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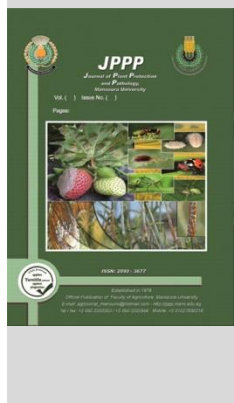
Toxicity Evaluation of Foliar Extracts of some Medical Plants on Fruit Fly *Ceratitis capitata* (Wiedemann) and Peach Fly *Bactrocera zonata* (Saunders), Diptera: Tephritidae

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ABSTRACT

This study aimed to evaluate the toxicity of foliar extracts of four medical plants, harmal (*Peganum harmala* L.), laurel (*Laurus nobilis* L.), marmaria (*Salvia officinalis* L) and zizyphus (*Zizyphus spina-christi*. Willd) on fruit fly *Ceratitis capitata* (Wiedemann) and peach fly *Bactrocera zonata* (Saunders). The effect of these extracts on larvae pupal duration, percent of malformation, percent of reduction in infestation and percent reduction in number of punctures /fruit. The results proved that, harmal, laurel and zizyphus show toxic effect on both *C. capitata* and *B. zonata* larvae. harmal was more toxic to the larvae of both flies than the other three compounds, with LC₅₀ values 8.52 and 10.5 ml/L for both flies, respectively. Harmal recorded the highest mean of malformation percent 48.79 and 43.62% for *C. capitata* and *B. zonata*, respectively followed by laurel and zizyphus in case of *C. capitata* and zizyphus and laurel in case of *B. zonata*. Harmal recorded the highest mean of reduction percent of infestation followed by laurel and marmaria in case of *C. capitata* and laurel and zizyphus in case of *B. zonata*. the highest mean of percent reduction in number of punctures/fruit was recorded after treatment with harmal and laurel for both *C. capitata* and *B. zonata*. The tested extracts show toxic effect on larvae of both flies, cause changes in some biological measurements and reduce both percent of infestation and number of punctures/fruit, so these extracts could be incorporated in management programmes of both fruit flies.

Keywords: plant extracts, biological measurements, *Ceratitis capitata*, *Bactrocera zonata*, punctures

INTRODUCTION

Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and Peach fruit fly *Bactrocera zonata* (Saunders) [Diptera: Tephritidae] are serious and destructive pests of fruits and vegetables in tropical and subtropical regions. These pests cause severe damage to the fruits resulting in a great loss of fruit production annually (Khan *et al.*, 2005).

Frequent and indiscriminate use of chemical insecticides for controlling these pests resulting in harmful consequences such as, environmental pollution, residual toxicity on food and development of more resistant strain of pests to chemicals. This has encouraged studies related to novel tactics in pest control to avoid these hazards, among them the use of natural products, Plant extracts are from several non- chemical control alternatives that take great interest due to their availability, efficacy and degradability, low human and mammalian toxicity and friendly to the environment (Aqil *et al.* 2010). More than 2000 plant species are known to have potential activity against insects (Klocke 1989; Souza *et al.* 2017).

The present investigation has been carried out in the laboratory to evaluate the effect of four plant extracts: harmal (*Peganum harmala* L.), laurel (*Laurus nobilis* L.), marmaria (*Salvia officinalis* L) and zizyphus (*Zizyphus spina-christi*. Willd) on:-

- 1- Toxicity of larvae, of fruit flies, *C. capitata* and *B. zonata* in treated soil.
- 2- Pupal duration and percent of malformation of both fruit flies.

- 3- Percent reduction of infestation and percent reduction of number of punctures of infestation on guava fruits treated with the four plant extracts.

MATERIALS AND METHODS

Fruit flies:

Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and Peach fruit *Bactrocera zonata* (Saunders) were obtained from the laboratory colony reared in the Horticulture Insects Department, Plant Protection Research Institute, Dokki, Giza, Egypt. Both flies were reared under laboratory conditions of 25 ± 3 °C and 60 ± 5 % R.H. both the insect larvae were reared using artificial larval rearing diet (consisting of 84.50 g sugar, 84.50 g brewer's yeast, 330 g wheat bran 500 ml water, 3 g sodium benzoate and 3 g citric acid,) according to the technique of (Afia, 2007). Full grown larvae were used in the experiment immediately after they had jumped from the artificial rearing medium for pupation. The adult flies were transferred to the adult rearing cage with wooden frames, supplied with metal screen from all sides. The adult flies were fed on sugar and protein hydrolysate at a ratio of 3:1, respectively. The eggs were collected by plastic vials that had several small pores to allow the adult females laid their eggs inside these vials which provided with water in its base.

