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Evaluation of some insecticides on whitefly *Bemisia tabaci* (Genn.) (Homoptera: *Aleyrodidae*) and monitoring of pesticide residues in random samples of cabbage plants.

Azza I. Mohamed
Central Agric. Pesticide Laboratory, Agricultural Research Center, Dokki, Egypt.

ABSTRACT

The present investigation aims to study the monitoring of the whitefly *B. tabaci* adult susceptibility (Genn) in five Governorates, Giza, Behira, Gharbia, Faiyoum and Dakahlia. by evaluation the efficacy of some insecticides from organophosphorus compounds which still used in controlling some pests, such as Pirimiphos-methyl, Profenofos, Chlorpyrifos-methyl, Cyanofenphos and Diazinon. Dakahlia Governorate strain was the most susceptible one among all, followed by Giza, strain while Gharbia strain was the most resistant one.

In addition survey of pesticide residues in cabbage samples collected at random from local markets of the same Governorates in Egypt showed that, some pesticides were detected, however, others were not detected. The results revealed that cabbage samples were contaminated with different amount of pesticide residues. No pesticide exceeded the MRL in samples.

Keywords: Insecticides, whitefly, pesticide residues, cabbage plants

INTRODUCTION

In the recent years the whitefly *Bemicia tabaci* (Homoptera: *Aleyrodidae*) has become one of the most devastating worldwide agricultural pests. Several biotype of this species damage cotton and a broad range of vegetables especially cabbage which is considered as one of the most economically important crop and plays a very useful role in nutritional aspects for humans. There for the chemical control becomes the dominant way of checking insect pest populations of agriculture.

It is important to construct a good system that allows one or more whiteflies to be studied without affecting their life span and reproductive characteristics, for this purpose Clip-cage technique has been used (Muniz and Nombela 2001). The distribution of whiteflies on the underside of leaves, their high reproductive potential, and their increasing resistance to pesticides contribute to the difficulty in controlling the insect with conventional spray applications (Johnson *et al.*, 1982 and Dittrich *et al.*, 1985). The pesticide is to be accepted for controlling the pests on plants, it must be highly effective in reducing pest populations, very specific toward target species through specific mode of action, induce no adverse effect on the plants with a low persistence harvested crops, in addition to low mammalian toxicity. There for we had to study some of traditional insecticides to control this pest.

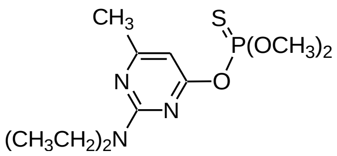
Monitoring programs of pesticide residues in the food at local markets of different governorates are currently carried out in all countries in order to generate a flow of data that serves in following up the regulation of pesticides use and evaluate the situation of food contamination with pesticide residues. Pesticide residues analysis in market samples requires methods capable of identifying and measuring more than one pesticide residue in samples of unknown history.

In response to this requirement, a number of methods has been developed and applied routinely for the control of pesticide residues in food.

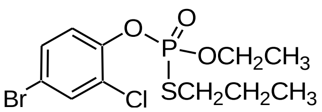
MATERIALS AND METHODS

Pesticides used:

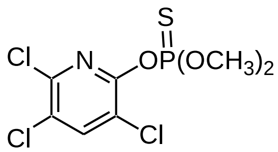
1-Pirimiphos-methyl:*O*-2-diethylamino-6-methylpyrimidin-4-yl*O,O*-dimethyl phosphorothioate



2-Profenofos - *O*-4 bromo-2-chlorophenyl *O*-ethyl *S*-propyl phosphorothioate.

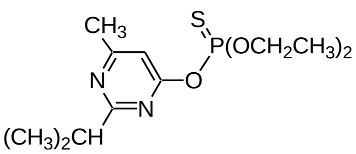


3-Chlorpyrifos-methyl *O,O*-dimethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate



4-Cyanofenphos: *O*-4-cyanophenyl *O*-ethyl phenylphosphonothioate

5- Diazinon: *O,O*-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate



Strains:

Field populations:

The whitefly adults of *Bemisia tabaci* used in this study were collected from cabbage fields of Five Governorates, Giza, Behira, Gharbia, Faiyoum and Dakahlia Collection was done in September 2008 ,and then adults SPWF (sweet potato whitefly) were collected from field with custom mad battery operated suction sampler (Dittrich *et al*.,1990). Adults were randomly collected across a representative collage growing area from two or more fields. Samples collected were pooled in wide mouth glass jars and kept in cool box during the transport from the field to the laboratory. Before bioassay testes, the jars were taken out of the box and inverted upside down, no that the healthy individuals would move to the top due to positive light, weak and dead individuals were discarded.

