

MORPHOGENETIC EFFECTS OF CRUDE EXTRACTS AND ESSENTIAL OILS OF CERTAIN MEDICINAL AND AROMATIC PLANTS AGAINST SOME IMMATURE STAGES OF *Bactrocera zonata* (SAUNDERS) (DIPTERA: TEPHRITIDAE)

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(Manuscript received 9 September 2018)

Abstract

The morphogenetic effects of crude extracts and essential oils of the four tested plants (long leaved Eucalyptus, Jojoba, Lavender and Neem) on adults of *Bactrocera zonata* which emerged from treated larvae or pupae (the 3rd larval instar and pupae of 1- and 3-days old) as contact poisons was studied. Results revealed that the values of effective concentrations (EC₅₀%) differed according to the tested formulation and used plant as well as the treated individuals. The essential oils were more effective than crude extracts against the tested stages. The full grown larvae of *B. zonata* were the most adversely-affected to Lavender (as essential oils or crude extract) recording the lowest values of EC₅₀ (1.05 and 13%, respectively), whereas Jojoba recorded the highest values (EC₅₀ was 18% for crude extract and 3 % for essential oils). In case of one-day old pupae, Jojoba crude extract and Lavender essential oils were the most efficient showing EC₅₀ values of 9.13 and 1.25%, respectively. Pupae of 3-days old were the most tolerable stage to the crude extracts of the four used plants showing EC₅₀ values > 20%, but the same stage was differently susceptible to essential oils of the tested plants, where Eucalyptus was the most efficient (EC₅₀= 3.10%). On the other hand, the increment of concentrations, the increase of effectiveness, where the malformed individuals and degree of deformities gradually and sharply increased with the increase of concentrations of all used formulations.

INTRODUCTION

Fruits are one of the most important crops in the whole world because of its nutritional and economic importance as well as for local utilization or exportation. Fruit trees are mostly infested with many pests which affect its quantitative and qualitative productivity such as some species of scale insects, mealy bug, mites, wood borers and others. Fruit flies such as the peach fruit fly, the Mediterranean fruit fly and other species are dangerous, multivoltine, polyphagous and cosmopolitan insect pests which considered the most destructive pests, since they infest fruits directly causing a great damage to them. These species of fruit flies belonging to Order Diptera, Family Tephritidae are important pests of major economic significance in most of the temperate, subtropical and tropical countries because they infest fleshy fruits of wide range hosts mainly peach, apricot, mangoes, guava, figs and citrus. The peach fruit

fly, *Bactrocera zonata*, is a native polyphagous South and South-East Asian pest that is of major quarantine importance for exports to the U.S.A., Japan and European countries and considered one of the most destructive fruit pests which spread in several regions of the world (White & Elson-Harris, 1992 and Allwood *et al.*, 1999). In nineteenth of the last century, this pest was officially identified and recorded for the first time in Egypt where it attacks some of the main fruit varieties causing severe damage for several hosts in the Near East (e.g. mangoes, peach and others) (Al-Eryan, 2008).

Many researchers studied the malformations or deformities as morphogenetic effects of pesticides or plant extracts for several insect species (Socha & Sehnal, 1972; Madhavan, 1973; El-Tantawy & Khider, 1981; El-Fishawi & Kelany, 1985; Mosallam, 1993; Tayeb, 1993; Saleh, 1995; Mediouni-Ben Jemâa & Boushih, 2010; Rizwan-ul-Haq, 2012 and Pavela, 2015).

This work aimed to study the morphological adverse effects of certain formulations (crude extracts and essential oils) of four medicinal and aromatic plants (Long leaved eucalyptus, Jojoba, Lavender and Neem) against some immature stages (larvae and pupae) of the peach fruit fly, *B. zonata*.

MATERIALS AND METHODS

1- Rearing Technique

The experiments were conducted on full grown larvae and pupae of *B. zonata* that obtained from a laboratory strain that continuously reared in Horticultural Insects Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza under conditions of $25\pm 3^{\circ}\text{C}$ and $60 \pm 5\%$ R.H. Adults were reared in a wooden cage (40x40x40 cm) which covered from all sides with plastic screen. Adult flies were fed with fortified protein hydrolysate mixed with sugar at a ratio of 1:3, respectively. Also, a wick saturated with water was added in a plastic bottle. Plastic bottles as false fruits that had many fine pores (as oviposition receptacles) were put in the cage. These false fruits were filled with 3cm water to receive and prevent eggs dehydration. Larvae were reared on the artificial diet which consisted of 500 ml tap water, 4.00gm sodium benzoate, 4.00gm citric acid, 84.50gm sugar, 84.50gm brewer's yeast and 330gm wheat bran. These constitutes were carefully mixed in large plastic container. Then, eggs were scattered on the diet that was placed in plastic trays of 25x15x5cm and tightly covered with muslin clothes using rubber bands. After that, these trays were placed in a large plastic cage containing fine sand at the bottom to allow the jumping larvae to pupate. All pupae were separated by sieving from sand (Shehata *et al.*, 2006).