Tested Plant leaves:-

Dried leaves of four plant species, harmal (*Peganum harmala* L.), laurel (*Laurus nobilis* L.), marmaria (*Salvia officinalis* L) and zizyphus (*Zizyphus spina-christi*. Willd) were used in the present study.

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Extraction process:-

Dried leaves of four plant species harmal (*P. harmala* L.), laurel (*L. nobilis* L.), marmaria (*S. officinalis* L.) and zizyphus (*Z. spina-christi* Willd) were washed under tap water followed by distilled water. leaves were powdered in mixer. 100 g of each species were used and mixed with 250ml of 95% acetone as organic solvent according to Salari (2011), then covered and shaken well for 30 minutes and left for 48 hr with repeat shaken again every 12 hr and filtered through narrow colander, Then the solution filtered again over a filter paper. The filtrate was transferred to wide petri dishes and left until evaporation of the solvent and left for 72hr then 100% plant extraction was prepared and become ready to be used.

Experiment (1):-**Toxicity effect and some biological measurements:**

Five different concentrations, 5, 10, 15 and 20 ml/L of the tested plant extracts, harmal, laurel, marmaria and zizyphus with water were prepared and 10 ml of each were added separately to 100 gm of sandy soil, in plastic cups of 7cm diameter and 9cm height. Ten recently jumped larvae (full grown) of each fruit fly species were added separately to the surface of the treated soil. three replicates of each concentration for each foliar plant extract were carried out and three replicates with untreated soil (soil treated with water only) were made as a control. The experiment was maintained under laboratory conditions with humidity of approximately 75 % and temperature 30 ± 1 °C and left till adult emergence. Number of dead insects, pupal duration (since start of the treatment till adult emergence) and number of deformed insects of both fruit flies were recorded.

Experiment (2):-**Effect of foliar plant extracts on percent reduction of infestation:-**

In this bioassay 54 fully ripe guava fruits were washed with water then dried and immersed separately in five different concentrations 2.5, 5, 10, 15 and 20 ml/L of the four tested foliar plant extracts for ten seconds and left to dry for 2 hr. Three replicates for each concentration were applied (3fruits for each replicate). The treated fruits were placed in a rearing cage with ten mature males and ten mature females flies separately for *C. capitata* and *B. zonata* and subjected to infestation for a period of 72 hr. Untreated fruits were subjected to infestation in a similar rearing cage were used as a control. Rearing cages were supplied with adult's food (3: 1 sugar: protein hydrolyzed). Each fruit was examined separately for infestation, number of infested fruits and number of punctures for each fruit for each concentration was recorded for both flies and percent reduction in infestation and percent reduction in number of punctures were calculated.

Data analysis:-

Percentages of Mortality of both flies were corrected according to (Abbott, 1925) then results were analyzed to calculate LC_{50} and LC_{90} according to Finney (Finney 1971) using LDP line soft wear (Ehab soft). Percentage reduction in infestation and percentage reduction in number of punctures of infested fruits were calculated: % reduction = (control - treated) ÷ control × 100. IBM SPSS21 software package was used for statistical analysis to find out the significance of each compound. Analysis of variance (ANOVA) two ways was used to determine the significance between means.

