EFFECT OF SOME INSECTICIDES ON RESISTANCE LEVEL AND CERTAIN BIOCHEMICAL ASPECTS IN Ceratitis Capitata (DIPTERA: TEPHRITIDEA) FROM DIFFERENT LOCALITIES IN EGYPT

[4]

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ABSTRACT

To estimate toxic actions of two insecticides on {lambda-cyhalothrin (Karilot gold 5%EC) and the biopesticide spinosad, (Conserve 0.24%CB)}, adults of the laboratory strain of Mediterranean fruit fly, Ceratitis capitata (Wied.) were treated with different concentrations. Discriminating value (LC₉₉) was selected for monitoring resistance in three field populations collected from different governorates (Qaluobia, Behira and Sharkia). Data showed that, males of laboratory strain as well as field colony strains were more susceptible than females. Qaluobia field colony exhibited the highest resistance level to lambda-cyhalothrin followed by Sharkia and Behira colonies in both male and female flies comparing with laboratory strain. Sharkia colony showed remarkable higher level of resistance to spinosad in males and females more than Behira and Qaluobia colonies. On the other hand, Ceratitis capitata flies of the different field strains showed remarked higher levels of the enzymes activity than the laboratory strain. Males showed the highest levels of acid phosphatase in Qaluobia, whereas Sharkia showed the highest level in females. The levels of alkaline phosphatase were higher in Qaluobia followed by Behira and Sharkia in both sexes. Behira had the highest enzymatic activity of AChE in male and female flies. The levels of total protein were higher in Behira than the other field colonies in males, but Sharkia exhibited the highest level in case of female flies.

This means that the enzymatic activities play an important role in the insect resistance to spinosad and lambda-cyhalothrin, and there were positive correllation between resistance levels and enzymatic activity in the different field colonies.

Keywords: Mediterranean fruit fly, *Ceratitis capitata*, Pesticides, Biopesticides, Resistance, Discriminating, Biochemical, lambda-cyhalothrin, spinosad.

INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) is one of the most harmful pest in order Diptera: Tephiritidea. It is the most widespread in many countries all over the world and considers one of the most dangerous insect pests for horticultural crops (White & Elson-Harris, 1992 and Solari *et al.*, 2016). This cosmopolitan and polyphagous species of fruit flies invades about 350 hosts such as apple, apricot, citrus, figs, guava, mangoes, peach, pear, quince and others (White and Elson-Harris, 1992). Females deposit eggs under the peel of semi-ripening and ripening fleshy fruits. The hatched larvae feed on the pulp of the infested fruits causing deleterious damage throughout 3 larval instars. After that, the full grown larvae (3rd larval instar) leave the fallen fruits to pupate inside soil at 2-5 cm depth. Under Egyptian conditions, the Mediterranean fruit fly have about 8 - 10 generations (Hafez and Fares, 1967). It causes losses directly and indirectly in the crops, resulting in significantly low productivity per feddan as well as reducing fruit quality (Joomaye *et al.*, 2000).

Some chemical insecticides which belong to different groups have been used to control this harmful insect. These insecticides had side effects on the environment, human health and had toxic effects on non-target organisms The wide-spread use of chemical pesticides causes adverse effects on pest.

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Because of excessive repeated use resulting in create of resistance strains of pests and thus disrupt the ecological balance, The existence of resistant strains to pesticides will have an impact in increasing the use of such pesticides with high concentrations or rates. The use of a high concentration of pesticide had negative impact on the natural enemies and the ecosystem, rising pesticides residues in fruits, leading to increase costs for farmers and rejected fruit export shipments (Carante and Lemaitre, 1980; Rossi & Rainaldi, 2000 and Magana *et al.*, 2007).

This work aimed to estimate, in laboratory experiments, the resistance of certain insecticides (Spinosad and Lambda-Cyhalothrine) against the adults of *C. capitata* (Wied.) of three field strains (Qaluobia, Behira and Sharkia) compared to the laboratory strain.

