



The effect of different Mediterranean plant extracts on the development of the great wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) and their toxicity to worker honeybees *Apis mellifera* L. (Hymenoptera: Apidae) under laboratory conditions

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Abstract

Ethanollic extracts of twenty one medicinal and health plants were used to examine their effects on the development of the greater wax moth *Galleria mellonella* and on honeybee workers. The results indicated that feeding the moth larvae on most of the extracts, prolonged the larval stage duration 2-40 days more than the control. Six extracts prolonged pupation period 2-5 days more than the control. Extracts of *Abrus precatorius*, *Laurus nobilis*, *Petroselinum sativum* and *Plantago psyllium* had insecticidal effect against the moth; they killed 100 or 95% of the tested wax moths respectively without adverse effects on worker bees except in the case of *A. precatorius*. Worker honeybees were also affected adversely by few of the used extracts, the most poisonous was *Cicer arietinum*, followed by *Myristica fragrans* and *Raphanus sativus*. These extracts killed 80, 70 and 55% of the experimental bees respectively. Some of the used plant extracts seem to act as insect growth regulators and toxicants and can be used effectively to control populations of wax moth.

Key words: Plant extracts, wax moth, *Galleria mellonella*, honeybees.

Introduction

The greater wax moth (GWM) *Galleria mellonella* (Lepidoptera: Pyralidae) is the most serious pest of honeybee wax combs in storage places and can cause substantial losses to combs, hive material and bees in beehives all over the world^{6,7}. The larval stage (with its 8-9 instars) is the only feeding stage of GWM (the mouthparts of the adult stage are atrophied) and the most destructive stage. During its development it builds silk-lined tunnels in the honey comb and feeds on honey, pollen, wax, faeces and cocoons of the bee larvae. This leads to destruction of honeycombs and subsequent deterioration of the weakened colonies³¹. Wax moth occurs in all apiaries and beekeeping areas but is more active in warm areas at temperature higher than 27°C where it spreads very rapidly⁶.

In the last three decades the control measures that are available for combs and other equipments not occupied by bees are physical methods involving exposure to heat⁴ and store combs in full light²⁷. Biological methods involve limited use of microbial insecticide *Bacillus thuringiensis*⁵ and the use of juvenile hormone^{36,37}. Actually the most used methods are chemical treatments, which involve the use of different chemicals, such as ethylene oxide and paradichlorobenzene²⁵, carbon dioxide¹⁶, sulfur dioxide⁹ and methyl bromide⁴, to kill the different stages of the moth. Recently several researches were conducted on the wax moths to develop and improve biological control methods using a parasitic wasp (*Apanteles galleriae*), a bacterium (*Bacillus thuringiensis*), a protozoan (*Nosema galleriae*) and a nuclear polyhedrosis virus^{1,5,8,17,30}. Only one strain of *B. thuringiensis* has been made commercially available as a biological insecticide inside the hive. Unfortunately, the commercial production of *B. thuringiensis* has been stopped.

In fact, an effective and harmless method of control of this pest has not been developed. Physical, chemical and biological methods are imperfect^{4,5}. In addition, continuous use of chemicals will increase the hazard of residue accumulation in the treated wax and consequently in the honey stored in the combs. Therefore, further studies are needed to find more effective, low cost, low residue control methods. A good candidate could be the use of natural products which are at hand to the beekeeper such as plant extracts. Recent studies showed that many plant extracts have an effect on insects and mites¹¹⁻¹⁵. Insecticidal effects of plant essential oils are evidenced in many cases when used against coleopteran insects, specially stored product insects. Among 22 essential oils tested as fumigants against the bean weevil *Acanthoscelides obtectus* (Bruchidae) those of *Thymus serpyllum* and *Origanum majorama* were the most toxic²⁸. Studies on the insecticidal effects of essential oils on Lepidopterous insects is still limited. Kanat and Alma¹⁸ revealed that nine essential oils from Turkish forests and sulfate turpentine were effective against the larvae of *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae). Muckensturm *et al.*²⁶ found that eugenols from *Laurus nobilis* (Lauraceae) essential oils decreased the feeding of *Mythimna unipuncta* (Lepidoptera: Noctuidae). *Acorus calamus* (Araceae) oil and its active ingredients, asarone and analogues, were potent growth inhibitors and antifeedants to the variegated cutworm *Peridroma saucia* (Lepidoptera: Noctuidae)²⁰. Such results are promising in the case of wax moth. As a member of Lepidoptera wax moth may be susceptible to essential oils and plant extracts. Since the main target in beekeeping operation is the high quality low residue product, it is worthwhile to try such environment safe substances against the wax moth.