2- Botanicals Used

Both crude extracts and essential oils of different parts of four medicinal and aromatic plants were used to determine their effects on the different stages of the peach fruit fly, *Bactrocera zonata* under laboratory conditions. These used plants were shown in the following table:

Plant	Scientific name	Family	Selected part
Long leaved eucalyptus	<i>Eucalyptus longifolia</i>	Myrtaceae	Leaves
Jojoba	<i>Simmondsia chinensis</i>	Simmondsiaceae	Seeds
Lavender	<i>Lavendula angustifolia</i>	Lamiaceae	Plants as a whole
Neem	<i>Azadirachta indica</i>	Meliaceae	Seeds

These crude extracts and essential oils were obtained from El-Abgy Factory for Extracting Oils. The selected parts of used plants were carefully washed with distilled water and left individually to dry in shade for three days under airy room conditions of $25\pm 3^{\circ}\text{C}$ and $63\pm 5\%\text{RH}$. After that, they were separately collected to grind using electric grinder. Then, to prepare the crude extracts, the grinded materials were soaked at ratio 1 plant : 1 Ethyl alcohol (97%): 1 distilled water for 48 hours. The soaked materials was filtered and kept to use. But, to obtain essential oils, the soaked materials were rotary-evaporated, where the evaporated oils were condensed and collected.

3- Procedure

The full grown larvae (the 3rd larval instar) and pupae of 1- and 3-days old were used to study the morphological effects of the above mentioned materials as surface contact poisons for larvae and pupae of the peach fruit fly under laboratory conditions. Serial concentrations of both oils and extracts were prepared (0, 1, 2.5, 5, 10 and 20% V/V) were prepared in acetone as a solvent. One cm³ was homogenously dispersed in a Petri-dish (9 cm in diameter) which was left for 5 minutes till acetone evaporated. The check individuals treated with acetone only and other control individuals were left untreated. Then, larvae or pupae were confined as 75 individuals/ concentration in three replicates for each (25 larvae or pupae/ each Petri-dish as a replicate) that were left till adult emergence.

The morphological abnormalities of larval-pupal or pupal-adult transformations were assessed. The resulted malformations were assessed as graded scoring system (Tables, 1-4). The degree of deformities (score) was calculated by multiplying the number of individuals by their numerical activity ratings and dividing the sum of total number of treated larvae or pupae. The unaffected individuals should a zero score, maximum observed responses should set the highest number with the intermediates arbitrarily fixed in between according to Redfern *et al.* (1970) (for example, if 4 pupae

or adult rated 2 and 7 individuals rated 3, the score = $4 \times 2 + 7 \times 3 / 11 = 2.64$). The total score can then be calculated and plotted on semi-logarithmic paper as a log-dose response curve. The intersection of this curve with the 50% response line yields the EC₅₀ (Median Effective Concentration) response figure. The probit conversion of this type of curves is not likely to result in straight line, because of the fact that the grading system is arbitrary (Staal, 1972). The graded scoring system was used to evaluate the bioactivity of the tested compounds and the obtained data were plotted in semi-logarithmic paper.

RESULTS AND DISCUSSION

The morphological effects (as larval-pupal and pupal-adult malformations as well as scoring systems that were illustrated in Tables 1- 4) of crude extracts and essential oils of the four tested plants (Eucalyptus, Jojoba, Lavender and Neem) on adults of *B. zonata* emerged from treated larvae or pupae as contact poisons was obtained in Table (5) and Figures (1-6).

Table 1. Illustration of malformations resulted in certain medicinal and aromatic plants applied as surface contact poisons for third instar larvae or pupae of *Bactrocera zonata*.

