

∆ Journal TOXICOLOGICAL AND PERSISTENCE STUDIES OF IMIDACLOPRID AND PIRIMIPHOS-METHYL AGAINST WHITEFLY (Bemisia tabaci) ON / IN GABBAG LEAVES.

Azza I. Mohamed and Nevein S. Ahmed

J. Biol. Chem. Environ. Sci., 2008, Vol. 3(4): 293-307 www.acepsag.org Central Agricultural Pesticides Laboratory, Agric. Res. Center, Dokki, Giza, Egypt.

ABSTRACT

The effects of imidacloprid (novel insecticide) and pirimiphosmethyl (organophosphorus insecticide) to control the sweet potato whitefly(SPWF) insect which feed on cabbage were carried out using adult stage in Dakhlia Governorate in our study. This study was perused for two successive periods in July and October in the same year.

Generally, In October, the results were different where LC_{50} was 2.51 and 5.57 ppm for imidacloprid and pirimphos methyl while it was 0.43 and 1.45 ppm in July for the two insecticides, respectively. In case of LC_{90} , it was 30.31 and 123.76 ppm in October and 9.65 and 42.81 ppm in July, respectively. Highly resistance ratio (RR) was observed at the end of the season (October) with 23.87 and 48.16 fold for imidacloprid and pirimiphos-methyl than the early season (July) with 7.60 and 16.66 fold, respectively.

In addition, this work aimed to study the behavior of imidacloprid and pirimiphos-methyl insecticides in cabbage under field conditions. Cabbage plant was treated at recommended rate with tested insecticides i.e. 125 ml/100 L water & 375 ml/100L water for imidacloprid and pirimiphos-methyl, respectively.

The analytical data showed a gradual decrease of tested insecticides residues with time, the initial deposit of imidacloprid from the 2nd application was 1.43 ppm, this value dropped to 0.31 ppm at 12 days after treatment. In 15th day, sampling process was carried out by taken outer and inner leaves. Residues of imidacloprid were 0.16 ppm

for outer leaves and were 0.06 ppm for inner leaves. These values were decreased to 0.05 ppm and 0.01 ppm for outer and inner leaves after 21 days. Also the data showed that; the initial deposit of pirimiphos-methyl on cabbage leaves after the second application was 1.22 ppm in outer leaves and 0.37 ppm in inner leaves which decreased to 0.11 ppm and 0.01ppm at 12 days from application for outer and inner leaves respectively. In 21st day primiphos-methyl became undetectable in both outer and inner leaves.

INTRODUCTION

Cabbage is a popular crop in Egypt, which is grown in more than one season and usually attacked by aphids, whiteflies and several species of lepidopterous larvae. Some of the most serious pests of steadily growing importance on cotton, vegetables and ornamentals are the different biotypes of the cotton whitefly *Bemisia tabaci* (Cahill et al 1995). This pest cause direct and indirect damage to their host crop, general weaking of the plants due to whitefly infestation could cause serious reduction of cotton yield due in part to a decreased number of bolls, in part to a decline in weight of seeds and lint per boll (Mound 1965). The larval feeding of *B. tabaci* reduced leaf chlorophyll content and photosynthetic capacity per unit of remaining chlorophyll (Buntin et al 1993).

Adults and Nymphs of whiteflies removed sap from the phloem, resulting in spotting, yellowing and abscission, induced plant death (Schuster and Stansly, 1994). Indirect damage is occurring due to transmitting of virus diseases to host crop. Tomato leaf curl is a disease caused by tomato yellow leaf curl virus (TYLCV) (Cohen and harpaz 1964, Cohen and Nitzany 1966 and Cohen and Marco 1970). Therefore, the need of an effective control for sweet potato whitefly prompted us to conduct the present studies.

Insecticides of various groups are being used to control this pest. Two insecticides are recommended for use on this crop such as imidacloprid and pirimiphos-methyl. Residues after the application should be followed and the waiting periods between application and harvesting should also be recommended to be sure that the residues are below tolerance levels before marketing.

The present work was designed to evaluate the toxicity of both insecticides against the whitefly and also to investigate the residues of imidacloprid and pirimiphos-methyl on and in the outer and inner leaves of cabbage plants so as to determine the interval between spraying and harvest required for the safe use of this crop for human consumption.

