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Research Article

Insecticide Resistance of a Field Strain of Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in Egypt

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Abstract

Background and Objective: Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is one of the most destructive insect-pests of vegetable and horticultural crops in Egypt. Conventional insecticides, especially malathion have been applied extensively to control this pest. The present study aimed to monitor whether the field-strain of *C. capitata* has developed a resistance to certain insecticides mostly used in the field to control this pest. **Materials and Methods:** The study was conducted under laboratory conditions on the 2nd generation of the wild flies of *C. capitata*. Feeding bioassay of malatox, malathion, fenitrothion and spinosad pesticides was assayed at 24 and 48 h post-treatment. All data were statistically subjected to the LDP line program. **Results:** Bioassay results proved occurrence of resistance of *C. capitata* to the tested insecticides, with a moderate to very high level of resistance in male flies ranged from 24.24-115.56 fold and females ranged from 18.79-112.81 fold, 24 h post-treatment. Resistance factor increased to high level of resistance in males; 89.19-100.8 fold and moderate to high level in females; 29.34-99.45 fold, 48 h post-treatment. **Conclusion:** The results showed that the least resistance insecticide was recorded with malathion for *C. capitata* males and females at 24 h. In this study, resistance levels found her are considered a good indicator of possible field control failures if one of the tested products is used. Thus, this study suggests that resistance management programs must be planned in control this pest.

Key words: Tephritidae, insecticide resistance, spinosad, conventional insecticide, organophosphorus insecticides, mediterranean fruit fly, toxicity index

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INTRODUCTION

Mediterranean fruit fly (medfly), *Ceratitis capitata* (Diptera: Tephritidae) is one of the most harmful pests worldwide[1]. It poses a serious threat to fruit and vegetable production throughout the world[2]. Significant efforts are made to suppress this pest within fruit-growing areas[3] or making medfly-free areas. Malathion is a usual choice of insecticides for this purpose; this is usually combined with protein hydrolysate for fruit flies control[4], such as *C. capitata*, *Anastrepha suspensa*[5], *Bactrocera zonat*, *B. cucurbitae* and *Carpomyia vesuviana*[6]. Researchers throughout the world are seeking alternative methods to control fruit flies by replacing conventional insecticides with safe control methods and friendly environment insecticides such as bio-pesticide, spinosad. This pesticide was conducted on *C. capitata*[7-13], *A. fraterculus* (wiede.)[9] and *A. suspensa*[7].

Under laboratory conditions, development of insecticide resistance has been studied in various fruit flies pests around the world. In the late 1950s and 1960s, in Hawaii, the laboratory strains of *B. dorsalis* Hendel and the *B. cucurbitae* Coquillett developed resistance to DDT but not to malathion, while, *C. capitata* was susceptible to these insecticides. Four decades later under laboratory selection in Taiwan, resistant lines of *B. dorsalis* were produced to malathion and other Ops, as well as to methomyl and pyrethroid[14]. Furthest, previous studies have been reported that malathion-resistant strain of *C. capitata* has an across-resistance to trichlorophon, diazinon, phosmet and methyl-chlorpyrifos, carbarayl, lambada-cyhalothrin, lufenuron and spinosad[15].

Under field conditions, before 1990, despite those insects exposed significantly to insecticides, there was no clear evidence of fruit flies pesticide resistance under field conditions[16]. The first field recording of *C. capitata* resistance has been reported by Magana *et al.*[17].

In the current study area, as well as surrounding wide areas, malathion, fenitrothion and malatox have been used extensively to control most insect-pests, of which *C. capitata*, since a long period of time (Table 1). In the meantime, spinosad has not been used yet.

The regular and random use of insecticides can cause development of resistance to insecticides, hence failure in control the target pest. Therefore, the main objective of this study was to monitor occurrence of resistance against the field strain of *C. capitata* towards malathion, fenitrothion and malatox, as well as the toxicity bioassay of spinosad, as currently a proved bio-pesticide in integrated pest management.