RESULTS AND DISCUSSION**Toxicity effect: -**

Mortality percentages of Mediterranean fruit fly *C. capitata* and Peach fruit fly *B. zonata* larvae after treatment with different concentrations of the four tested plant extractions are indicated in Table (1). The results indicated that, mortality percentages of both insects were increased as the concentration of the plant extracts increased and the highest mortality percentages of *C. capitata* larvae (86.36 %) were recorded after treatment with harmal (*P. harmala* L.) followed by laurel (*L. nobilis* L.) recorded 70.46% mortality and zizyphus (*Z. spina-christi*) recorded 49.99% mortality and marmaria (*S. officinalis* L.) recorded the lowest mortality percentage. While, after the treatment of *B. zonata* larvae 93.33% mortality percentage was obtained after the treatment with harmal followed by zizyphus recorded 57.78% and laurel recorded 37.8%. So harmal, laurel and zizyphus show toxic effect on both *C. capitata* and *B. zonata* larvae. The obtained results are in agreement with that obtained by Moretti (1998) who reported that marmaria (*Salvia officinalis*) oil, characterized by relatively high concentrations of monoterpenic ketones (camphor and thujone), and produced mortality of close to 50% to *C. capitata* adults, and Abbassi et al., (2003) found under laboratory conditions the toxic effect of *P. harmala* on the survival of the desert locust, *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae). The results also agree with Jbilou et al., (2006) who found insecticidal effects of methanolic extracts from different medicinal plants, including *P. harmala* seeds on the larvae and adults of the stored grain pest *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) after a period of time. The results also agree with Ghada et al., (2020) who showed that *Moringa oleifera* oil, Lemon peel oil and *M. oleifera* extract causing toxic effect to *B. zonata* pupae when mixed in yellow, sandy and clay soil.

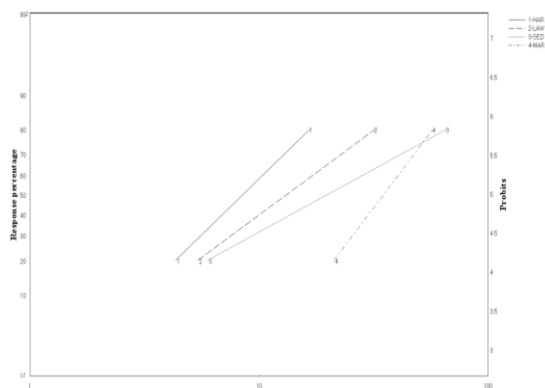
Table 1. Mortality percentage of *C. capitata* and *B. zonata* larvae after treatment with different concentrations of leaf extracts of harmal, laurel, zizyphus and marmaria.

Insect	Tested extracts	Concentration ml/L				
		2.5	5	10	15	20
<i>C.capitata</i>	Harmal	9.08	22.7	49.9	81.8	86.36
	Laurel	4.54	20.44	43.17	44.76	70.46
	Zizyphus	6.82	18.17	29.53	43.23	49.99
	Marmaria	0	0	2.3	6.8	18.22
<i>B.zonata</i>	Harmal	0	17.18	35.33	66.67	93.33
	Laurel	0	2.23	8.9	28.9	37.8
	Zizyphus	0	6.7	51.12	55.55	57.78
	Marmaria	0	0	2.23	4.5	13.46

The toxicity values of the four plant extracts based on LC_{50} and LC_{90} are indicated in Table (2) and Fig. (1 and 2), the data show that, when *C. capitata* larvae were exposed to the tested extracts, harmal (*P. harmala* L.) recorded the lowest LC_{50} value (8.51 ml/L) followed by laurel (*L. nobilis* L.) recording 13.209 ml/L and zizyphus (*Z. spina-christi*) recording 19.89 ml/L then marmaria (*S. officinalis*) (35 ml/L). While when *B. zonata* larvae were exposed, harmal also has the lowest value of LC_{50} (10.53 ml/L) followed by zizyphus 13.68ml/L and laurel 24.64ml/L then marmaria 45.62 ml/L. So harmal was more toxic to both *C. capitata* and *B. zonata* larvae than the other three compounds.

Table 2. Toxicity of leaf extracts harmal, laurel , zizyphus and marmaria on *C. capitata* and *B. zonata* larvae.

Insect	Tested extracts	Lethal concentration		Slope \pm S.E.	Toxicity index
		LC ₅₀ (ml)	LC ₉₀ (ml)		
<i>C.capitata</i>	Harmal	8.519	23.841	2.88 \pm .227	100
	Laurel	13.209	51.346	2.174 \pm .220	64.494
	Zizyphus	19.898	124.133	1.612 \pm .021	42.813
	Marmaria	35.008	74.7	3.891 \pm 1.044	24.334
<i>B.zonata</i>	Harmal	10.53	23.385	3.7 \pm .346	100
	Laurel	24.64	64.695	3.057 \pm .473	42.757
	Zizyphus	13.68	43.64	2.538 \pm .320	76.993
	Marmaria	45.629	114.358	3.212 \pm 1.104	23.088

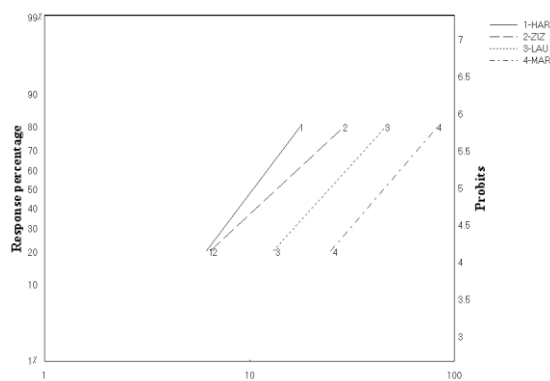


1- Harmal, 2- Laurel ,3- Zizyphus, 4- Marmaria.