MATERIALS AND METHODS

1. Toxicological Study

1.1. Laboratory susceptible strain:-

The susceptible reference strain of *Bemisia tabaci* (Genn), Originated from field collection from over vegetables and ornamentals, growing in the Governorate under study. This strain has been reared in laboratory culture for 30 generation under standard conditions at 26° C \pm 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 (10×200 cm and 80 height). Each chamber was provided by fluorescent lamps and each stand surrounded by muslin. Insects were fed on untreated cabbage plants 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.

1.2 Field populations

The whitefly adults of *B. tabaci*, used in the present study, were collected from cabbage fields of Dakhlia Governorate. Adults of SPWF were collected from field with custom mad battery operated suction sampled (Dittrich *et al* 1990). Adults were randomly collected across a representative collage growing area from field. 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 tests, the jars were taken out of the cool box and inverted upside down. The healthy individuals would move to the top due to positive light, weak and dead individuals were discarded.

2. The insecticides used:

a)Imidacloprid:1-[(6-chloropyridin-3-yl)methyl]-N-nitro-4,5-dihydroimidazol-2-amine, is a systemic chloronicotinyl insecticide used in the form of admire 20% SC in the recommended rate of 125cm³/100L water.

b) Pirimiphos-methyl O-(2-Diethylamino-6-methylpyrimidin-4-yl-O,O-dimethylphosphorothioate was used in the form of the E.C. formulation under the trade name of actellic 50% E.C. at the recommended rate of 375cm³/100L.

3. Bioassays by using leaf-cage technique:

The bioassay method was done to evPaluate the insecticidal activity of sub lethal concentrations. The method used for experiments similar to that by Probhaker *et al* 1989 with slight modifications:

- a) Preparation of stock solution from each formulated insecticides on the active ingredient basis, by diluting each formulation in water (v/v) subsequently several dilutions were made to give the needed concentrations against the adult stage of each population.
- b) Cabbage leaves were dipped into these concentrations for each insecticide.
- c) Small cages were fixed on the lower side of treated and control leaves, not less than 30 healthy adults were placed in each cage (Muniz *et al* 2001).
- d) Each concentration was replicated three times and the control was tested with water only.
- e) The test cages were maintained at 27° C ± 2 , 70-85% RH, and a photoperiod of 16 hours light was used during the experiments, (De Coke. et al 1990).
- f) Mortality counts were made for the treated and the control after 24 hours

4. Persistence of insecticides

4.1. Field experiment design:

Cabbage plants *Brassica oleracea* were cultivated as nurslings in Dakhlia Governorate on the first of August 2006. The whole area of experiment was 225 m². The land received the normal agronomic practices throughout the experimental period. The whole area of the experiment divided to two parts, each part was 112 m² for the application of two insecticides.

4.2. Insecticidal application:

On the 18th of October application of insecticides was carried out as a first spray, the cabbage plants still immature (not rounded), the cultivated plants were highly infested by whitefly when the

cabbage plants were immature and so application of insecticides was necessary. The second spray was applied after 27 days from the first spray on 13th November and plants were mature and rounded. Knapsack motor sprayer equipped with one nozzle was used. Control samples were collected before application of insecticides.

4.3. Sampling

After the second spray, sampling was carried out randomly at interval times i.e. samples were taken at two hours after application (zero time), 1, 3, 6, 9, 12, 15 and 21 days after treatment. Samples were carried out by taken a whole plant (rounded cabbage) which was divided into two portions (outer and inner leaves) and each portion was kept in plastic bag (poly ethylene bags) and put in cool box during the transport from the field to the laboratory, then treated carefully with clean sharp blades into three replicates each one containing 50gm (three replicates each), all samples were kept in the freezer at -20 °C till analysis.

4.4. Extraction

Methanol was found to be the best solvent for extracting the insecticides from cabbage leaves according to the method of Molhoff (1975). Frozen samples (50 gm) were left until reach room temperature then blended with 200 ml methanol for 3 minutes and then filtered through a funnel with a dry pad of cotton into a graduated cylinder where a known volume (100 ml) was taken.

a) Imidacloprid:

A known volume of the extract was concentrated on a water bath at 40^{0} C to remove methanol and then partitioned with 100 ml n-hexane which was discarded, the aqueous layer was partitioned with three times 50 ml methylene chloride. The extract was dried through a cotton pad and anhydrous sodium sulphate and evaporated at 40^{0} C (Hend 2004).

b) Pirimiphos-methyl

After water separation from methanol extract, the extract was partitioned with methylene chloride (100, 50 and 50ml) in a 500 ml separatory funnel after adding 40 ml of saturated solution of sodium chloride. The methylene chloride phase was filtered through a pad of clean cotton and anhydrous sodium sulphate and then evaporated just to dryness using a rotary evaporator at 40°C EI-Baki *et al* (2000).