MATERIAL AND METHODS

1- Rearing Technique

1-1-Laboratory strain: Laboratory strain of C. capitata were continuously reared under laboratory conditions of 25 ± 3 °C and $60 \pm 5\%$ R.H in Horticulture Insects Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. Adults of C. capitata were reared in a cage (60x40x40 cm) with wooden frames and covered from each side with metal screen, except one side that covered with muslin clothes for oviposition. Flies in rearing cage were fed with sugar and fortified protein hydrolysate at ratio of 5:1, respectively. Also, water was added in a plastic bottle with cotton plug. Eggs were received

- in plastic pans filled with 3cm water to prevent eggs dehydration (Awadallah and El-Hakim, 1987).
- 1-2-Field strains: To obtain mass production, large quantities of infested Guava fruits were collected from heavily infested orchards in the three examined governorate (Qaluobia, Sharkia and Behira). These fruits were separately incubated in large plastic pans with fine sand to allow jumping larvae to pupate. Pupae of each strain were separated via sieving and introduce to separate cages for each strain. Adults of these strains were dealt or processed as the laboratory strain.
- 1-3- Larval diet content: The larval artificial diet consists of tap water (500 ml), wheat bran (330 gm), sugar (84.50gm), Brewer's yeast (84.50 gm), sodium benzoate (3.00gm) and citric acid (3.00gm). These ingredients were carefully mixed in large plastic container. Then, eggs were scattered on the surface of the diet which was placed in plastic trays of 20x10x8cm that were tightly covered with muslin clothes using rubber bands. After that, these trays were placed in large plastic pans with sand at the bottom to allow the jumping larvae to pupate. All pupae were separated by sieving from sand (Awadallah and El-Hakim, 1987).

2- Pesticides Used:

- 1- Karilot gold 5% EC (Lambda-Cyhalothrin).
- 2- Conserve 0.24% CB (Spinosad).
- **3- Method of Application:** A discriminating concentration technique was used for rapid monitoring insecticidal resistance in field-collected, *Ceratitis capitata* flies. Roush and Miller (1986) revealed that testing an insect sample at a discriminating concentration is more efficient than

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estimating dose-response regression lines in monitoring for resistance. The diagnostic concentration is a single concentration that can discriminate between susceptible and resistant individuals. Gunning et al. (1984) suggested that the discriminating concentration was the LC₉₉ values for susceptible insects. The LC99 of the susceptible C. capitata flies were estimated previously from the adult via technique by probit analysis and used to estimate a diagnostic concentration for the two compounds tested, based on considerations of Roush and Miller (1986). To estimate a concentration that would reliably discriminated, sixty males and 60 females were separately placed in small cages in three replicates (10 pairs / each cage) that were exposed to Petri-dishes treated with the tested insecticides. Two ml. of each compound was pipetted into a Petri-dish. Petri-dishes were rolled for approximately 2 min. to ensure that all surfaces received the insecticidal treatment. Petri-dishes were air dried (vertically) at room temperature for 24 hours. Cages with flies were placed at room temperature, and the mortality was assessed after 24 hours.

Resistance percentages in the tested insecticide in the field colonies were determined by calculating mortality percentage of the discriminating concentration of Spinosad and Lambda-Cyhalothrin, the correcting control mortality and percentage of survival in laboratory insect at the discriminating concentration used. To calculate the resistance percentage, the following formula adopted by McCutchen *et al.* (1989) was used.

Resistance percentage = 100-(MF/MSx100), where:

MF = % mortality at discriminating concentration in field collected flies.

MS = % mortality (constant) at discriminating concentration in laboratory flies.

4- Biochemical Assays

- **4.1. Determination of total proteins activity:** Total proteins were determined by method of Bradford (I976). Protein reagent was prepared by dissolving 100mg of Coomassie Brilliant blue G-250 in 50ml 95% ethanol. To this solution 100ml 85% (W/V) phosphoric acid were added. The resulting solution was diluted to a final volume of 1 liter.
- **4.2. Determination of acid and akaline phosphatase activity:** Acid and alkaline phosphatases were determined according to the method described by Powell and Smith (1954). In this method, the phenol released by enzymatic hydrolysis of disodium phenylphosphate reacts with 4-aminoantipyrine and by the addition of potassium ferricyanide, the characteristic brown color is produced.
- **4.3. Determination of acetylcholine esterase activity:** AChE (acetylcholine esterase) activity was measured according to the method described by Simpson *et al.* (1964), using acetylcholine bromide (AchBr) as substrate.