Fig. 1. LC-P lines of four plant extracts harmal, laurel, zizyphus and marmaria against *C. capitata* .**Table 3. Effect of leaf extracts of harmal, laurel, zizyphus and marmaria on pupal duration (in days) of *C.capitata* and *B. zonata* larvae.**

Insect	Tested extracts	Concentration in ml/L					Mean	F	P
		2.5	5	10	15	20			
<i>C.capitata</i>	Harmal	7.6	8	8.66	10	10.33	8.65	6.14	0.008
	Laurel	7.33	6.33	6	5.66	5.33	6.32		
	Zizyphus	7	7	6	8.66	9.66	7.60		
	Marmaria	7	6.33	7.66	8.66	9	7.76		
	Mean	7.23	6.9	7.08	8.24	8.58			
	F				2.11				
	P				0.14				
<i>B.zonata</i>	Control	7.3	7.3	7.3	7.3	7.3	7.3	19.3	< .01
	Harmal	5.6	6.33	7	7	8	6.54		
	Laurel	5	4.66	4.66	5.66	6	5.21		
	Zizyphus	5.66	5.66	6.66	7.33	7.33	6.32a		
	Marmaria	5.3	5.6	5.6	7	7	5.97		
	Mean	5.39	5.56	5.98	6.75	7.08			
	F				17.28				
	P				0.01				
	Control	5.3	5.3	5.3	5.3	5.3	5.3		

Comparing the mean pupal duration recorded with the four leaf extracts, the results show that in case of *C. capitata*, harmal significantly ($p < 0.001$) elongated the duration (8.91d) while zizyphus ($p = 0.17$) and marmaria ($p = 0.06$) insignificantly increase duration recorded (7.66 d) and (7.73d), respectively and laurel significantly ($p = 0.001$) decrease the duration recorded (6.325d) compared to control (7.3 d). In case of *B. zonata* the results show that, all plant extracts used significantly elongated the pupal duration with increasing the concentration except laurel insignificantly ($p = 0.27$) decrease the duration. The obtained results agree with that recorded by Ghada *et al.*, (2020), who cleared that *M. oleifera* leaf extract, lemon peel oil and *M. oleifera* oil elongated the pupal duration for *B. zonata* when the concentrations were increased.

**Fig. 2). L C-P lines of four plant extracts harmal, laurel , zizyphus and marmaria against *B. zonata*.**

Comparing values of LC₅₀ obtained for the two insects, data revealed that, in case of *C. capitata*, all values except that obtained with zizyphs were lower than that obtained in case of *B. zonata*, so *C. capitata* larvae may be more susceptible to these extracts than *B. zonata*.

Effect on some biological measurements:

1-Effect on pupal duration:-

Data in Table (3) show the effect of four plant extracts on pupal duration (since start of the treatment till adult emergence) of both insects. Data obtained cleared that, the percentage of pupal duration increase with increasing concentration for both *C. capitata* and *B. zonata* after treatment with harmal compared to control, so harmal elongated the pupal duration.

2-Effect on percent of malformation:-

The results in Table (4) clear that, the percentage of malformation was concentration dependent, as it reached the highest values 86.67, 86.12, and 73.55 % with harmal, laurel and zizyphus, respectively after treatment of *C. capitata* larvae with the highest concentration 20 ml /L compared to control (untreated) 0 %, while after treatment of *B. zonata* larvae with the same concentration it reached the highest values 93.33, 48.7 and 73.68% with the same extracts, respectively compared to control 0 %, so the tested extracts may affect on the hormonal system of the insect. The results agree with Kelany *et al.*, (1991) who reported a different grades of deformities in emerged flies of *M. domestica* after treatment with aqueous neem seed kernel extract. The results also agree with Selem (2005) who found

abnormalities in emerged adults of *M. domestica* after the treatment of 3rd larval instar with NeemAzal-T and galangal extract, and Khalil *et al.* (2010) who reported that, Pyriproxyfen caused high percentage of malformations in the adult fly of *M. domestica*, and Ghada *et al.*, (2020), who stated that treatment of *B. zonata* pupae with *Moringa oleifera* oil, lemon peel oil and *M. oleifera* leaf extract

induced different scores of deformities in all parts of the body of emerged adult flies.