Laboratory- susceptible strain:

The susceptible reference strain of *B. tabaci* (Genn.), originated from over vegetables and has been reared in laboratory culture for 30 generations under standard conditions at 26 ±1 C^0 and 70± 5 R.H, and a photoperiod of 16:8 (light: dark) hrs. Rearing method was described by Coudriet *et al*., 1985 with some minor modification as follows: whitefly was reared in chambers on metallic stands (70×200 cm. and80 heights). Each chamber was provided by fluorescent lamps and each stand surrounded by muslin. Insects were fed on cabbage plants *Brassica oleracae* that were grown in plastic pots (15 cm. diameter). The pots were maintained in another chamber under laboratory conditions without exposure to any insecticides until needed.

Bioassays by using Leaf-cage technique:

This bioassay method was done to evaluate the insecticidal activity of sub lethal concentrations. The method used for experiments similar to that described by Prabhaker *et al*., (1989) with slight modification:-

1-Preparation of stock solution from each formulated insecticide by diluting to give the needed concentrations against the adult stage of each population.

2- Castor leaves were dipped into these concentrations for each insecticide.

3- The cage was fixed on these leaves.

4- Not less than 30 healthy adults were placed in each cage.

5-Each concentration was replicated three times and the control was tested with water only.
6- The test cages were maintained at 27^0±C,70-85 % RH, and a photoperiod of 16 hours light was used during the experiment s, (De cock *et al.*, 1990).
7- Mortality counts were made for the control and the treated after 24 hours. The criterion for mortality was no visible movement.

Data analysis:

Concentration of mortality, LC_{50} and LC_{90} values, their 95% confidence intervals and slops were determined using a software package "LD-P line", copyright of Dr.Ihab M.Bakr, Plant Protection Research Institute.

Monitoring of pesticide residues in cabbage plants:-

Pesticide residue analysis in local market samples necessitates a method capable to identify and measure more than one pesticide residue in sample of unknown history. Multiple residue analysis is the most appropriate too far that purpose. Not only the number of pesticides detected but also the percentage of recovery of each and the applicability are the criteria controlling the choice of such method. The multiple residue analysis method of the AOAC official method of analysis (AOAC, 2000) was found to be the most appropriate tool for that purpose.

1- Sampling:-

Samples were collected at random from the different local markets of five Egyptian Governorates, Giza, Behira, Gharbia, Faiyoum and Dakahlia. Samples process was carried out by taken a whole plant (rounded cabbage) which was divided into two halves and each half was divided vertically into small sections. Samples collected were kept in cool box during the transport from the markets to the laboratory. Laboratory samples were comminuted and homogenized to sub-samples and reserved in polyethylene packages labeled by names of Governorate, date of collection, then these packages divided to three portions, identify them, respectively as(i) Original (ii) Check (iii) Reverse. Each package contains 50 gm of samples. All three portions reserved in freezer until the time of analysis.

2-Extraction of plant samples: - (Luke *et al*., 1981)

Plant samples "50 gm of sub samples" are blended with Acetone and filtered. Most nonionic residues are extracted into aqueous acetone solution. Residues are transferred from aqueous acetone to Methylenecholoride ⁄ Petroleum ether by partitioning, with salt added to aqueous layer after the first partitioning to aid transfer. The organic layer was passed over anhydrous sodium sulfate and the aqueous layer was transferred into another Separatory funnel and partitioned again with two consequent portions of Methylene chloride then filtered through the same anhydrous sodium sulfate. The used reagents were (i) Solvents-acetone, Petroleum ether, was pesticide residue analysis grade. (ii) Anhydrous sodium sulfate was heated at 250 ^{0}C for 3 hour and cooled in desiccators until used ‹‹Activation process››.