Table 1: Commonly used insecticides to control Mediterranean fruit fly in tested area

Insecticide (%)	Application rate	Remarks
Malatox (57)	150 cm^3/100 L water	Cover spray
Fenitrothion (50)	150 cm^3/100 L water	Cover spray
Malathion (57)	500 cm^3/20 L water	Partial spray with protein hydrolysate
Malatox (57)	500 cm^3/20 L water	Partial spray with protein hydrolysate

MATERIALS AND METHODS

The present study was carried out in the Laboratory of Eradication of the peach fruit fly at Damanhour, El-Beheira Governorate, Egypt.

Insect strains of *C. capitata*

Field strain (FS): The field strain of *C. capitata* was obtained from infested navel orange and mandarin fruits by fruit flies from orchards which have been sprayed periodically by the farmers with malathion and malatox, in Hosh-Esaa district, El-Beheira Governorate, Egypt, in Nov 2016. The infected fruits were incubated under laboratory conditions (25±2°C and 60-70% RH) in plastic jars (50 cm diameter×17 cm height) furnished with fine sand and covered with a muslin sheet. After completing pupation, the sand was sieved and the pupae were collected. Pupae were placed in a Petri dish and transferred to the natural adult rearing cage (40×40×60 cm) includes a source of water and an adult flies' diet (sugar and protein hydrolysate, 3:1 v:v). Two sides of each cage were made of glass, one side has a sleeve for inspection, front side has muslin cloth, floor is made of wood and ceiling of fiber wire 0.2 mm, as well as a rack of mesh metal (1 cm in diameter) was in the mid height of the cage. Fly mass culture was obtained by exposure of clean navel orange and mandarin fruits to the flies inside the cage on the rack along 24 h and then fruits were incubated as above-mentioned to obtain pupae.

Susceptible strain (SS): Initial culture of the susceptible strain of *C. capitata* was obtained as pupae from the lab medfly colony rearing at the Department of Horticulture Insects, Plant Protection Institute, Doki, Giza. The pupae were kept under the laboratory conditions in an adult rearing cage (30×30×30 cm) according to El-Gendy[18]. Eggs were collected in a water tray every 48 h and placed on an artificial diet[18]. Artificial diet trays (20×10×10 cm) were covered with thick cloth lids for the first three days. The fourth day, trays lids were removed and the trays were incubated in the plastic jars to obtained the pupae. Collected pupae were transferred to the adult rearing cage to start a new generation[19].

Selection of insecticides and food attractant: Four formulated insecticides belonging to two groups; organophosphate (malatox (50% EC, Help Comp), malathion 57% EC (Zaiat Comp.) and fenitrothion 50% EC (Adwia Comp.)) and bionatural insecticide (Spinosad 24% SC (Dow AgroSciences Comp.) were selected for the present study. The attractive food was Buminal 37% (hydrolysate protein, Bridge trade comp.).

Lab assay: Toxicity bioassay was conducted with 2nd generation flies. Five males and females (5-6 days-old) of each SS and FS of *C. capitata* were confined in plastic cups (250 cc) covered with a muslin cloth, without food for 12 h before treatment. Insecticides treatments and a water control were applied by a feeding bioassay[20]. Six to eight concentrations of each insecticide, with four replicates for each concentration were prepared with tap water and mixed with 5% hydrolysate protein. Concentrations considered in this study had a mortality >0 and <100%. Mortality (%) of each sex was observed and recorded at 24 h, dead flies were removed and 48 h post-treatment.

Statistical analysis: All data obtained in all experiments were subjected to an analysis by the LDP line program (Bakr Software)[21], which corrected to control mortality by Abbott's formula[22]. The concentrations mortality line of the tested adults was estimated and LC_{50} was calculated according to Finney[23]. The relative toxicity (toxicity index; TI) of the tested insecticides was determined according to Sun[24]. The resistance ratios (RR) for all tested compounds were estimated by dividing LC_{50} values of FS by LC_{50} of SS and categorized as a resistance factor (RF)[25], susceptibility (RF = 1), low resistance (RF = 2-10), moderate resistance (RF = 11-30), high resistance (RF = 31-100) and very high resistance (RF>100).