4.5. Clean Up.

a) Imidacloprid

The chromatographic column was prepared by adding 20ml ethyl acetate followed by 4.5 gm 5% deactivated florisil and 2 gm of anhydrous sodium sulphate and washed with ethyl acetate. Elution with 20 ml ethyl acetate and eluted from the column with 25 ml acetonitrile and then evaporated at 40°C (Hend 2004).

b)Pirimiphos-methyl

The concentrated extract was transferred quantitatively to a glass beaker with 20 ml n-hexane and mixed with 5-10 gm of activated charcoal and 2 gm of anhydrous sodium sulphate and the slurry was allowed to settle. The clear layer of the slurry was transferred to a suitable chromatographic column fitted and packed with silica gel and allowed to pass slowly through the column. The charcoal was washed 6 times with 20 ml n-hexane each and passed through the column. The combined extract was evaporated to dryness and subjected to GC-determination (Abo-El-Seoud et al 1995).

4.6. Determination

a) HPLC Determination

Imidacloprid residues were dissolved in HPLC grade methanol and analyzed by the (HPLC) Agilent (1100) equipped with UV diode ray detector and a C18 column (150mm x 4.6 mm i.d. x 0.5 μ m film thickness, mobile phase methanol 75% and water 25%, the flow rate was 0.9ml/min, The retention time of imidacloprid was 2.09 minutes.

b) GC determination:

Gas chromatographic analysis by using A Hewlett Packard, series 6890 plus gas chromatograph equipped with a flame photometric detector operated in the phosphorus mode (526 nm filter) was used for pirimiphos-methyl determination using peak area under the following conditions: the column was DB-608 (30 m length x 0.32 mm i.d. x 0.25 μ m film thickness). Temperatures were 245, 200 and 250°C for injector, column and detector, respectively. Gases flow rates were 3.75, 100 and 100 ml / min for nitrogen, hydrogen and air, respectively. At these conditions, the retention time of pirimiphosmethyl was 2.91 minutes.

An external standard analysis was used to calculate the recovery rate of pesticide residue by adding known amounts of imidacloprid and pirimiphos-methyl to the untreated cabbage leaves samples. Results were corrected according to the rates of recovery following the same technique previously mentioned for the extraction and cleaning up and quantitation. The rates of recovery in cabbage leaves were 91.47 and 97.54 % for imidacloprid and pirimiphos-methyl, respectively.

RESULTS AND DISCUSSION

1. Toxicity of imidacloprid and pirimiphos-methyl insecticides to the whitefly *Bemisia tabaci* on cabbage

Susceptibility of adults' whitely *B. tabaci* to two chemical insecticides was studied for the field collected populations of Dakhlia Governorate through the cotton season 2006 in July and again in October following the intensive spray application of insecticides to control the cotton pests and other pests on different crops.

To evaluate the effect of used insecticides to response of susceptibility laboratory strain and field population of the whitefly *B. tabaci*, the feeding technique for adults was used.

The LC₅₀ and LC₉₀ values, their 95% confidence limits, slope values and resistant ratio RR were calculated [based on data of field collected whiteflies relative to susceptible laboratory strain].

1.1 Effect of insecticides on the susceptible laboratory strain whitefly adults *Bemisia tabaci*

Results concerning the toxicity of imidacloprid and pirimiphosmethyl insecticides used against *B. tabaci* susceptible strain on the adult stage were tabulated in table (1).

The obtained results in table one indicated that imidacloprid had slightly higher toxicity than pirimiphos-methyl. The LC_{50}^{s} were 0.42 and 0.84 ppm, while LC_{90}^{s} was 1.27 and 2.57ppm for imidacloprid and pirimrphos-methyl respectively; so imidacloprid was the most potent insecticide against the adult of susceptible strain. The slope values for regression lines of two insecticides were slightly high 2.67 for imidacloprid and 2.63 for pirimiphos-methyl i.e. at most extent the slope values for both insecticides be equal and reflect homogeneity of tested susceptible strain.

1.2 Effect of insecticides tested against field collected B. tabaci adults

1.2. A. Field strains collected early in cotton season 2006

Data concerning the effect of the used insecticides (imidacloprid and pirimiphos-methyl) on the field strain *B. tabaci* adults collected in July 2006 are tabulated in table (2).