Although plant extracts were examined against many Lepidopterous insects, no studies were conducted to find plant extracts which may affect development and life span of wax moth. Identifying a natural compound with high insecticidal activity against the wax moth and with low toxic effects to honeybees is essential. This will enable their use in integrated pest management programs to control wax moth in honeybee colonies as well as in storage areas without contaminating honey bee products with pesticide residues. Therefore, the objective of this study was to investigate the effect of twenty one different extracts of medical and health plants grown in the Mediterranean region of Jordan on the development and life span of wax moth (*Galleria mellonella*). Such treatment methods with non-harmful products could contribute considerably to control this pest and reduce the risks of beehive product contamination and may give a possible solution for this apicultural problem.

Materials and Methods

An experiment was carried out under controlled laboratory conditions at the Jordan University of Science and Technology Campus in Jordan, during spring months of 2006.

Experimental plants collection and identification: Twenty one plants belonging to thirteen different plant families (Table 1) commonly used in Jordan for medical and/or health purposes were used in this experiment. The tested plants were collected from their natural habitats in Jordan. Taxonomic identification was performed by botanists from the Yarmok University. Voucher specimens were deposited in the Faculty of Agriculture, Jordan University of Science and Technology.

Preparation of plant extracts: The seeds of the collected plant materials were dried and ground in a Wiley grinder with a 2 mm diameter mesh. Sample of 50 g of the ground material was extracted by cold percolation with 95% ethanol. The ethanolic extract was concentrated under vacuum, weighed and the residue was used for the tests.

The effect of the extracts on honeybees: Honeybee workers of *Apis mellifera syriaca* were used in this experiment. Sister nurse bees were collected from Langstroth colony and kept in special designed small cages (5 cm x 10 cm x 10 cm) at the beginning of the experimental period. Each cage was closed at the bottom with a net (meshes 2 mm x 2 mm) with an insertion and two safety glass windows to allow watching the bees. Each cage was supplied with forty worker bees. The bees were feed with 10 ml of water sugar solution (1:1) mixed with 0.1 g water-dissolved plant extract. As a control the bees were feed with sugar solution only. All cages were inspected daily to record the number of dead bees. The experiment was conducted in a complete randomized design with six replications and incubated at 35°C for six days.

Experimental wax moths: To rear wax moth larvae, newly emerged wax moth females and males were collected in small cages. After mating females were left to deposit eggs. The eggs were incubated in a warm room at 30°C, relative humidity of ca. 70% and 24 hours darkness until emergence of adult stages of great wax moth. Directly after emergence twenty adults, ten males and ten females of the same age, were collected in another cages (50 cm x 50 cm x

50 cm) and incubated under the same conditions to get the next generation. The larval stages were provided as needed with a cultural media (an artificial food mixture) prepared according to Balazs².

Testing the effect of plant extracts on the wax moth: The fifth larval instars were chosen to make the investigations. Identification of the instars was made depending on the calculated age of the larva, width of the head capsule and/or its weight²⁹. The larvae were collected from the cages and transferred to plastic Petri dishes, so that only one larva was in each Petri dish. The Petri dishes were supplied with the same feeding mixture mixed with 0.1 mg of the studied plant extract so that for each larva only one extract was added. A control with no extract was used. Each plant extract was replicated six times in a complete randomized design. The experimental larvae were incubated at 30°C, relative humidity of ca. 70% and 24 hours darkness. The Petri dishes were daily inspected for the dead or unusual signs appear on the larvae or pupae until emergence of the adult stage. The duration of the larval and pupal stages after addition of the plant extract was also recorded.

Statistical analysis: Collected data were subjected to analysis of variance (ANOVA), and means were compared using Fisher's least significant differences³².

Results

Extracts of twenty one different plants belonging to thirteen plant families were used in this experiment (Table 1) to examine their effects on the larval and pupal stage durations and on the mortality of wax moth as well as mortality of honey bee workers. Unless it is clearly stated all values in this work are given as mean \pm SE. The results revealed that most of the investigated plant extracts had an effect on the larval and pupal stages duration, but only six of them had direct killing effect on the wax moth (Table 2).

The results illustrated in Table 2 show that the influence of the extracts on the duration of the larval stage (DLS) was variable from extract to another, but a general trend of increasing the duration

Table 1. List of scientific names and families of the plants used against *Galleria mellonella*.

