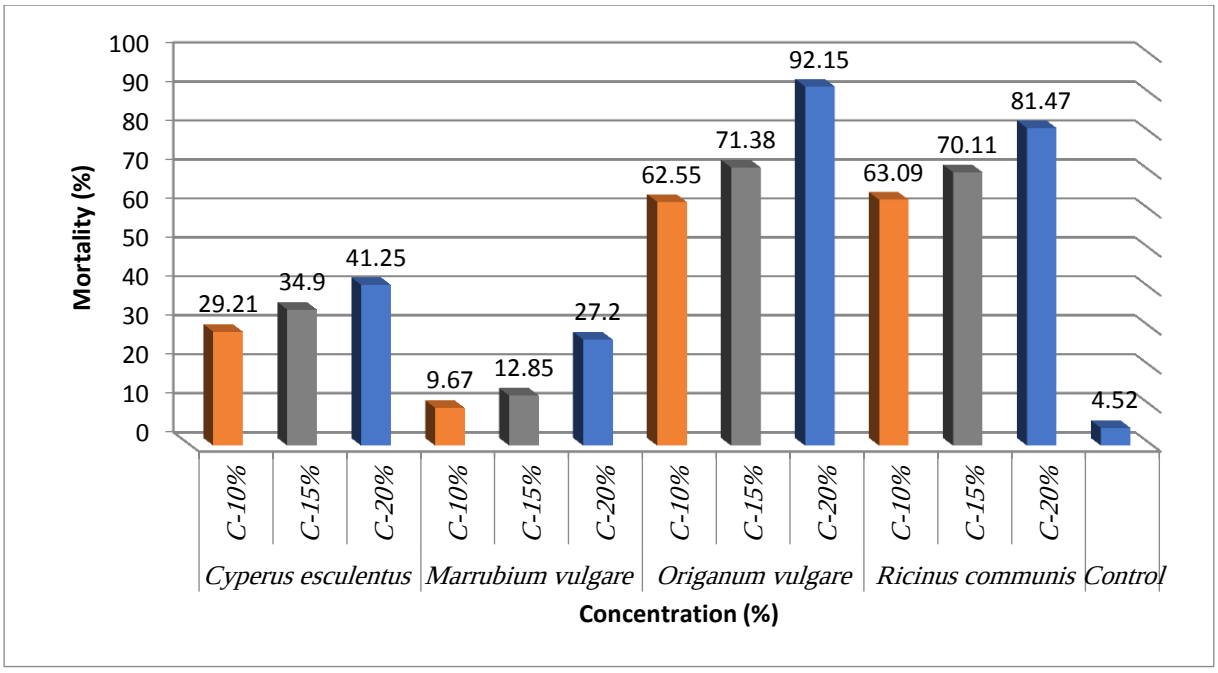

Fig. 1. Mortality Percentage of greater wax moth as affected by plant extracts

Table 3. LC_{50} of some Sinai's Flora extracts against larvae of greater wax moth, *Galleria mellonella*

Plant extract	LC_{50}	Slope	Toxicity Index*
Cyperus esculentus	26.66	2.119 ± 2.322	26.51
Marrubium vulgare	43.19	3.841 ± 3.708	16.36
Origanum vulgare	7.07	0.989 ± 1.367	100
Ricinus communis	8.45	1.119 ± 1.501	83.66