RESULTS AND DISCUSSION

1- Mortality Percentages: Data in Table (1) indicate effectiveness of the different concentration of the two used pesticides (Spinosad and Lambda-Cyhalothrin) against males and females of *C. capitata* laboratory strain. The tested concentrations ranged between 2500- 12500 and 25- 200 ppm for Spinosad and Lambda Cyhalothrin, respectively. The mortality percentages ranged between 21.40- 94.23, 21.00- 83.73% (for Spinosad)

and 15.95- 83.05%, 12.54- 78.88% (for Lambda Cyhalothrin) for males and females, respectively. The slope values of Spinosad were 2.22 and 2.16 for males and females, whereas those obtained for the second pesticide (Lambda Cyhalothrin) were 3.08 and 2.43 for males and females, consecutively. The values of LC_{50} for males and females were 5420.42, 5952.90 and 94.14, 92.31 ppm for Spinosad and Lambda-Cyhalothrin, respectively. But, the respective LC_{99} were 30549.21, 53249.61 and 1042.73, 1094.39 ppm.

Table (1): Efficiency of two pesticides against males and females of *C. capitata* laboratory strain

	Lambda-Cyhalothrin			Spinosad		
Mortality (%)		Concentration (ppm)	Mortality (%)		Concentration (ppm)	
Female	Male		Female	Male		
78.88	83.05	200	83.73	94.23	12500	
67.29	69.42	150	68.51	73.99	10000	
46.36	46.07	100	54.25	55.46	7500	
34.43	34.20	75	38.77	37.18	5000	
25.30	20.29	50	21.00	21.40	2500	
12.54	15.95	25	-	-	1	
2.43	3.08	Slope	2.16	2.22	Slope	
92.31	94.14	LC ₅₀	5952.90	5420.42	LC ₅₀	
1094.39	1042.73	LC99	53249.61	30549.2	LC99	

2- **Resistance Percentages:** Mortality and resistance percentages as well as relative resistance formales and females of three different field collections (Qaluobia, Behira and Sharkia) to discriminating concentrations of the two used pesticides were shown in Table (2). The percentages of mortality for males and females of the three examined field colonies ranged between 29.41- 40.74%, 6.66- 34.61% and 0.0- 28.57%, 0.0-21.05% for Spinosad and Lambda-Cyhalothrin, respectively. The discriminating concentration (LC99) of the baseline caused 59.59, 58.84 and 70.29% resistance to spinosad for males collected from Qaluobia, Behira and Sharkia, respectively. Males of Sharkia field colony exhibited the highest values of resistance to Spinosad (70.29%). Females of the three tested field colonies showed differently values of % resistance to Spinosad of 79.79, 65.04 and 93.27 for Qaluobia, Behira and Sharkia, respectively. The relative resistance of the three tested field colonies to spinosad ranged between 1- 1.18 and 1- 1.43% for males and females, successively.

Table (2): Response of males and females of *C. capitata* collected from three governorates to discriminating concentrations of pesticides

Pesticide	Strain	Mortality (%)		Resistance (%)		Relative resistance	
		Male	Female	Male	Female	Male	Female
Chinagad	Qaluobia	40	20	59.59	79.79	1.17	1.16
Spinosad (Conserve)	Behira	40.74	34.61	58.84	65.04	1.18	1.43
(Conserve)	Sharkia	29.41	6.66	70.29	93.27	1	1
Lambda Cyhalothrin (Karilot)	Qaluobia	0	0	100	100	1	1
	Behira	28.57	21.05	71.14	78.73	1.40	1.27
	Sharkia	25	10	74.74	89.89	1.33	1.11

^{*}Resistance = $100 - \frac{\%$ mortality at discriminating concentration in field strain mortality at discriminating concentration in laboratory strain $\times 100$

Respecting the second compound (Lambda-Cyhalothrin.) according to the discriminating concentration (LC₉₉), Qaluobia field colony showed the highest values of % resistance (100) for each males and females. The other tested field colonies (Sharkia and Behira) recorded % resistance to Lambda-Cyhalothrin of 74.74, 71.14 and 89.89, 78.73 for males and females of *C. capitata*, respectively. The resistance relatively ranged between 1- 1.40 and 1-1.27 for males and females, consecutively.