No.	Scientific name	Family
1	<i>Apium graveolens</i> L.	Apiaceae
2	<i>Petroselinum sativum</i> Hoffman.	Apiaceae
3	<i>Brassica alba</i> L.	Brassicaceae
4	<i>Raphanus sativus</i> L.	Brassicaceae
5	<i>Brassica rapa</i> L.	Brassicaceae
6	<i>Cucurbita maxima</i> Duches.	Cucurbitaceae
7	<i>Abrus precatorius</i> L.	Fabaceae
8	<i>Phaseolus vulgaris</i> L.	Fabaceae
9	<i>Medicago sativa</i> L.	Fabaceae
10	<i>Vicia sativa</i> L.	Fabaceae
11	<i>Cicer arietinum</i> L.	Fabaceae
12	<i>Pisum sativum</i> L.	Fabaceae
13	<i>Hordeum sativum</i> Pers.	Gramineae
14	<i>Laurus nobilis</i> L.	Lauraceae
15	<i>Linum usitatissimum</i> L.	Linaceae
16	<i>Malva sylvestris</i> L.	Malvaceae
17	<i>Myristica fragrans</i> Houtt	Myristicaceae
18	<i>Sesamum indicum</i> L.	Pedaliaceae
19	<i>Plantago psyllium</i> L.	Plantaginaceae
20	<i>Nigella sativa</i> L.	Ranunculaceae
21	<i>Corchorus olitorius</i> L.	Tiliaceae

Table 2. The effect of different plant extracts on duration of larval and pupal stages and mortality of wax moth.

Plant scientific name	Duration of larval stage * days	Duration of pupal stage days	Mortality of wax moth
<i>Apium graveolens</i> L.	15.00 ± 3.46h	5.67 ± 0.58h	0.00±0.00d
<i>Petroselinum sativum</i> Hoffman	43.67±16.1bcd	9.33 ±2.52bcd	5.67±0.58a
<i>Brassica alba</i> L.	24.00±5.57fgh	6.67±0.58efgh	0.00±0.00d
<i>Raphanus sativus</i> L.	19.00± 5.29 gh	6.67 ±0.58efgh	0.00±0.00d
<i>Brassica rapa</i> L.	16.00 ±3.61h	5.67 ±0.58h	0.00±0.00d
<i>Cucurbita maxima</i> Duches.	15.67 ±5.69h	6.00 ±0.00gh	0.00±0.00d
<i>Abrus precatorius</i> L.	47.00±5.57abc	8.67 ±1.53bcde	6.00±0.00a
<i>Phaseolus vulgaris</i> L.	18.00±7.55gh	6.33 ±0.58fgh	0.00±0.00d
<i>Medicago sativa</i> L.	50.00±8.00ab	10.33 ±2.08abc	0.00±0.00d
<i>Vicia sativa</i> L.	38.00±4.00bcde	10.67 ±0.58ab	0.00±0.00d
<i>Cicer arietinum</i> L.	19.00±2.00gh	6.00 ±0.00gh	0.00±0.00d
<i>Pisum sativum</i> L.	19.00±8.89gh	5.67 ±0.58h	0.00±0.00d
<i>Hordeum sativum</i> Pers.	33.00±6.24def	8.33 ±0.58cdef	4.67±0.58b
<i>Laurus nobilis</i> L.	16.00±1.73h	8.00 ±0.00defg	6.00±0.00a
<i>Linum usitatissimum</i> L.	30.00±10.44efg	7.00 ±2.65efgh	0.00±0.00d
<i>Malva sylvestris</i> L.	24.00±5.29fgh	6.33 ±1.53fgh	0.00±0.00d
<i>Myristica fragrans</i> Houtt	19.00±7.55gh	6.00 ±1.00gh	0.00±0.00d
<i>Sesamum indicum</i> L.	18.00±4.58gh	6.67 ±1.53efgh	0.00±0.00d
<i>Plantago psyllium</i> L.	30.00±11.53efg	7.33±1.53defgh	5.67±0.58a
<i>Nigella sativa</i> L.	37.67±11.24cde	7.67 ±1.53defgh	2.67±1.15c
<i>Corchorus olitorius</i> L.	56.00±6.24a	11.67 ±2.08a	0.00±0.00d
Control	16.00±2.00h	6.67 ±0.58h	0.00±0.00d