3- Clean-up of plant samples

The Nitrogen Phosphorous Detector (NPD) used in determination of organ phosphorous and nitrogen residues is a specific detector which detects only phosphorous and Nitrogen containing compounds. According to this specificity it is not preferred to clean up samples in order to avoid loss of residues during clean-up process.

4- Determination:-

Identification, quantitation and confirmation of pesticide residues were carried out on using Gas Chromatograph (GC) Hewlett Packard 5890 series II equipped with double nitrogen phosphorous detectors (NPD) and two capillary columns with the following specifications column A with : composition 5% diphenyl and 95% dimethylpolysiloxane ,film thickness 0.25 um, length 25 m, internal diameter 0.32 mm and phase ratio 150, while column B with: composition 14% cyanopropylphenyl and 86% methylpolysiloxane, film thickness 0.25 um, length 25 m, internal diameter 0.32 mm and phase ratio 320. The gas chromatograph instrument was adjusted for; injector temperature: 225°C, detector temperature A and B; 280°C, flow rate of hydrogen: 3.5± 0.1 ml ⁄ min, flow rate of air:100-120 ml ⁄ min, column head pressure:75 KPa, Carrier gas: nitrogen, flow rate of carrier gas + detector auxiliary gas :25 ml ⁄ min, septum purge:5 ml ⁄min .,split vent 70 ml ⁄min, spletless injection mode with injection volume: 1µl and oven temperature program as follows 90:270°C with total run time:40 min.

RESULTS AND DISCUSSION

Susceptibility of adult's whitefly *B. tabaci* to insecticides was studied for the field collected populations of five Governorates through September 2008. The LC_{50}'s slope values and resistance ratio RR were calculated (based on data of field collected whiteflies relative to the susceptible laboratory strain), five insecticides were tested against adults of field collected *B. tabaci* from strains (Giza, Behira, Gharbia, Faiyoum and Dakahlia Governorates).

The results concerning the effect of the used insecticides against *B. tabaci* adults are tabulated in table (1) which indicated that the Dakahlia population was the most susceptible one among the other populations, with lowest LC_{50}'s 88.54, 81.88, 48.68, 53.16 and 23.10 ppm for Pirimiphos-methyl, Profenofos, Chlorpyrifos-methyl, Cyanofenphos and Diazinon, respectively and also with lowest R.R (resistance ratio, compared to susceptible strain) except LC_{50} and resistance ratio of Gharbia Governorate for Pirimiphos-methyl and LC_{50}'s of Behira and Giza populations and so resistance ratio for the same populations. The data showed that Cyanofenphos insecticide had very high resistance in all field populations under tests specially Giza and Behira populations while Diazinon insecticide showed the less resistance ratio among the tested insecticides and lowest LC_{50}'s compared with others. These data refers to that organophosphorus are still working and have dramatic impact on agriculture which agree with Badawy and El-Arnaouty(1999) who demonstrated that organophosphorus insecticides were more toxic than others and still play a central role on agricultural protection. Data also agree with Abd El-Gawad,(2003) who reported that organophosphorus and organonitrogen pesticides are widely used in agriculture and animal production for controlling of various insects. In contrast to chlorinated pesticides, organophosphorus and organonitrogen have the advantage of being more rapidly degraded in the environment. Generally, resistance is most likely to develop in insects having high fecundity, short generation time and history of repeated exposures of a large proportion of its population to insecticides Farghaly (2005). Nauen *et.al*., (1997) recommended that the *Myzus nicotianae* has developed a high degree of resistance against several chemical classes of insecticides especially organophosphorus which agree with this study.

From this study, it's clear that we have to throw some light on the insecticidal activities resulted from the effect of some traditional used insecticides in controlling different pest.

Table (1): The Effects of the used insecticides to adults' *B. tabaci* from different Governorates.