Comparing the effect of the tested extracts on the two flies, the data cleared that, harmal has the highest mean of malformation percent 48.79 and 43.62% for *C. capitata* and *B. zonata*, respectively followed by laurel and zizyphus in case of *C. capitata* and zizyphus and laurel in case of *B. zonata*.

Table 4. Effect of leaf extracts of harmal, Laurel, zizyphus and marmaria on percent of malformation of *C. capitata* and *B. zonata* after exposure of larvae to treated soil.

Insect	Tested extracts	Concentration in ml /L					Mean	F	P
		2.5	5	10	15	20			
<i>C. capitata</i>	Harmal	26.11	38.44	52.21	82.22	86.67	57.13	23.05	0.01
	Laurel	6.67	25.25	52.31	59.8	86.12	46.03		
	Zizyphus	8.9	27.9	47.11	60.24	73.55	43.54		
	Marmaria	0	2.23	9.26	21.18	30.75	12.68		
	Mean	10.42	23.45	40.22	55.86	69.27			
	F				28.68				
	P				0.01				
<i>B. zonata</i>	Control	0	0	0	0	0		9.006	0.002
	Harmal	0	35.98	56.23	76.17	93.33	52.34		
	Laurel	0	2.23	8.9	35.91	48.7	19.14		
	Zizyphus	0	6.7	59.45	70.65	73.68	42.09		
	Marmaria	0	0	4.45	11.32	21.16	7.39		
	Mean	0	11.22	32.25	48.5	59.2			
	F				10.4				
	P				0.0007				
	Control	0	0	0	0	0			

Effect on percent reduction of infestation:-

Results obtained in Table (5) cleared that, after treatment of both insects with the tested extracts, percentages in reduction of infestation increased significantly as the concentration increased and 100% reduction of infestation was obtained at a concentration 15ml/l when harmal and laurel were used while, when marmaria was used the same result was attained at the highest concentration 20ml/l in case of *C. capitata* and at 15ml/l in case of *B. zonata*. After treatment with zizyphus, the highest percent reduction at the highest

concentration 20ml/l was 71.4% and 87.5 % in case of *C. capitata* and *B. zonata*, respectively.

Comparing the mean percent reduction of infestation for all extracts, analysis show that, in case of *C. capitata*, harmal recorded the highest mean of percent reduction followed by laurel and marmaria. in case of *B. zonata* harmal also recorded the highest mean of reduction percent followed by laurel and zizyphus.

Table 5. Effect of leaf extracts of harmal, Laurel, zizyphus and marmaria on percent reduction of infestation by *C. capitata* and *B. zonata*.

Insect	Tested extracts	Concentration in ml /L					Mean	F	P
		2.5	5	10	15	20			
<i>C. capitata</i>	Harmal	42.85	71.4	85.7	100	100	79.99	13.58	0.0004
	Laurel	42.85	57.14	85.71	100	100	77.12		
	Zizyphus	14.28	28.57	28.57	71.4	71.4	42.84		
	Marmaria	14.28	14.28	57.14	85.7	100	54.26		
	Mean	28.54	42.84	64.84	89.27	92.85			
	F				26.67				
	P				0.01				
<i>B. zonata</i>	Harmal	37.5	50	75	100	100	72.5	2.9	0.078
	Laurel	37.5	37.5	75	100	100	70.0		
	Zizyphus	12.5	50	75	75	87.5	60.0		
	Marmaria	0	12.5	50	100	100	52.5		
	Mean	21.8	37.5	68.5	93.75	96.87			
	F				30.34				
	P				0.01				

Effect on percent reduction in number of punctures /fruit:

Results in Table (6) showed that, the percent reduction in number of punctures /fruit that treated with the tested extracts was significantly increase with increasing concentration for both insects, *C. capitata* and *B. zonata*. 100% percent reduction was recorded at a concentration 15 and 20 ml/l after treatment with all tested extracts except zizyphus which recorded 90% percent reduction at the same concentrations in case of *C. capitata* and 82.63 and 91.3% respectively, in case of *B. zonata*.