All values in this table are given as mean ± SD. Means followed by the same letter are not significantly different ($P \leq 0.05$) as determined by the least significant difference. *Duration of larval stage after addition of plant extracts.

was recorded except in the case of *Apium graveolens* L. and *Cucurbita maxima* Duches. Both extracts decreased larval duration 1.00 and 0.33 day less than the control respectively, with no significant difference ($P \leq 0.05$) from the untreated control. Furthermore, as a result of feeding the moth larvae on the extracts, the DLS was either approximately similar to the control with no significant difference ($P \leq 0.05$) between them as in the case of *Brassica rapa* L. and *Laurus nobilis* L., or prolonged compared to the control as in the case of the rest of the used extracts. Regarding the extracts which extended the DLS; eight of them expanded DLS 2-8 days more than that recorded in the control with no significant difference ($P \leq 0.05$) between them and the control as in the case of *Brassica alba* L., *Raphanus sativus* L., *Phaseolus vulgaris* L., *Cicer arietinum* L., *Pisum sativum* L., *Malva sylvestris* L., *Myristica fragrans* Houtt and *Sesamum indicum* L. Three extracts extended the DLS from 14-17 days with significant difference from the control ($P \leq 0.05$) as observed by *Hordeum sativum* Pers., *Linum usitatissimum* L. and *Plantago psyllium* L. Five extracts, *Medicago sativa* L., *Petroselinum sativum* Hoffman., *Abrus precatorius* L., *Vicia sativa* L. and *Nigella sativa* L. significantly increased larval duration 21-33 days compared to the control ($P \leq 0.05$). Finally *Corchorus olitorius* L. was able to increase the larval duration 3.5 times more than that of the control ($P \leq 0.05$).

During the experiment, no larvicidal activity of the extracts was recorded. The experimental larvae continued their metamorphosis normally with relatively normal size except in the case of *A. precatorius*, *L. nobilis*, *Petroselinum sativum*, *H. sativum*, *P. psyllium* and *V. sativa* extracts, which had smaller larvae than that of the control. In addition the larvae treated with *L. nobilis*, *V. sativa*, *P. psyllium* and *H. sativum* extracts pupate without forming normal cocoon, though they spanned an extra ordinary thick irregular silk web in the case of *H. sativum* and *V. sativa*. The extra ordinary silk production was also practiced by the larvae treated with *P. vulgaris* and *C. arietinum* even though normal cocoons were formed.

The effect of the plant extracts on the pupal stage duration is

presented in Table 2. *C. olitorius*, *V. sativa* and *M. sativa* increased the pupal stage duration between 4-5 days, while *Petroselinum sativum*, *A. precatorius* and *H. sativum* prolonged the pupation period between 2-3 days with significant difference ($P \leq 0.05$) from the control. The rest of the used plant extracts influenced differently the pupation period with no significant differences from the control ($P \leq 0.05$). Most of the formed pupae were of normal size except in the case of *L. nobilis*, *Petroselinum sativum*, *H. sativum* and *V. sativa*, where the size of the pupae were much smaller than that of the control.

Concerning the effect of the plant extracts on the mortality of wax moth (Table 2), only six extracts successfully killed the wax moth with significant difference ($P \leq 0.05$) from the untreated control. Four of them showed the highest killing rate. Moth mortality was 100% with *A. precatorius* and *L. nobilis*, 95% with *Petroselinum sativum* and *P. psyllium*, 78.3% with *H. sativum* and 45% of the experimental population with *N. sativa* (Fig. 1). *G. mellonella* treated with *L. nobilis* extract continued their metamorphosis giving

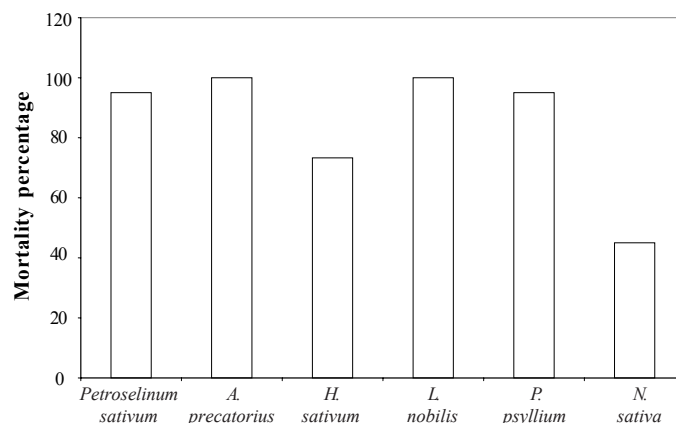


Figure 1. Percentage of the dead *Galleria mellonella* as a result of treating its larvae with seven different plant extracts.

abnormal small size adults which die shortly after emergence. On the other hand, *A. precatorius*, *P. psyllium*, *Petroselinum sativum*, *N. sativa* and *H. sativum* extracts caused death of the insects during the pupal stage. The color of the pupae became darker and no further development was observed later. The other fifteen studied extracts did not knock down the wax moth.