3- Effect on Acid Phosphatase: Statistical analysis of variance of data revealed that the differences in amounts of acid phosphatase between the examined colonies were highly significant in both males and females of the Mediterranean fruit fly (Table 3). The amounts of acid phosphatase insignificantly varied in males of the three examined field colonies and

^{**}Relative resistance = The highest resistance percentage corresponding resistance percentage of each strain

higher than that of laboratory strain. The highest amount of acid phosphatase (29.07 mU/g.b.w.) was shown in males of Qaluobia field colony followed by Behira and Sharkia field colonies (28.50 and 26.90 mU/g.b.w., respectively) in case of females, the highest

Table (3): Acid phosphatase activity in the whole homogenates of different field colony of *Ceratitis capitata* compared to the laboratory strain.

Etald aslama	(mU/ g.b.wt) Ac	Activity ratio		
Field colony	8	9	3	9
Qaluobia	29.07±0.95 a	23.1±0.95 b	1.36	1.11
Behira	28.5±1.37 a	20.83±0.25 °	1.33	1.003
Sharkia	26.9±1.77 ^a	33.9±1.28 a	1.26	1.63
Laboratory	21.43±0.60 b	20.77±0.57 °	1	1
F test	23.17**	160.75 **	-	-
L.S.D	2.36	1.61	_	-

^{*} Enzymatic activity was expressed as µg naphthol released / min / g.b.w.

Value of acid phosphatase amount (33.9 mU/g.b.w.) was significantly observed in individuals of Sharkia field colony. But, the lowest value of 20.83 mU/g.b.w. was recorded with females of Behira colony that insignificantly varied with those shown with females of laboratory colony (20.77 mU/g.b.w.). The activity ratios of the three tested field colonies were 1.36, 1.33, 1.26 fold (for males) and 1.11, 1.003, 1.63 fold (for females) of the laboratory colony.

^{**} Activity ratio = enzymatic activity of filed colony strain/ enzymatic activity of the laboratory strain

4- Effect on Alkaline Phosphatase: As shown in Table (4) the differences in Alkaline phosphatase between the examined colonies were statistically high significant. Qaluobia field colony significantly recorded the highest amounts of Alkaline phosphatase in both males and females (932.67 and 1074.33 mU/g.b.wt) showing the highest values of activity ratio 3.63 and 5.63fold of laboratory colony, respectively. The amounts of Alkaline phosphatase in males and females of both Behira and Sharkia colonies were insignificantly differed (603.33, 463.0 and 553.67, 430.67 mU/g.b.wt., respectively. The respective activity ratios were 2.35, 2.43 and 2.15, 2.25 fold of laboratory colony, successively.

Table (4): Alkaline phosphatase activity in the whole homogenates of different field colony of *Ceratitis capitata* compared to the laboratory strain

Field colony	(mU/ g.b.wt) Alk	Activity ratio		
	8	9	3	9
Qaluobia	932.67±39 ^a	1074.33±23.8 ^a	3.63	5.63
Behira	603.33±25.17 ^b	463±12.53 ^b	2.35	2.43
Sharkia	553.67±21.22 ^b	430.67±43.14 b	2.15	2.25
Laboratory	256.67±15.27 °	190.67±9.02 °	1	1
F test	324.11**	638.53**	-	-
L.S.D	50.16	48.61	-	-

5- Effect on Acetylcholine Esterase: Amounts of acetylcholine esterase in males and females of the tested colonies high significantly differed (Table, 5). The highest amounts of enzyme (396.67 and 317.67 AchBr/min/g.b.w.) were shown with males and females of Behira field colony recording the Vol. 47, No. 3, Sept. 2019

highest ratios of activity of 1.23 and 1.61 fold of the laboratory colony. The activity ratios of males and females of the other two used colonies (Qaluobia and Sharkia) were 1.16, 1.60 and 1.14, 1.59 fold of the laboratory colony, successively.