These results throw light on the effectiveness of the tested insecticides against the adults of whitefly which were collected from the fields in July 2006 (early cotton season), these data showed a low level of resistance toward imidacloprid. The resistance ratio (RR) were 1.02 and 1.73 fold with LC_{50} and LC_{90} respectively, while in case of primiphos- methyl the data showed medium R.R with 7.60 fold for LC_{50} , and high level of resistance 16.66 fold with LC_{90} .

Table (1): Toxic effects of Pirimiphos-methyl and Imidacloprid on susceptible strain of whitefly *Bemisia tabaci* adults under laboratory condition after 24 hours of treatment.

Rarameters Insecticides	LC _M (ppm)	F.L.*	L.C ₉₎ (ppm)	II.	Slope ±SE	X,	
Imidacloprid	0.42	(0.37-0.48)	1.27	(1.05-1.66)	2.67±0.238	5.27	
Pirimiphos-methyl	0.84	(0.74-0.95)	2.57	(2.1-3.36)	2.63±0.236	5.88	

Table (2): Toxic effects of Pirimiphos-methyl and Imidacloprid on field strain of whitefly *Bemisia tabaci* adults collected from Dakahlia Governorate in July 2006 under laboratory condition after 24 hours of treatment.

Parameters Insecticides	(ppm)	FL*	RR at LC ₄ (Fold)	(ppm)	f.L**	RR at LC ₉ (Fold)	Slope <u>+</u> SE	X [*]
Imidacloprid	F. 0.43 S. 0.42	(0.24-0.65)	1,02	F. 9.65 S. 1.72	(6.47-17.64)	7.60	0.95±0.12	1,44
Pirimiphos-methyl	F. 1.45 S. 0.84	(0.91-2.05)	1.73	F. 42.81 S. 2.57	(27.84-78.83)	ló.66	0.87 <u>+</u> 0.092	1,92

^{*: 95%} fiducial limit for LC50

RR= LC to of LC to of field strain

RR= LC to or LC to of susceptible strain

^{**: 95%} fiducial limit for LC90

F.: field strain

S.; susceptible strain

1.2.B. Field strains collected at the end of cotton season 2006

Examination of obtained results in table (3) clearly indicated a great effect of the imidacloprid (novel insecticide) with LC_{50} 2.51 and LC_{90} 30.31ppm followed by pirimiphos-methyl with LC_{50} 5.57 and LC_{90} 123.76 ppm, the obtained results indicated that imidacloprid had higher toxicity than pirimiphos-methyl.

From calculation the resistance ratios for both insecticides at LC₅₀ and LC₉₀ levels proved that; adults field strain of whitefly *Bemisia tabaci* show slightly higher resistance toward pirimiphosmethyl than imidacloprid. i.e. resistance ratio at LC₅₀ for imidacloprid and pirimiphosmethyl are 5.98 and 6.63 fold respectively while high resistance ratio (R.R) at LC₉₀ for imidacloprid and pirimiphosmethyl with 23.87 and 48.16 fold respectively.

The slope values for regression lines of two insecticides were slightly low 1.3 ± 0.11 for imidacloprid and 0.95 ± 0.14 for pirimiphos-methyl i.e. the slope values for both insecticides reflect heterogeneity of tested field strain, whatever from all tests imidacloprid was the most potent insecticides, these results agree with those of Solaiman (2005), Farghaly (2005) who demonstrated that neonicotinoid insecticides were more toxic than the tested insecticides from different classes for all strains of whitefly. Farman *et al.*, (2004) revealed that all the insecticides (chlorpyrifos, endosulfan, ethamidophos, imidacloprid), tested controlled the cotton whitefly, imidacloprid was the most efficient in controlling whiteflies.

Ulaganathan and Gupta (2004) who evaluated the efficacy of different insecticidal spray i.e. acetamipride, imidacloprid and beta – cyfluthrin against the sucking pests of cotton i.e. jassids, whiteflies, aphids and trips, and they found that imidacloprid was more effective in reducing the number of the population. Also Weichel and Nauen (2004), recorded that imidacloprid was the most important insecticide in hop cultivation in Germany.