Larval score	Characteristics	Pupal score
0	Adults morphologically seemed to be normal	0
1	Adults with wings slightly curled	1
-	Adults with wings severely curled and malformed legs	2
2	Partial emergence of adults with head and thorax	3
-	Partial emergence of adults with head only	4
3	Intact dead pupae	5
4	Dead larvae	

Tale 2. Scoring system of larval-pupal and pupal-adult transformations of *B. zonata* resulted in certain medicinal and aromatic plants applied as surface contact poisons to full grown larvae.

Score	Eucalyptus		Jojoba		Lavender		Neem	
	Extract	Oil	Extract	Oil	Extract	Oil	Extract	Oil
0	+	+	+	+	+	+	+	-
1	+	-	+	+	+	-	+	+
2	+	+	+	+	+	-	+	-
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+

+ Refers to appearance of score in the treatment.

- Refers to disappearance of the score in the treatment.

Table 3. Scoring system of pupal-adult transformations of *B. zonata* resulted in certain medicinal and aromatic plants applied as surface contact poisons to one-day old pupae.

Score	Eucalyptus		Jojoba		Lavender		Neem	
	Extract	Oil	Extract	Oil	Extract	Oil	Extract	Oil
0	+	+	+	+	+	+	+	+
1	+	-	+	+	-	+	+	+
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	-	+
4	-	-	+	-	-	-	-	-
5	+	+	+	+	+	+	+	+

Table 4. Scoring system of pupal-adult transformations of *B. zonata* resulted in certain medicinal and aromatic plants applied as surface contact poisons to three-day old pupae.

Score	Eucalyptus		Jojoba		Lavender		Neem	
	Extract	Oil	Extract	Oil	Extract	Oil	Extract	Oil
0	+	+	+	+	+	+	+	+
1	+	+	+	+	-	+	-	-
2	-	+	-	+	-	+	+	+
3	-	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+

Table 5. Effective concentrations (EC₅₀%) of crude extracts and essential oils of certain medicinal and aromatic plants against different developmental stages of *B. zonata*.

Botanicals	EC ₅₀ (%)					
	3 rd larvae instar		Pupae			
			1-day		3-day	
	Extract	Oil	Extract	Oil	Extract	Oil
Eucalyptus	15.60	1.12	15.30	5.15	> 20	3.10
Jojoba	18.00	3.00	9.13	4.60		10.75
Lavender	13.00	1.05	16.10	1.25		12.00
Neem	15.30	1.25	18.70	6.90		13.80

Data in Table (5) indicate the adverse effect of crude extracts and essential oils of the used plants against the 3rd larval instar and pupae (1- and 3-days old) of the peach fruit fly represented as Effective Concentrations (EC₅₀%). The values of effective concentrations (EC₅₀) differed according to the tested formulation and used plant as well as the treated individuals. Generally, the essential oils were more

effective than crude extracts against the tested stages showing the lowest values of EC_{50} s%. Respecting the full grown larvae of *B. zonata*, Lavender (as essential oils or crude extract) was the most adversely-effective showing the lowest values of EC_{50} (1.05 and 13%, respectively), whereas Jujube was the worst (EC_{50} values were 18.00% for crude extract and 3.00% for essential oils). But, in case of one-day old pupae, Jojoba crude extract and Lavender essential oils were the most efficient showing EC_{50} values of 9.13 and 1.25%, respectively. On the other side, both crude extract and essential oils of Neem were the lowest effective against 1-day old pupae of the peach fruit fly recording EC_{50} values of 18.70 and 6.90%, respectively. Finally, pupae of 3-days old were the most tolerable stage to the crude extracts of the four used plants showing EC_{50} values $> 20\%$, but the same stage was differently susceptible to essential oils of the tested plants, where Eucalyptus was the most efficient (EC_{50} = 3.10%) followed by Jujube (EC_{50} = 10.75%) and Lavender (EC_{50} = 12.00%), while Neem was the least efficient showing the highest value of EC_{50} of 13.80%.

On the other hand, as shown in Figures (1- 6), the increment of concentrations, the increment of effectiveness, where the malformed individuals and degree of deformities gradually and sharply increased with the increase of concentrations of all used formulations.