RESULTS

Results of lcp-lines slope values in Table 2 and 3 and Fig. 1-4 of the tested insecticides at 24 h, ranged from 1.11-2.58 and 1.32-3.05 for SS and FS males, 1.13-1.95 and 1.55-4.05 for SS and FS females. Sam trend achieved at 48 h, lcp-lines slope values ranged from 1.16-3.09 and 1.95-4.18 and 1.16-2.49 and 1.65-3.34 for SS and FS males, these results

Table 2: Toxicity of Spinosad, malatox, fenitrothion and malathion insecticides against fly's males and females of *C. capitata* and their resistant toward these insecticides at 24 h post-treatment

	FS				LS				
Insecticide	LC_{50}	χ^2	Slope ±SE	TI	LC_{50}	χ^2	Slope ±SE	TI	RR
Male									
Spinosad	157.5 (123.83-269.56)	0.52	3.02±0.88	100.00	3.72 (1.91-6.91)	1.02	1.20±0.18	75.53	42.34
Fentro	324.73 (151.01-580.34)	0.59	1.32±0.46	48.50	2.81 (1.94-3.81)	3.96	2.58±0.48	100.00	115.56
Malatox	414.92 (262.70-600.05)	3.17	2.39±0.43	37.96	5.78 (1.66-10.88)	0.86	1.11±0.29	48.61	71.78
Malathion	450.24 (322.33-561.03)	2.15	3.05±0.84	34.98	18.57 (8.46-32.32)	2.61	1.51±0.39	15.12	24.24
Female									
Spinosad	203.98 (143.08-628.93)	0.54	2.26±0.70	100.00	8.18 (4.91-29.06)	00.11	1.13±0.36	50.85	24.93
Fentro	469.29 (287.18-862.62)	0.51	1.55±0.44	43.47	4.16 (2.75-6.16)	30.41	1.95±0.42	100.00	112.81
Malatox	524.21 (261.15-830.120	2.43	1.77±0.49	38.91	20.41 (9.91-35.19)	00.49	1.50±0.38	20.38	25.68
Malathion	571.46 (133.96-703.27)	0.27	4.05±1.26	35.69	30.41 (16.64-54.97)	30.35	1.55±0.38	13.69	18.79

FS: Field strain, TI: Toxicity index, RR: Resistance ration, LS: Lab assay

Table 3: Toxicity of Spinosad, malatox, fenitrothion and malathion insecticides against fly's males and females of *C. capitata* and their resistant toward these insecticides at 48-hr post-treatment

	FS				LS				
Insecticide	LC_{50}	χ^2	Slope ±SE	TI	LC_{50}	χ^2	Slope ±SE	TI	RR
Male									
Spinosad	73.59 (49.47-89.14)	0.86	4.18±1.33	100.00	0.73 (0.5-1.58)	0.27	1.16±0.37	100	100.80
Fentro	172.05 (85.87-236.88)	0.27	2.05±0.56	42.78	1.78 (1.22-2.41)	0.42	3.09±0.66	41.01	96.66
Malatox	194.45 (112.12-363.63)	2.46	1.95±0.49	37.85	2.18 (1.14-3.42)	2.32	1.66±0.38	33.48	89.19
Malathion	239.45 (158.06-338.80)	0.23	2.37±0.55	30.74	2.42 (1.40-3.50)	2.31	1.75±0.38	30.17	98.95
Female									
Spinosad	96.68 (78.28-121.04)	1.45	3.34±0.70	100.00	1.20 (0.17-2.31)	1.28	1.16±0.36	100..00	80.57
Fentro	209.83 (96.70-324.21)	1.05	1.65±0.43	46.07	2.11 (1.36-2.98)	1.56	2.49±0.58	56.87	99.45
Malatox	227.51 (130.67-317.13)	0.74	1.85±0.38	42.49	3.52 (1.69-5.96)	2.53	1.34±0.32	34.09	64.63
Malathion	296.04 (122.58-408.27)	0.63	2.75±0.86	32.65	10.09 (6.62-16.64)	0.07	2.07±0.47	11.89	29.34