Table (5): Acetylcholin esterase activity in the whole homogenates of different field colony of *Ceratitis capitata* compared to the laboratory strain

Field colony	ugAchBr/min/	Activity ratio		
-	3	9	3	9
Qaluobia	374±5.29 ab	315.67±4.04 ^a	1.16	1.60
Behira	396.67±15.27 ^a	317.67±5.86 ^a	1.23	1.61
Sharkia	368.67±20.13 ^b	314.33±5.51 ^a	1.14	1.59
Laboratory	323.33±7.64 °	197.33±6.43 ^b	1	1
F test	15.62**	344.87 **	-	-
L.S.D	25.35	10.41	-	-

6- Effect on Total Proteins: Data compiled in Table (6) reveal that the amounts of total proteins in males and females of the used colonies high significantly varied. Males of Behira colony significantly showed the highest amount of total proteins (95.77 mg/g.b.wt) recording the highest value of activity ratio (1.19) that insignificantly differed with those obtained with Qaluobia colony (93.8 mg/g.b.wt). The latest colony showed activity ratio of 1.17 fold of the laboratory one. The differences in amounts of total proteins in males of Sharkia colony (87.63 mg/g.b.wt) insignificantly varied with those of Qaluobia colony showing the lowest value of activity ratio of 1.09 fold of the laboratory one. But, females of

Sharkia colony significantly recorded the highest amount of total proteins (97.23 mg/g.b.wt.) showing the highest value of activity ratio of 1.49 fold of the laboratory colony. The other tested colonies of Qaluobia and Behira showed insignificantly and nearly equal amounts of total proteins of 80.47 and 83.90 mg/g.b.wt, respectively. The respective activity ratios were 1.24 and 1.29 fold of the laboratory colony.

Table (6): Total proteins activity in the whole homogenates of different field colony of *Ceratitis capitata* compared to the laboratory strain.

Field colony	Total proteins	Activity ratio		
	3	9	8	9
Qaluobia	93.8±3.9 ab	80.47±1.6 b	1.17	1.24
Behira	95.77±2.96 ^a	83.9±1.34 ^b	1.19	1.29
Sharkia	87.63±2.69 ^b	97.23±2.42 ^a	1.09	1.49
Laboratory	79.87±3.5°	64.87±1.9°	1	1
F test	14.12 **	152.91**	-	-
L.S.D	6.22	3.51	-	-

The results obtained in this investigation clear that there were significant differences between the response of male and female of *Ceratitis capitata* spinosad. Valtierra-de-Luis.*et al.* (2019) evaluated the toxicity dose of Spinosad and determined the presence of resistance traits in *Ceratitis capitata* (Tephritidae) and *Drosophila suzukii*. The concentration-mortality response did not differ between the sexes or among three different batches of insects. Lethal dose values were calculated based on mean ingested volumes. For *C. capitata* LD₅₀ values were 1.462 and 1.502 nga.i./insect for males and

females, respectively, equivalent to 0.274 and 0.271 nga.i./mg for males and females of the second species of *D. suzukii*, respectively.

Data in the present study are accordance with those published by Abou-Elsoud. *et al* (2013). who monitored resistance in different field strains of *Bactrocera zonata* flies. The obtained results revealed that the discriminating concentration of the organophosphorus insecticide; Malathion (LC₉₉) caused 62.32, 58.20,42.40 and 40% mortality for the flies collected from Qaluobia, Behira, Menoufia and Ismailia governorates; respectively. Flies of Ismailia field colony exhibited the highest resistance value to Malathion toxicity. The corresponding resistance value was 59.60%; whereas Qaluobia strain recorded the lowest resistance value to Malathion action (37.05%). On the other hand, Behira and Menoufia governorates occupied the middle situation of resistance in this pest strain among the two strains that mentioned previously.