The obtained results are in agreement with those recorded by Saleh (1995) who reported that crude extracts of the different parts (roots, stems, leaves and flowers) of the goat weed (*Ageratum conyzoides*) with several solvents showed different types of adult deformities in *Culex pipiens pipiens* when added to larvae and pupae in water. On other side, Mediouni-Ben Jemâa and Boushah (2010) stated that Cyromazine (an insect growth regulator that mainly used to control several dipteran insects) had deleterious effects on the second instar larvae and adults of the Tunisian laboratory strain of Mediterranean fruit fly *C. capitata*. Effects were recorded as larvae and adult mortality together with the evaluation of larval and pupal body deformities, female fecundity and adult emergence rate. The toxic effect investigated as the appearance of body deformities for larvae and pupae was recorded. The percentage of deformities or abnormalities was positively correlated to cyromazine doses. Rizwan-ul-Haq (2012) investigated *B. zonata* for 48 hours post treatment, under the effects of different concentrations of lead acetate, and observed that under the effects of lead, abnormalities and malformation were developed in the larvae of the peach fruit fly. Pavela (2015) evaluated essential oils of many botanicals (such as *Mentha longifolia*, *Ocimum basilicum*, *Thymus* spp., *Eucalyptus* spp. and others) for larvicidal and biological potentiality against many species of mosquitoes.

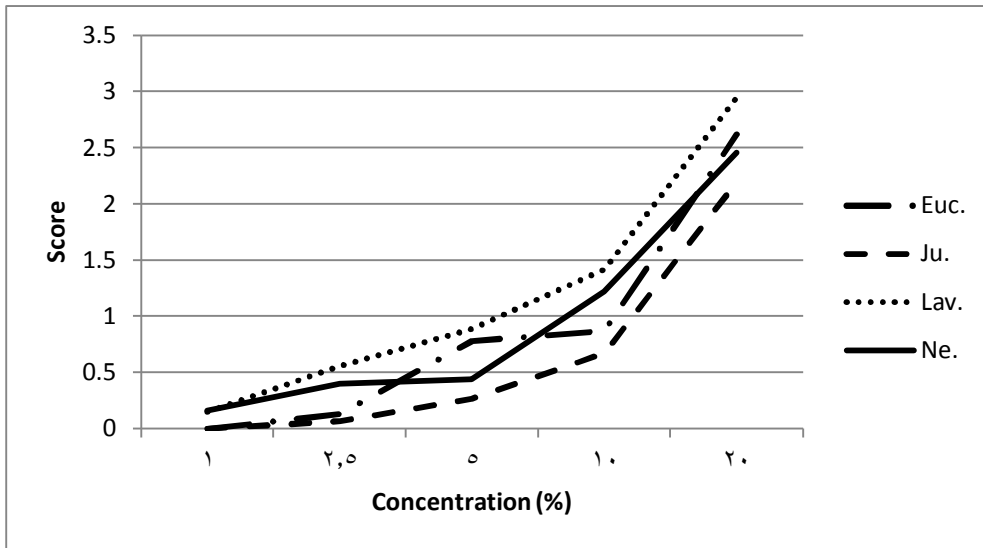


Fig. 1. Morphogenetic action of crude extract of certain medicinal and aromatic plants for the 3rd larval instar of *B. zonata*.

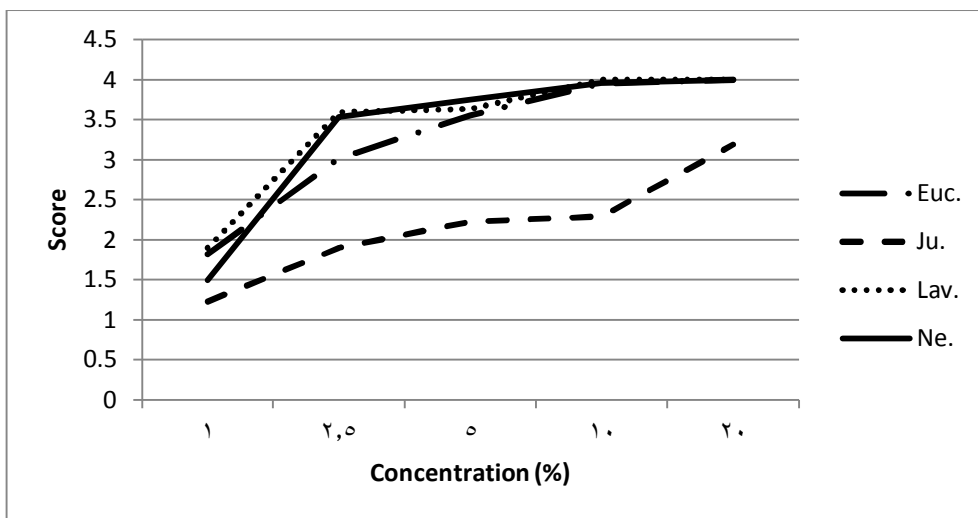


Fig. 2. Morphogenetic action of essential oils of certain medicinal and aromatic plants for the 3rd larval instar of *B. zonata*.