FS: Field strain, TI: Toxicity index, RR: Resistance ration, LS: Lab assay

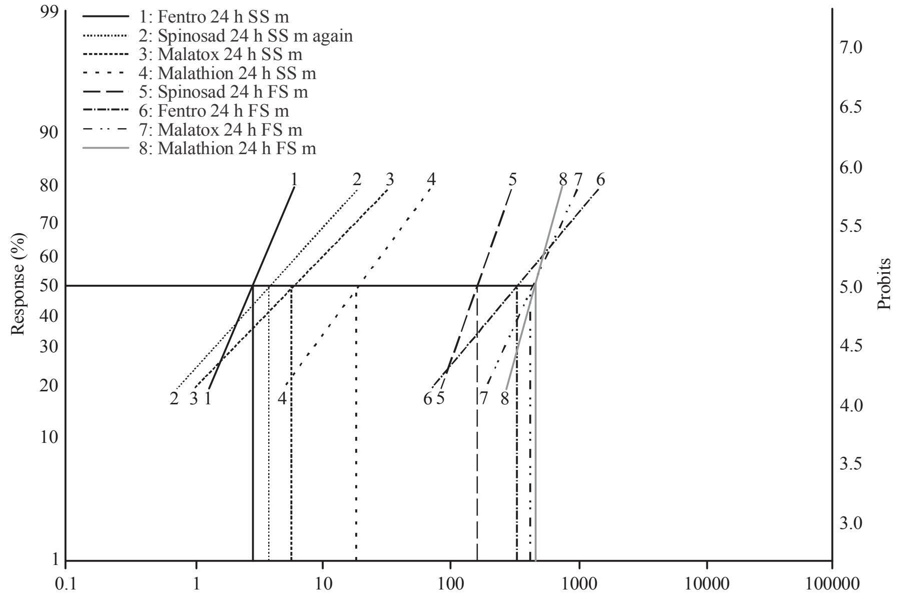

Fig. 1: Lcp-lines of field and susceptible strains of *B. zonata* males treated with insecticides 24 h post treatment