The effect of the different plant extracts on the mortality of honeybees is illustrated in Table 3 and Fig. 2. The results showed that worker honey bees were also affected adversely by some of the used extracts. The extracts of *C. arietinum*, *M. fragrans*, *R. sativus*, *V. sativa*, *A. precatorius* and *A. graveolens* killed 80, 70, 55, 30, 29.2 and 12.5% of the experimental bees respectively, which was significantly different from the control ($P \leq 0.05$). The rest of

the used extracts had low killing effect on the worker bees with no significant difference ($P \leq 0.05$) from the control.

Discussion

The extracts obtained from *A. precatorius* and *L. nobilis* were found the most active among those tested against *G. mellonella* followed by those obtained from *Petroselinum sativum*, *P. psyllium* and *H. sativum*. Finally *N. sativa* was the least effective. The results proved for the first time presence of insecticidal properties of the former mentioned extracts against *G. mellonella*. The insecticidal effects of extracts from plant origin have been well demonstrated by many workers against insects other than wax moth. Commonly plant extracts can be inhaled, ingested or skin absorbed by insects. *Thymus serpyllum* and *Origanum majorama* were found to be toxic to bean weevil *Acanthoscelides obtectus* (Bruchidae)²⁸. Contact, fumigant and antifeedant effects of extracts of *Syzygium aromaticum* and *Illicium verum* against the red flour beetle *Tiobolium castaneum* and maize weevil *Sitophilus zeamais* were also proved¹¹⁻¹⁵. The mechanisms of toxic effect of plant extracts are not presently well known and probably attributable to the chemical composition¹⁸.

The used effective plant extracts did not show larvicidal effects on the larval stages; the experimental larvae continued their development although in smaller size in some cases until reaching the pupal stage. This behavior may be accredited to antifeedant effect of the effective plant extracts. This agrees with results obtained by Muckensturm *et al.*²⁶ who found that eugenols from *L. nobilis* extracts decreased the feeding of *Mythimna unipuncta* (Lepidoptera: Noctuidae). The antifeedant effect of plant extracts is also reported by many researchers¹¹⁻¹⁵. Moreover most of investigated plant extracts prolonged the LSD, this emphasis the assumptions and results of other researchers. Borrer *et al.*³ mentioned that the biological activity of the plant extracts is comparable to that of insect growth regulators and toxicants, others elucidate that insect growth regulators (IGR) and toxic

Table 3. The effect of the different plant extracts on the mortality of honeybees.

Scientific name	Dead bees	Killing rate %
<i>Apium graveolens</i> L.	5.00±1.41ef	12.5
<i>Petroselinum sativum</i> Hoffman	3.00±1.79fgh	7.50
<i>Brassica alba</i> L.	1.00±0.89hi	2.50
<i>Raphanus sativus</i> L.	22.0±2.83c	55.0
<i>Brassica rapa</i> L.	2.00±1.41ghi	5.00
<i>Cucurbita maxima</i> Duches.	0.00±0.00i	0.00
<i>Abrus precatorius</i> L.	11.67±2.34d	29.2
<i>Phaseolus vulgaris</i> L.	4.00±1.26efg	10.0
<i>Medicago sativa</i> L.	2.00±1.10ghi	5.00
<i>Vicia sativa</i> L.	12.0±2.83d	30.0
<i>Cicer arietinum</i> L.	32.00±3.41a	80.0
<i>Pisum sativum</i> L.	0.00±0.00i	0.00
<i>Hordeum sativum</i> Pers.	2.00±1.41ghi	5.00
<i>Laurus nobilis</i> L.	1.00±1.26ghi	2.50
<i>Linum usitatissimum</i> L.	3.17±1.47fgh	7.93
<i>Malva sylvestris</i> L.	0.00±0.00i	0.00
<i>Myristica fragrans</i> Houtt	28.0±4.60b	70.0
<i>Sesamum indicum</i> L.	3.00±1.79fgh	7.5
<i>Plantago psyllium</i> L.	1.00±1.10hi	2.5
<i>Nigella sativa</i> L.	3.00±1.55fgh	7.5
<i>Corchorus olitorius</i> L.	6.00±2.28e	15.0
Control	1.00±0.89hi	2.5

Dead bee values are given as mean ± SD. Means followed by the same letter are not significantly different ($P \leq 0.05$) as determined by the least significant difference.