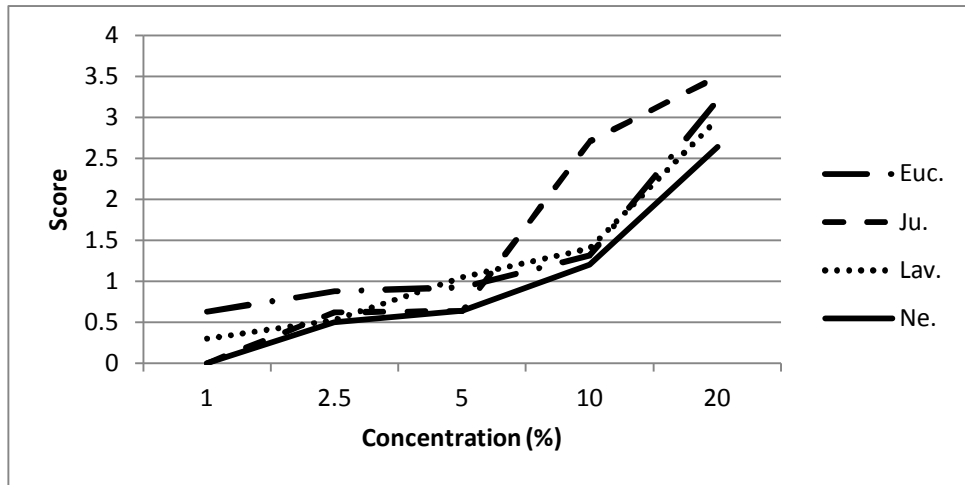


Fig. 3. Morphogenetic action of crude extract of certain medicinal and aromatic plants for 1-day old pupae of *B. zonata*.

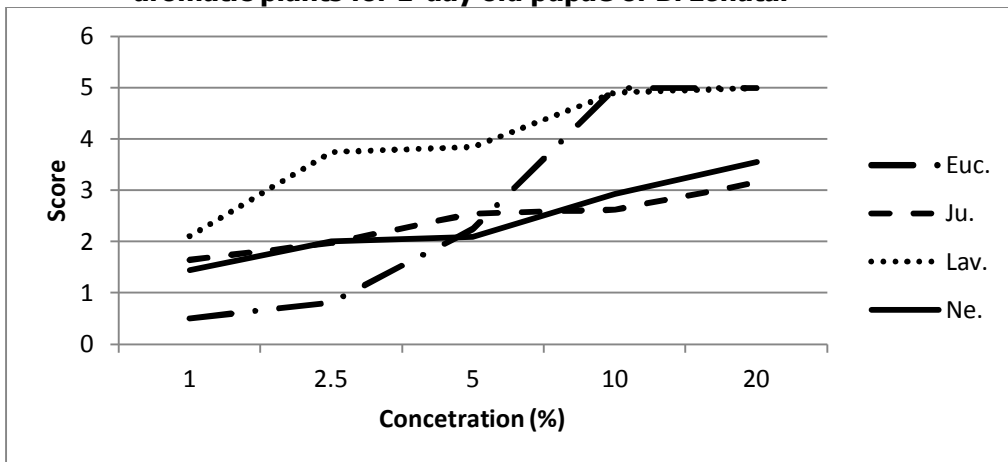


Fig. 4. Morphogenetic action of essential oils of certain medicinal and aromatic plants for 1-day old pupae of *B. zonata*.