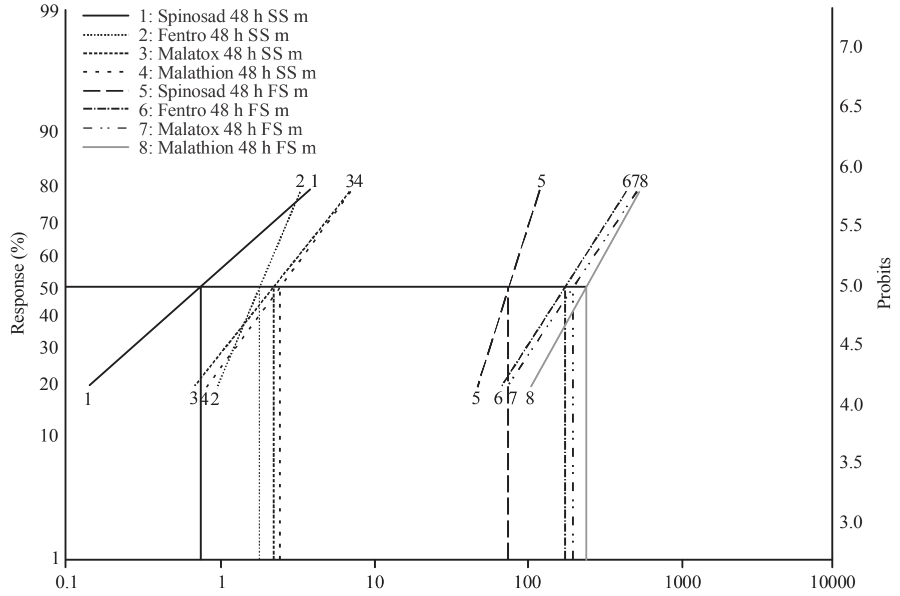

Fig. 2: Lcp-lines of field and susceptible strains of *B. zonata* males treated with insecticides 48 h post treatment

proved the homogeneity of the tested *C. capitata* population. In addition, the calculated Chi square values were less than the tabulated ones at 0.05 probability level for all tested insecticides on both fly's males and females.

The results revealed variation in sensitivity of both *C. capitata* FS and SS to the tested insecticides, with lower sensitivity for FS than SS according to LC_{50} values at 24 and 48 h post-treatment. TI values of the tested insecticides on FS males of *C. capitata* showed superior efficiency of spinosad with 100% TI at LC_{50} at both 24 and 48 h post-treatment. Followed by, fenitrothion, malatox and malathion with respective values; 48.50, 37.96 and 34.98% and 42.78, 37.85 and 30.74% at 24 and 48 h post-treatment, respectively. On the other hand, TI values at 24 h on SS showed that fenitrothion was superior in efficiency with 100% TI, followed by spinosad, malatox and malathion with 75.37, 48.57 and

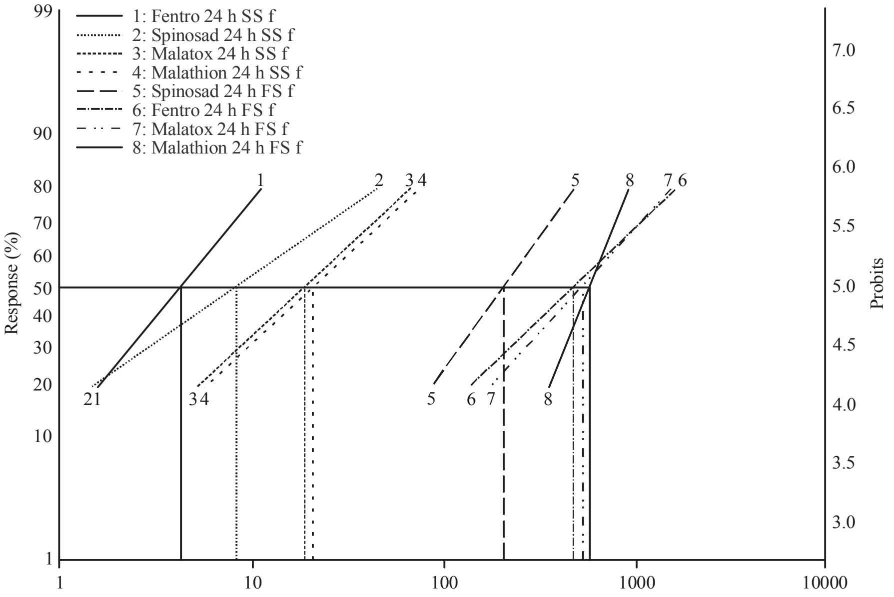

Fig. 3: Lcp-lines of field and susceptible strains of *B. zonata* females treated with insecticides 24 h post-treatment