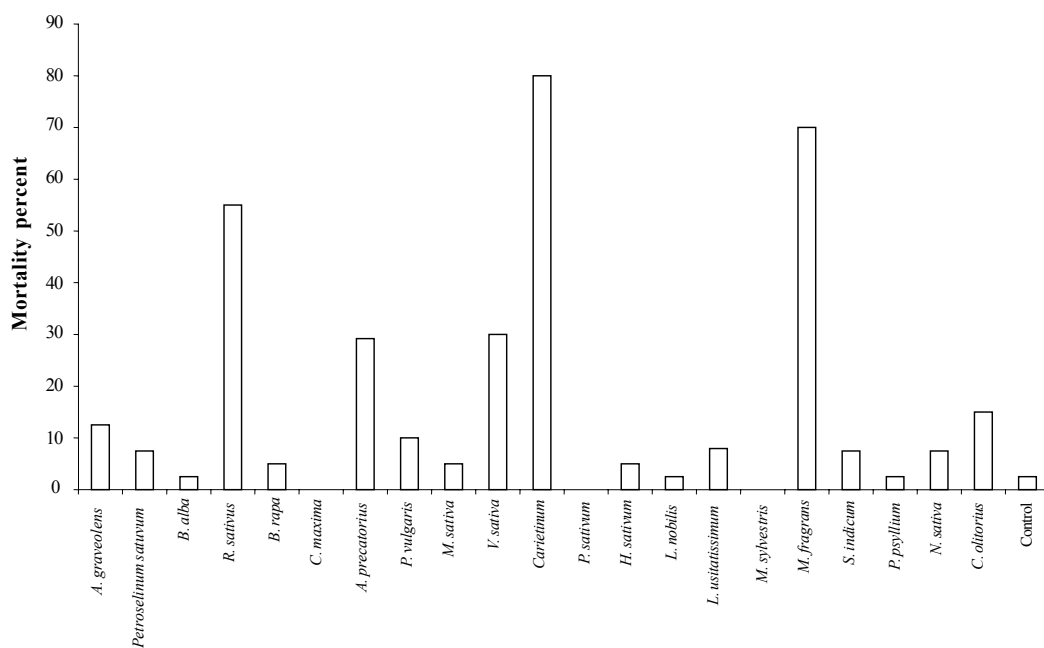


Figure 2. Percentage of the dead honey bee workers as a result of treatment with twenty one different plant extracts.

compounds/mixtures interfere with the form of the power time curve of insect development, even leading to abortion of metamorphosis^{10, 21, 22} and could act to inhibit, retard or even accelerate insect development processes³³. The biological activity of plant extracts on *G. mellonella* obtained in the present investigation fit with those that retard insect development. This agree with the results obtained by Koul *et al.*²⁰ who found that *Acorus calamus* (Araceae) oils were potent growth inhibitors and antifeedants to the variegated cutworm *Peridroma saucia* (Lepidoptera: Noctuidae).

In the present work, death of the treated wax moth was registered after formation of the pupa during the pupal stage or directly after emergence of the adult stage in the case of *L. nobilis* probably as result of affecting development during larval stage and pupal stage. Prevention of pupal development by plant extracts was also reported by Konstantopoulou *et al.*¹⁹, who found that extracts from Satureja, Origanum and Mentha prevented egg hatching and provoked prohibition or malformation of puparium of the flies *Drosophila auraria*. The IGR effects of the plant extracts are clear again in this case. It was also demonstrated by several researchers³³⁻³⁵ that the application of IGR at larval stage resulted in the disruption of pupal development and early adult emergence. Additionally, treated larvae may give rise to morphologically deformed adults that are unable to fly properly²⁴.

The toxicity of plant extracts to honeybees is not well studied. Lindberg *et al.*²³ mentioned that honeybees appear somewhat susceptible to plant extracts. In the current experiments and among the tested plant extracts only *A. precatorius* caused mortality of honey bee workers and wax moth. This means that the remaining five plant extracts could be used safely to control wax moth either in storage places or even in infested colonies without any future danger of injuring honey bees.

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