***** Toxicity Index % = (low LC_{50} /other LC_{50}) ×10

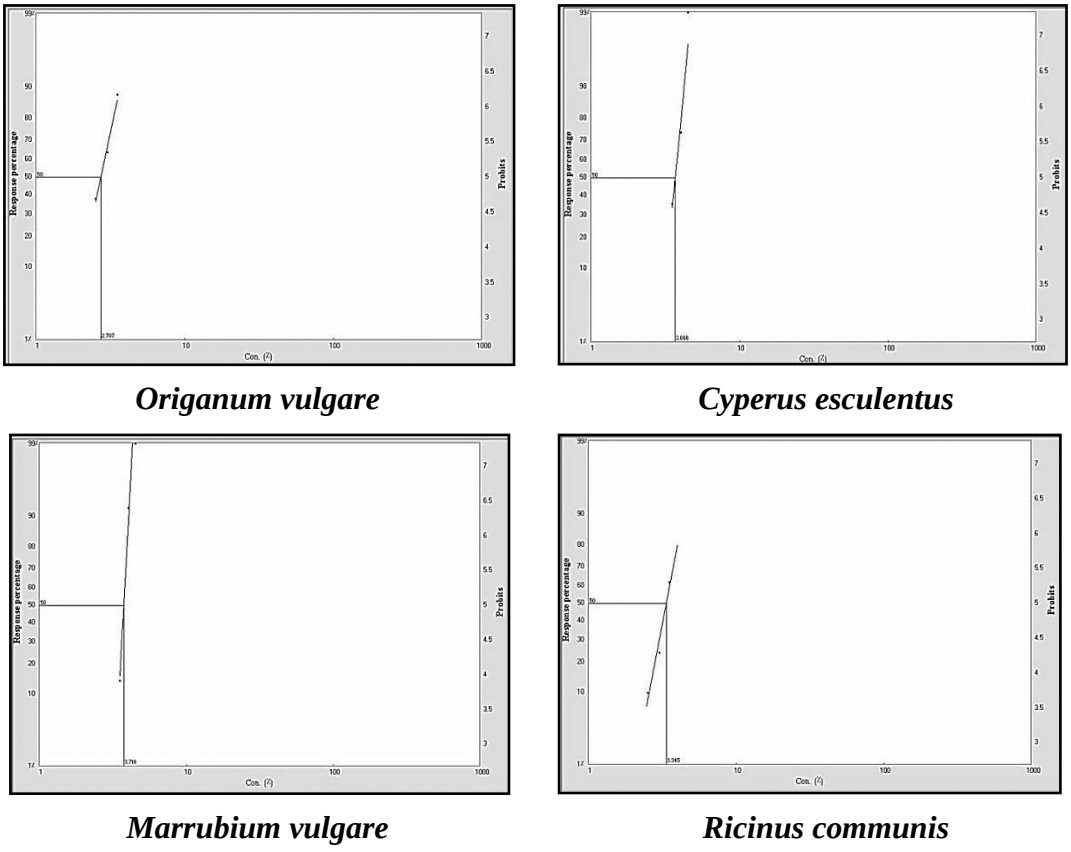

Fig. 2. Probit regression line of some Sinai's Flora extracts against larvae of greater wax moth, *Galleria mellonella*

Table 4. The effect of the wild plant extracts on honeybees' mortality

Plant extract	Conc. (%)	Mortality of bees (%)
	10	4.24±1.41g
Cyperus esculentus	15	6.12±1.79f
	20	8.34±1.26ef
	10	8.00±1.79ef
Marrubium vulgare	15	10.48±1.26de
	20	11.22±2.28d
	10	9.45±1.41def
Origanum vulgare	15	11.09±2.83d
	20	14.62±2.34c
	10	19.82±2.83b
Ricinus communis	15	22.19±4.60ab
	20	25.75±3.41a
Control		1.00±0.89h