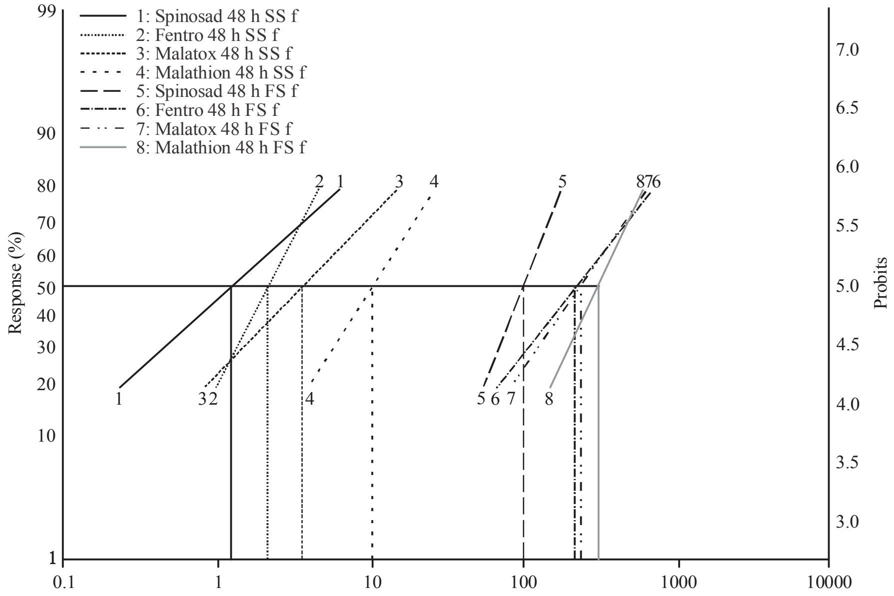

Fig. 4: Lcp-lines of field and susceptible strains of *B. zonata* females treated with insecticides 48 h post-treatment

15.12%, respectively. While, spinosad was the most effective (100% TI), followed by fenitrothion, malatox and malathion with 41.01, 33.48 and 30.17%, respectively at 48 h.

Regarding FS females, results of TI values of the tested insecticides showed superior efficiency of spinosad (100% TI) at both 24 and 48 h post-treatment, followed by fenitrothion, malatox and malathion with values of 43.47, 38.91 and 35.69% and 46.07, 42.49 and 32.65%, respectively at 24 and 48 h post treatment. On the other hand, fenitrothion was the superior in efficiency on SS females with 100% TI at 24 h, followed by spinosad, malatox and malathion with 50.85, 20.38 and 13.69%. While, spinosad was the most effective at 48 h with 100% TI, followed by fenitrothion, malatox and malathion with approximate values 56.87, 34.09 and 11.89%, respectively.

Resistance of the field strain of *C. capitata* towards malathion, malatox, fenitrothion and spinosad was evaluated under laboratory conditions at 24 and 48 h post-treatment. Results in Table 2 and 3 revealed a variation in RR for males of

C. capitata among the tested insecticides and among interval times; 24 and 48 h. Moderate to very high resistance ranged from 24.24-115.56 fold were obtained at 24 h, i.e. very high resistance, 115.56 fold, was obtained against fenitrothion, followed by a high resistance to malatox and spinosad with 71.78 and 42.34 fold and a moderate to malathion with 24.24 fold. At 48 h post-treatment, high resistance values, 89.19-100.8 fold, were obtained among the insecticides; 100.8, 98.95, 96.66 and 89.19 fold toward spinosad, malathion, fenitrothion and malatox, respectively.

Similarly, in fly's females, very high resistance (112.81 fold) was obtained against fenitrothion insecticide at 24 h, followed by moderate resistance to malatox, spinosad and malathion with RR values 25.68, 24.93 and 18.79 fold, respectively. The same trend was obtained at 48 h post-treatment, high resistance was obtained against fenitrothion with 99.45 fold, followed by spinosad and malatox with 80.57 and 64.63-fold and moderate to malathion with 29.34 fold.

Generally, the highest RR in fly's males and females was recorded for fenitrothion at 24 h, while it was the highest against spinosad and fenitrothion in fly's males and females at 48 h post-treatment, respectively.