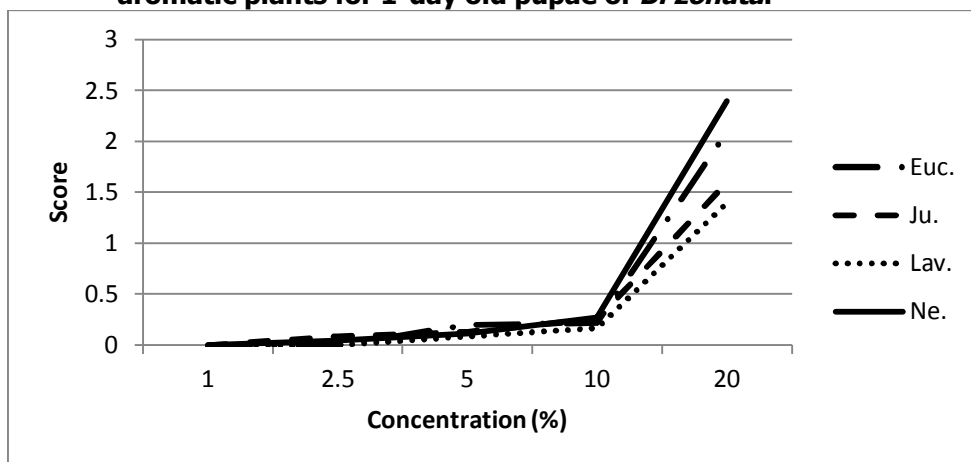


Fig. 5. Morphogenetic action of crude extract of certain medicinal and aromatic plants for 3-days old pupae of *B. zonata*.

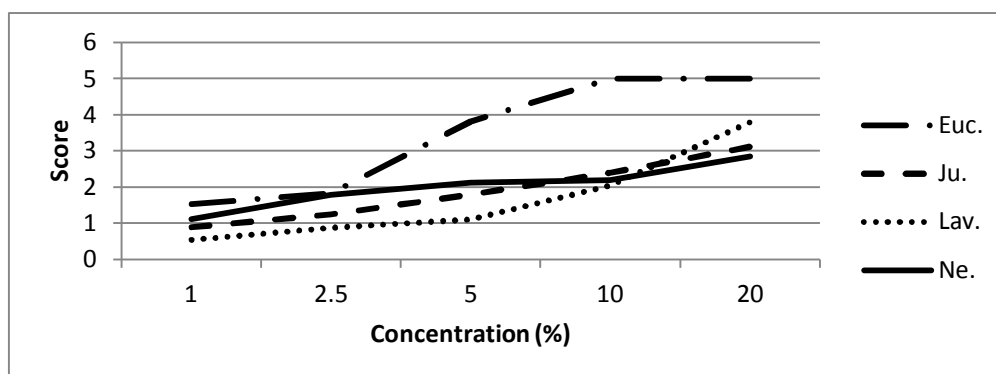


Fig. 6. Morphogenetic action of essential oils of certain medicinal and aromatic plants for 3-days old pupae of *B. zonata*.

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التأثيرات المورفولوجية لمستخلصات وزيت بعض النباتات الطبية والعطرية ضد بعض الأطوار غير الكاملة لذبابة ثمار الخوخ

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تم دراسة التأثيرات التشوهية فى الحشرات الكاملة لذبابة ثمار الخوخ الناتجة من يرقات العمر الثالث او عذارى عمر 1 و 3 يوم معاملة كسم باللامسة بمستخلصات وزيت نباتات الكافور والجوجوبا واللافندر والنعيم. أثبتت النتائج تباين قيم التركيزات الفعالة تبعا لنوع المستحضر المختبر والنبات المستخدم وكذلك الطور المعامل، حيث كانت الزيوت أكثر فعالية من المستخلصات النباتية ضد الأطوار المعاملة. ومن جهة أخرى كانت يرقات العمر الثالث لذبابة ثمار الخوخ الأكثر تأثرا لزيوت ومستخلص نباتات اللافندر حيث سجلت اقل قيمة EC_{50} (1.05 و 13%)، على التوالي) بينما كان نبات الجوجوبا اقلها فعالية (قيم EC_{50} 18% لمستخلص البذور، 3% للزيوت). وفى حالة العذارى عمر 1- يوم كان مستخلص بذور الجوجوبا وزيت نباتات اللافندر الأكثر كفاءة حيث اوضحت قيم EC_{50} 9.13 و 1.25%، على التوالي. أما بالنسبة للعذارى عمر 3- يوم فقد كانت أكثر الأطوار المعاملة تحملا لمستخلصات النباتات الأربع المستخدمة مسجلة قيم EC_{50} أكبر من 20%، الا أن الطور ذاته أظهر حساسية متفاوتة لزيوت النباتات المختبرة، حيث كان زيت الكافور الأكثر كفاءة (EC_{50} 3.10%). ومن جهة أخرى فإن زيادة التركيز أدت الى زيادة التأثير حيث زاد عدد الأفراد المشوهة أو درجة التشوهات بصورة تدريجية أو بصورة حادة بزيادة تركيز المادة المستخدمة.