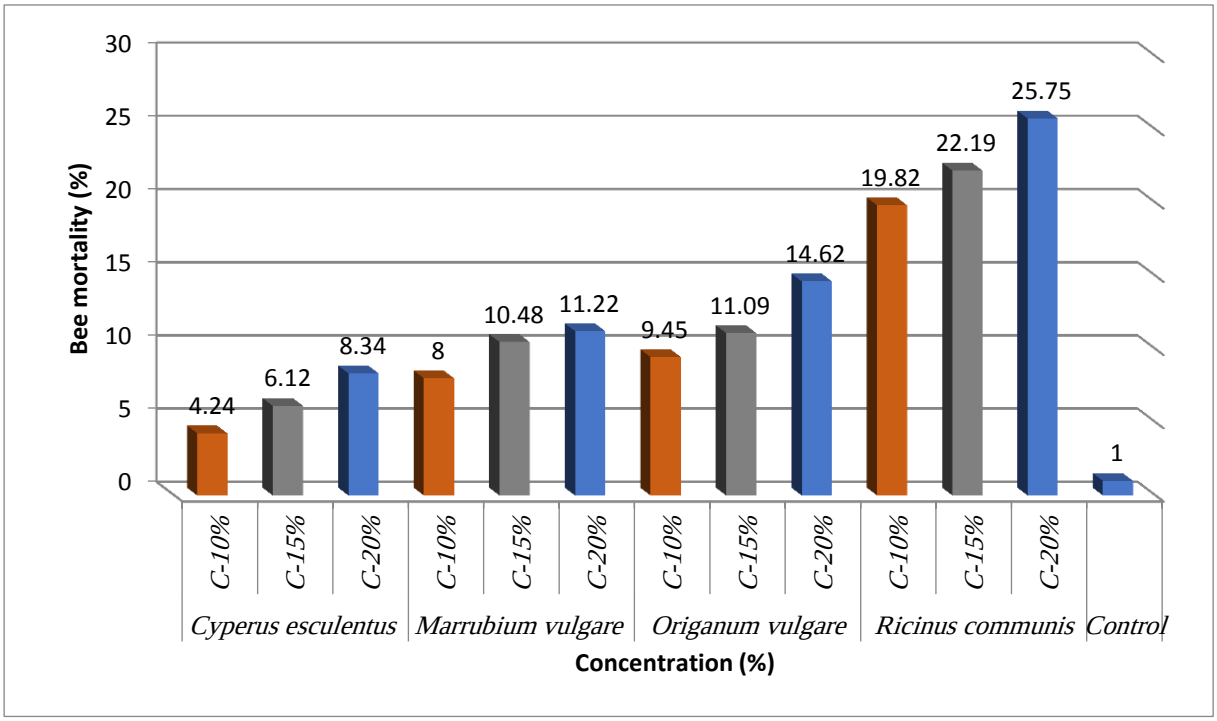

Fig. 3. Percentage of honeybee mortality as affected by Sinai's Flora extracts on mortality of bees

Results of comparative action of aqueous extracts of Sinai's Flora at lethal concentrations (LC_{50} levels) (Table 5 and Fig. 4) clearly showed that the lowest values were obtained for aqueous extract of *R. communis* 36.28% with high co-toxic factor (100%). While the highest values were achieved by aqueous extract of *M. vulgare* 48.46% with low co-toxic factor (66.09%) at LC_{50} lethal concentrations after exposure.

Table 5. LC_{50} of some Sinai's Flora extracts against adult honeybee workers

Plant extract	LC_{50}	Slope	Toxicity Index*
Cyperus esculentus	45.38	2.775 ± 2.607	79.94
Marrubium vulgare	48.46	2.497 ± 2.797	74.86
Origanum vulgare	44.42	2.551 ± 2.343	81.67
Ricinus communis	36.28	1.214 ± 1.119	100