DISCUSSION

In the present study, TI values of the tested insecticides revealed that spinosad was the highest efficiency of the tested insecticides against FS males and females of *C. capitata* at 24 and 48 h post-treatment. Fenitrothion was the second in efficiency, followed by malatox and malathion, respectively. On the other hand, fenitrothion was the superior efficiency on SS males and females, followed by spinosad, malatox and malathion, respectively at 24 h, while spinosad was superior in efficiency at 48 h. It is clear that the FS is more tolerant than SS to OP insecticides, as well as *C. capitata* females are more tolerant than males. These results are confirmed by those of Vargas *et al.*[26] on *C. capitata*, Burns *et al.*[7] on *C. capitata* and Caribbean fruit fly, *A. ludens* and El-Aw *et al.*[19] and Haider *et al.*[27] on *B. zonata*. Furthermore, the obtained LC_{50} values of the tested insecticides against *C. capitata* were higher not only on FS than SS, but also for females than males within the fly strain, whether at 24 or 48 h post-treatment. Confirmed results showed that LC_{50} values of *C. capitata* males (24 h LC_{50} 2.8 mg L^{-1} spinosad) were significantly more susceptible to spinosad than females (4.2 mg L^{-1})[28]. The LC_{50} values of spinosad and malathion were higher on females than males of *B. zonata*, in addition they were in a negative relation to time elapsed post-treatment[19]. Moreover, LD_{50} values of the tested insecticides against *B. cucurbitae* were higher in the FS than SS at 24, 48 and 72 h post-treatment, respectively[29]. The higher in LC_{50} values obtained in FS than SS of *C. capitata* and superior efficiency of spinosad, according to TI, than OP against FS, clear that FS is more tolerant than SS to OP insecticides, which may attribute to develop the resistance.

The present study demonstrated significant resistance levels of the fly to malatox, malathion, fenitrothion and spinosad, although spinosad has not been used in these areas before. It may be due to migration the resistant flies to these areas or because of genetic resistance occurring in the field race. Supported results suggested that resistance buildup in *C. capitata* is linked to organophosphate application in the field[30]. Moreover, several studies recorded resistance levels in the FS of *C. capitata* to malathion insecticide reached to 176 fold more than the SS[15,17,31]. Also, *Bactrocera* Genus exhibited resistance levels to insecticides; melon fly, *B. cucurbitae* and oriental fruit fly, *B. dorsalis* showed a resistance to both malathion and fenithion[32], *B. dorsalis* to trichlorphon, β-cypermethrin and avermectin[33] and *B. zonata* to malathion and spinosad[27,34].

Furthermore, the resistance levels were obtained not only among fly sex; fly's males and females, but also among investigated date, 24 and 48 h post treatment. It is clear that fly's females have been often less resistance than males; RR values of males were higher than females. RR values were higher at 48 than 24 h post-treatment for spinosad, malatox and malathion, whereas the RR values at 24 h were higher than at 48 h for fenitrothion. This may be due to insecticide degradation rate in the insects; it might be faster for spinosad, malatox and malathion than fenitrothion. Similar results in FS of *B. cucurbitae*, Pingtung, where RR values against spinosad were directly related to time post-treatment; 3.31 fold at 24 h, increased to 11.6 to reach 13.3 fold at 48 and 72 h post-treatment, respectively[29]. However, the toxicity bioassay of Greece strain of *C. capitata* did not reveal significant levels of resistance in spinosad and delatamethrin[13].

CONCLUSION

This study revealed occurrence the resistance of *C. capitata* to malathion, malatox, fenitrothion and spinosad. Furthermore, it shows probability of occurrence a cross-resistance to spinosad which needs further studies. The results indicated that the least insecticide resistance was observed with malathoin for *C. capitata* males and females at 24 h.

SIGNIFICANCE STATEMENT

This research discovered the resistance of *C. capitata* to fenitrothion and spinosad pesticides at 24 and 48 h. The results of this study are good indicator of possible field control failures. This study also suggested that the resistance management programs must be planned to control the pesticides.

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