The results obtained in this study exhibited the same as Akl (2016). who found that males of *Ceratitis capitata* of laboratory strain as well as field colony strain were more susceptible than females and the mortality percent increased by increasing insecticide concentrations. The results indicated that female flies of all governorates were more resistant than male flies to the insecticides toxicity. Biochemical assays of AChE, α - esterases, acid and alkaline phosphatase activity level were remarkable much higher in both sexes' of the field colony than the corresponding levels in the laboratory strain.

The results obtained in this study agree with those found by Young *et al* (2005) who reported that pyrethriod resistance in field populations of Vol. 47, No. 3, Sept. 2019

Australian *Helicoverpa armigera* is primarily asquence of the overproduction of esterases isoenzymes which metabolite and sequester pyrethriods. Hassan and Mostafa (2000) studied the effect of DEF an esterases inhibitor, N-propyl paraoxon a phosphatases inhibitor on the toxicity of three organophosphorus insecticides namely Lebaycid, Malathion and Fenitrotion against the 3rd instar larvae of *C. capitate*. They found that the synergistic rates were higher in the field colony as compared with the laboratory strain.

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تأثير بعض المبيدات المشرية على مستوى المقاومة وبعض المظاهر البيوكيميائية في حشرات ذبابة فاكسة البحر المتوسط من مناطق محتلفة في مصر

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المستخلص

تم معاملة الأطوار الكاملة من السلالة المعملية لذبابة فاكهة البحر المتوسط لتركيزات مختلفة من مبيدين حشريين (لمباداسيهالوثرين (كاريلوت جولد ٥%) و مركب حيوى، سبينوساد (كونسرف 0.24%)}. تم اختيار القيمة المميزة او التركيز المحدد (LC99) لتقدير درجة المقاومة لثلاث مجتمعات حقلية اختيرت من محافظات القليوبية والبحيرة والشرقية. وقد أظهرت النتائج أن ذكور السلالة المعملية كانت أكثر حساسية لكلا المركبين من الإناث. كذلك أظهرت حشرات محافظة القليوبية درجة مقاومة أعلى لمركب لمبادا—سيهالوثرين يليه حشرات محافظات الشرقية والبحيرة وذلك في كل من الذكور والإناث. أما ذكور واناث حشرات محافظة الشرقية فقد أظهرت درجة واضحة من المقاومة أعلى منها في حشرات محافظتي البحيرة والقليوبية. من جهة أخرى أوضحت الحشرات الحقلية للمحافظات الثلاث مستويات مرتفعة لنشاط الإنزيمات عنها في السلالة المعملية. فقد أظهرت ذكور حشرات محافظة القليوبية أعلى كمية لإنزيم الفوسفاتيز الحامضي في حين سجلت أعلى كمية لنفس الإنزيم في إناث حشرات محافظة الشرقية. أما انزيم الفوسفاتيز القاعدي فقد ظهر أعلى مستوى في ذكور واناث حشرات محافظة القليوبية يليها حشرات محافظات البحيرة والشرقية ، بينما سجل أعلى نشاط لإنزيم الأسيئيل كولين استيريز في ذكور واناث حشرات محافظة البحيرة. هذا وقد ظهرت أعلى كمية للبروتينات الكلية في ذكور محافظة البحيرة، في حين كانت الكمية أعلاها في إناث حشرات محافظة الشرقية . ونستخلص مما سبق أن النشاط الإنزيمي يلعب دورا رئيسيا في مقاومة الحشرات لفعل المبيدات وأن هناك ارتباط موجب بين درجة المقاومة للمبيدات والنشاط الإنزيمي في المجتمعات الحقلية للحشرات.

الكلمات المفتاحية: ذبابة فاكهة البحر المتوسط، الاسم العلمي لذبابة فاكهة البحر المتوسط، المبيدات التقليدية، المبيدات الحيوية، المقاومة، الضغط الإنتخابي، النظم البيوكيميائية، لمبادا سيهالورثرين، سبينوساد.