* Toxicity Index% = (low LC_{50} /other LC_{50}× 100

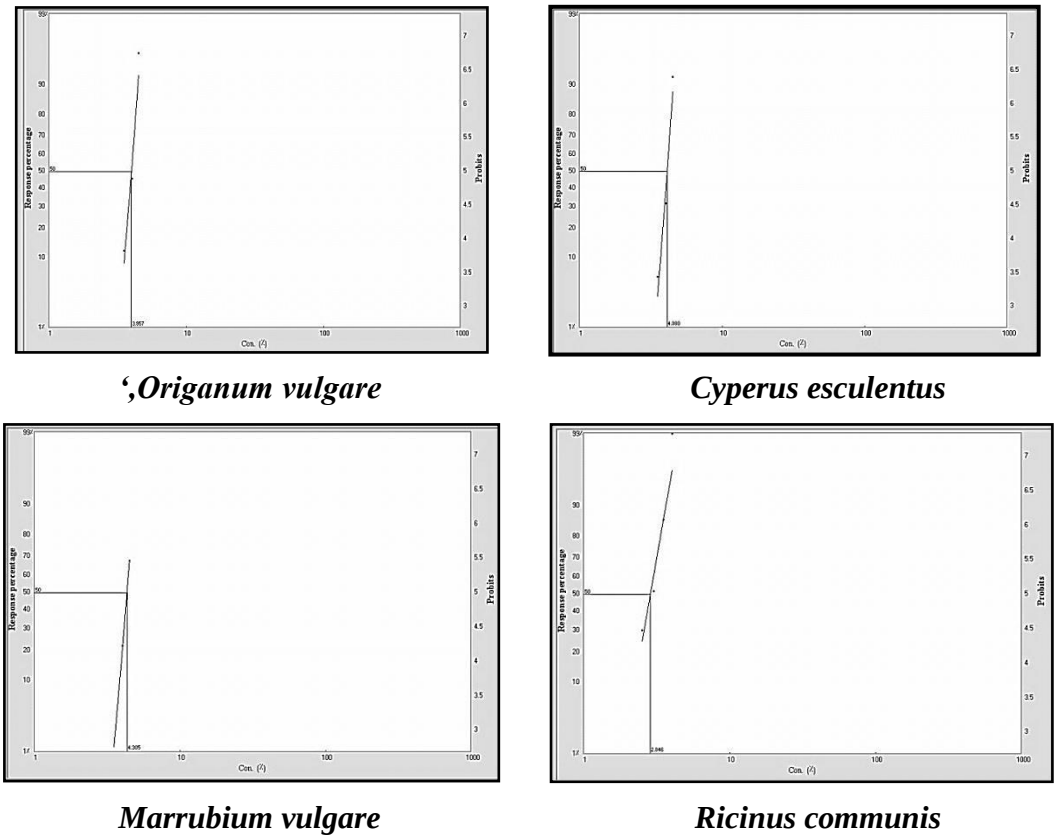

Fig. 4. Probit regression line of some Sinai's Flora extracts against adult honeybee workers

Conclusion

The greater wax moth is a major pest of the honeybee and can cause great losses to combs, hive material and bees because it feeds on wax and pollen stored in the combs of active honey bee colonies. This study was concerned with the control of this pest using wild plant extracts found in Sinai Peninsula. The results showed that the highest larval mortality percentage (92.15%) was showed with thyme extract at 20% concentration. Hence, it be concluded that thyme extract at 20% concentration is a good natural pesticide against greater wax moth and can be used in honeybees hives without high bee mortality.

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الملخص العربي

تأثير السمية لبعض مستخلصات النباتات البرية بسيناء ضد دودة الشمع الكبرى

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1. قسم حماية البيئة، كلية العلوم الزراعية البيئية، جامعة العريش، مصر.
2. قسم الإنتاج النباتي (تربية النحل)، كلية العلوم الزراعية البيئية، جامعة العريش، مصر.

أجريت الدراسة بهدف دراسة تأثير سمية المستخلصات المائية لبعض النباتات البرية النامية بشبة جزيرة سيناء على نسبة موت يرقات دودة الشمع الكبرى *Galleria mellonella*، وكذلك نشاط يرقات نحل العسل خلال الفترة من 2019/2018م بمنحل كلية العلوم الزراعية البيئية - جامعة العريش. تم تطبيق المستخلصات الإيثانولية لنباتات الزعتر*Origanum vulgare* والخروع *Ricinus communis* Linn والريحان *Marrubium vulgare* L. والسعد اللذيذ *Cyperus esculentus* L. بتركيزات 10%، و15%، و20% وأجري اختبار السمية التلامسية في المختبر على يرقات دودة الشمع الكبرى وصممت التجربة بنظام العشوائي الكامل وكل معاملة كررت عشرة مرات. وقد أوضحت النتائج أن المستخلصات النباتية التي تم اختبارها بتركيزات مختلفة أظهرت استجابات متغيرة ليرقات دودة الشمع الكبرى، *Galleria mellonellaas* مقارنة بالكنترول. سجلت أعلى نسبة موت لليرقات (92.15%) باستخدام مستخلص الزعتر *O. Vulgare* بتركيز 20%، يليه الخروع (81.47%) *R. comunis* بتركيز 20% مقارنة بالكنترول. على العكس من ذلك، تم تسجيل أقل نسبة موت لليرقات باستخدام مستخلص الريحان *M. vulgare* (9.67%) بتركيز 10% مقارنة بالمستخلصات النباتية الأخرى. لوحظ أن أقل نسبة موت لنحل العسل (24و4%) كانت مع نبات السعد بتركيز 10%

الكلمات الاسترشادية: مستخلصات نباتية، عثة الشمع، *Galleria mellonella*، حضنة، نحل.