Governorates► / Insecticides▼	Sus.		Giza			Behira			Gharbia			Faiyoum			Dakhlia		
	LC_{50}	Slope	LC_{50}	slope	R.R	LC_{50}	Slope	R.R	LC_{50}	Slope	R.R	LC_{50}	Slope	R.R	LC_{50}	Slope	R.R
Pirimiphos-methyl	1.23	1.74 ±04	92.34	1.37 ±0.81	75.04	102.15	2.04 ±0.77	83.04	82.12	2.03 ±0.40	66.76	172.02	1.05 ±0.23	139.85	88.54	2.56 ±0.11	71.98
Profenofos	0.96	1.05 ±0.01	69.22	0.78 ±0.03	72.08	59.78	1.06 ±0.83	62.27	112.25	0.97 ±0.32	116.92	87.11	2.16 ±0.23	90.73	81.88	0.99 ±0.50	85.29
Chlorpyrifos-methyl	0.64	2.31 ±0.04	123.01	1.76 ±0.34	192.20	126.81	1.09 ±0.21	198.14	149.51	2.12 ±0.12	233.60	116.95	1.86 ±0.1	182.73	48.68	2.18 ±0.10	70.55
Cyanofenphos	0.21	1.55 ±0.8	139.02	1.55 ±0.91	662	131.51	2.76 ±0.98	626.19	98.77	2.01 ±0.1	470.33	124.91	1.98 ±0.22	594.80	53.16	1.42 ±0.17	253.14
Diazinon	2.29	0.99	88.32	1.06 ±0.80	40.14	68.09	1.04 ±0.61	29.73	111.3 2	1.29 ±0.21	48.61	73.03	1.77 ±0.05	31.89	23.10	1.20 ±0.15	10.08

R.R: resistance ratio =$LC_{50}$of field strain/$LC_{50}$of susceptible strain

Table (2) demonstrated the data of monitoring pesticide residues obtained from the analysis of cabbage samples collected from the markets in Egypt. The results revealed that the amount of insecticide residues varied from Governorate to another, some insecticides were not detected and others were detected. Fourteen insecticides were tested as residues, eight insecticides were detected only but all of them did not exceed the MRL (maximum residue limits). In Giza market and Faiyoum markets Methamidifos was detected with 0.021 and 0.014 ppm, respectively. Acephate was detected only in Behira and Gharbia with 0.182 and 0.457 ppm, respectively while Bendiocarp, Atrazine, Diazinon Pirimiphos-ethyl, Profenofos and Tebuconazole were not detected at all .Behira and Faiyoum samples were the most contaminated samples while Gharbia and Dakhlia samples were the lowest. These data agree with Salama (1993) who found potato samples collected from local markets were contaminated with Chlorpyrifos, Dimethoate, Pirimiphos-methyl and other insecticides.

Also, Dogheim *et al.*, (1999) analyzed 397 samples of vegetables and fruits collected from eight local markets in Egypt. Of all analyzed of organophosphorus samples, 42.8% contaminated detectable residues of organophosphorus pesticides**,** of which 1.7% exceeded their MRL's. In (2002) they also collected a total of 2318 domestic samples of different types of fruits and vegetables from eight Egyptian local markets in six different regions of the country. All samples were examined for residues of 54 pesticides including organophosphorus, organonitrogen, organohalogen and certain pyrethroids. Overall, 18.5% of samples had no detectable pesticide residues. Of the contaminated samples, 79.6% contained detectable residues and 1.9% exceeded their Maximum Residue limits (MRL's). Also Tarek (2005) collected two hundred and eighty potato tubers samples at random samples from different locations of Egyptian markets, he detected the contamination of some organophosphorus, oraganochlorine and synthetic pyrethroid pesticide residues.

Table (2) Pesticide residues and maximumresidue limits in ppm monitored in cabbage samples collected from local markets in Egypt.

Markets► / Pesticides▼	Giza	Behira	Gharbia	Faiyoum	Dakahlia	MRL*
Methamidifos	0.021	*	*	0.014	*	0.5
Acephate	*	0.182	*	0.457	*	0.5
Bendiocarp	*	*	*	*	*	No MRLs established
Monocrotophos	0.128	0,045	*	0.043	*	No MRLs established
Atrazine	*	*	*	*	*	No MRLs established
Pirimicarb	*	*	0.005	*	*	1
Cyanofenphos	0.011	0.010	*	0.010	*	No MRLs established
Phosphamidone	*	0.007	*	*	0.006	No MRLs established
Pirimiphos-methyl	*	0.014	*	0.006	0.005	2
Diazinon	*	*	*	*	*	0.5
Pirimiphos-ethyl	*	*	*	*	*	No MRLs established
Chlorpyrifos	*	*	0.002	*	*	1
Profenofos	*	*	*	*	*	1
Tebuconazole	*	*	*	*	*	0.2

MRL* : Maximum residue limits

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ARABIC SUMMARY

تقييم بعض المبيدات فى مكافحة الذبابة البيضاء *Bemisia tabaci* (Genn.) وتقصى بقاء بعض المبيدات فى نبات الكرنب.