Comparing the mean percent reduction in number of punctures/fruit for all extracts, the data show that, in case of

C. capitata the highest mean of percent reduction in number of punctures 89.99 and 86.99 % was recorded by harmal and laurel, respectively followed by marmaria which recorded 74.01%, while in case of *B. zonata* 75.63, 74.77 were attained with the harmal and laurel, respectively and 72.19% percent reduction was recorded with zizyphus.

The obtained data are in full agreement with that obtained by Ortu *et al.*, (2009) who reported that more medfly punctures per fruit were observed on untreated (control) fruits than on fruits treated with the bio-insecticide *Naturalis* based *Beauveria bassiana* (Balsamo).

Table 6. Effect of leaf extracts of harmal, laurel, zizyphus and marmaria on percent reduction in number of punctures /fruit by *C.capitata* and *B. zonata*.

Insect	Tested extracts	Concentration in ml /L					Mean	F	P
		2.5	5	10	15	20			
<i>C.capitata</i>	Harmal	69.96	90	90	100	100	89.99	8.68	0.002
	Laurel	69.96	69.96	95.04	100	100	86.99		
	Zizyphus	30.03	39.93	44.95	90	90	58.98		
	Marmaria	34.98	60.06	80.03	95	100	74.01		
	Mean	51.23	64.98	77.5	96.25	97.5			
	F	13.84							
	P	0.0002							
<i>B.zonata</i>	Harmal	34.72	60.83	82.63	100	100	75.63	1.77	0.205
	Laurel	34.72	52.21	86.94	100	100	74.77		
	Zizyphus	43.47	65.27	78.32	82.63	91.3	72.19		
	Marmaria	8.61	43.47	65.27	100	100	63.47		
	Mean	30.38	55.44	78.29	95.65	97.82			
	F			37.57					
	P			0.01					

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تقييم سمية المستخلصات الورقية لبعض النباتات الطبية على ذبابة الفاكهة *Ceratitis capitata* (Wiedemann) وذبابة الخوخ *Bactrocera zonata* (Saunders)

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الملخص

تهدف هذه الدراسة الى تقييم سمية مستخلصات أربع نباتات طبية هم الهرمل *Peganum harmala*، اللورل *Laurus nobilis*، السدر *Zizyphus spina-christi* Willd والمرمريا *Salvia officinalis* L على يرقات ذبابة الفاكهة *Ceratitis capitata* وذبابة الخوخ *Bactrocera zonata* وتأثير هذه المستخلصات على العمر العذرى، نسبة التشوه، نسبة الخفض في الاصابة ونسبة الخفض في عدد الوخزات لكل ثمرة. وقد أثبتت النتائج أن كل من الهرمل، اللورل والسدر لهم تأثير سمي على يرقات كل من الذبابتين وأن الهرمل كان أكثر سمية من الثلاث مركبات الأخرى بجرعة نصف مميتة هي ٨,٥٢ و ١٠,٥٣ مل/لتر على الترتيب. كما سجل الهرمل أعلى متوسط نسبة تشوه ٤٨,٧ و ٤٣,٦٢% ليرقات *Ceratitis capitata* و *Bactrocera zonata* على الترتيب يليه اللورل والسدر في حالة *Ceratitis capitata* والسدر ثم اللورل في حالة *Bactrocera zonata*. وسجل الهرمل أيضاً أعلى متوسط نسبة خفض في الاصابة يليه اللورل ثم المرمريا في حالة *Ceratitis capitata* واللورل ثم السدر في حالة *Bactrocera zonata*. وكان أعلى متوسط نسبة خفض في عدد الوخزات تم تسجيله بعد المعاملة بكل من الهرمل واللورل مع كل من *Ceratitis capitata* و *Bactrocera zonata*. وقد أظهرت هذه المستخلصات تأثير سمي على يرقات كلا الذبابتين بالإضافة الى أحداث تغيرات في بعض القياسات البيولوجية وأحداث خفض في معدل الاصابة وعدد الوخزات في الثمرة لكل من الذبابتين، لذا يمكن ادراج هذه المركبات ضمن برامج مكافحة لكل من ذبابة الفاكهة وذبابة الخوخ.