عزة إسماعيل محمد سليمان .
المعمل المركزى للمبيدات – مركزالبحوث الزراعية – الدقى – الجيزة.

تمت دراسة تأثير السمية لخمس مبيدات من مجموعة المبيدات الفسفورية العضوية وهي : Diazinon, Profenofos, Pirimiphos-methyl وChlorpyrifos-methyl , Cyanofenphos لتقييم تأثيرها علي الحشرة الكاملة للذبابة البيضاء فى خمس محافظات مختلفة وهي :الغربيه , الدقهلية, البحيرة , الجيزة ,الفيوم .
وقد أظهرت النتائج أن هذه المبيدات علي الرغم من تداولها منذ وقت غير قصير إلا أنها مازال لديها الكفاءة و الفاعلية مع ظهور صفة المقاومة .
وعند دراسة التأثير الإبادى الفورى لحشرة الذبابة البيضاء *Bemisia tabaci* (Genn.) كانت قيمة التركيز القاتل لنصف العشيرة ١,٢٣ ، ٩٢,٣٤، ١٠٢,١٥، ٨٢,١٢ ، ١٧٢,٠٢، و٨٨,٥٤ جزء فى المليون بالنسبة لمبيد Pirimiphos-methyl في السلالة الحساسة،سلالات الجيزة،البحيرة،الغربية،الفيوم و الدقهلية علي التوالى .
وقد كانت قيمة هذا التركيز ٠,٩٦ ،٦٩,٢٢، ١١٢,٢٥، ٨٧,١١ و٨١,٨٨ جزء فى المليون بالنسبة الي مبيدProfenofos فى سلالات : الحساسة،الجيزة،البحيرة،الغربية،الفيوم والدقهلية علي التوالى .
وقد أظهرت النتائج أن سلالة محافظة الدقهلية كانت أكثر السلالات حساسية وأقل السلالات من حيث قيمة التركيز القاتل لنصف العشيرة .
بينما أظهرت سلالة محافظتى البحيرة و الغربية أعلي قيمة للتركيز النصفي القاتل وكان مبيد Diazinon هو أقل المبيدات من حيث قيمة التركيز القاتل لنصف العشيرة وأقلهم من حيث تكوين المقاومة حيث بلغ معامل المقاومة مقارنة بالسلالة الحساسة ٤٠,١٤، ٢٩,٧٠ ، ٤٨,٦١ ، ٣١,٨٩ و ١٠,٠٨ ضعف لسلالات محافظات الجيزة،البحيرة ، الغربية ،الفيوم والدقهلية علي التوالى .
وعند تقدير كمية متبقيات المبيدات في عينات الكرنب المجمعة عشوائيا من أسواق الخمس محافظات السابق ذكرها ..قد أوضحت النتائج أن بعض العينات تحتوى علي متبقيات لبعض المبيدات مثل : Methamidifos،Acephate، Monocrotophos ،Cyanofenphos ،Phosphamidone، Pirimiphos-methyl ولكن لم تظهر متبقيات هذه المبيدات في كل المحافظات المختبرة ، بينما لم تظهر متبقيات لمبيد Pirimicarb إلا فى عينات محافظة الغربيه ، وكذلك مبيد Chlorpyrifos بينما خلت عينات هذه المحافظة من أى متبقيات لبقية المبيدات المختبرة، فى حين أنه لم تظهر أى متبقيات للمبيدات الآتية: Atrazine، Diazinon ،Profenofos،Tebuconazole في أى من العينات تحت الدراسة . ومما يذكر أنه فى كل المتبقيات الموجودة لم تتخطي النسب الموجودة طبقا للحدود المسموح بها MRL’s .