Table (3): Toxic effects of Pirimiphos-methyl and Imidacloprid on field strain of whitefly *Bemisia tabaci* adults

Parameters Insecticides	LC ₆₉ (ррця)	F.L.÷	RR at LC38	LC∞ (ppm)	F.L.**	RR at LC ₉₀ (Fold)	Slope ± SE	X²
Imidacloprid	F. 2.51 S. 0.42	(2.46-3.97)	5.98	F. 30.31 S. 1.72	(23.06-43.31)	23.87	1.31 ±0.11	2.66
Pirimiphos- methyl	F. 5.57 S. 0.84	(2.61-8.73)	6.63	F. 123.76 S. 2.57	(82.53-241.14)	48.16	0.95 ±0,14	2.39

*: 95% fiducial limit for rLC50

**: 95% fiducial limit for LC90

RR = LC₁₀ or LC₂₀ of field strain

S.E.:Standard Error

LC30 or LC90 of susceptible strain

F.: field strain

S.:susceptible st

Generally, the previous observations could be summarized in the following points:

- 1- The resistance level of field strains at the end of cotton growing season (October field strain) table (3) was higher in tested insecticides than at the early season (July field strain). These results may be due to the frequent exposure of pest populations to insecticides.
- 2- Efficiency of imidacloprid insecticide toward adults of whitefly *Bemisia tab*aci is higher than pirimiphos-methyl insecticide.
- 3- Whitefly exhibited slightly higher level of resistance toward pirimiphos-methyl than imidacloprid, because of the continuous use of this insecticide through control the insect pest in horticulture crops.

2. Residues in the cabbage leaves

2.A. Residues in the outer leaves

Data in table (4) demonstrate the analysis of imidacloprid and pirimiphos-methyl residues in the outer leaves of cabbage up to 21 days after the second application. Samples before spraying for the second time was evaluated to show whether there were any residues of

the tested insecticides after the first spray but no residues were found. The difference in the initial deposits between the two tested insecticides was attributed to the variation in the physical and chemical formulation. As expected the degradation rates of pirimiphos-methyl was more than that in imidacloprid during the rest of the experiment. The initial deposits (two hours after application) in the outer cabbage leaves were 1.43 and 11.02 ppm for imidacloprid and pirimiphos-methyl, respectively. The high initial value of pirimiphos-methyl could be attributed to the high concentration of the spraying with actellic respect to that of admire. Following that period, the residues decreased to 1.18 and 7.23 ppm one day after the application indicating that the percentage loss were 17.48 and 34.39% reflecting the observed deterioration of pirimiphos-methyl (Abo-El-Seoud et al 1995) to imidacloprid (Gajbhiye et al 2004). A sharp decline of residues occurred for pirimiphos-methyl however, a comparatively slow rate of degradation was noticed for imidacloprid. Pirimiphos-methyl) became undetectable while the final residue at 21 days from the second spraying of imidacloprid was 0.05 ppm.

2.B. Residues in the inner leaves

Penetration of the two studied insecticides was attained to the inner leaves. Table (4) represents the residues of the tested insecticides in the inner leaves of the cabbage plants. The initial deposits were 0.53 ppm reduced to 0.03 ppm for imidacloprid after 21 days. In case of pirimiphos-methyl, the initial deposit was 5.37 ppm deteriorated till 0.02 ppm was detected at the end of the experiment.

From these data and according to the maximum residue limits MRL for imidacloprid and pirimiphos-methyl in cabbage plant were 0.5 and 1 ppm respectively. This indicates that PHI were 1 and 3 days for the outer leaves for both of two insecticides respectively while for the inner leaves PHI were 1day for imidacloprid and 10 day for pirimiphos—methyl, this also indicates that cabbage could be safely marketed 8 and 10 days after treatment with imidacloprid and primiphos-methyl, respectively.

Table (4):	Persisten	ce (of in	ida	eclopri-	d an	d piri	miphos	-methyl
insecticides	residues	on	and	in	outer	and	inner	leaves	during
autumn of y	ear 2006.								

Days after treatment (days)	Imida	cloprid res	idues on and	Pirimiphos-methyl residues on and in							
	Outer leaves		Inner le	aves	Outer Ie	aves	Inner leaves				
	Residues	% loss	Residues	% loss	Residues	Ð/o	Residues	%			
	(ppm)		(ppm)		(ppin)	loss	(ppm)	loss			
Initial*	1.43	0.00	0.53	0.00	11.02	0.00	5.37	0.00			
1	1.18	17.48	0.49	7.55	7.23	34.39	3.98	25.88			
3	0.96	32.87	0.37	30.19	4.68	57.53	1.79	66.67			
6	0.65	54.55	0.28	47.17	2.77	74.86	0.96	82.12			
9	0.44	69.23	0.20	62.26	1.17	89.38	0.50	90.69			
12	0.31	78.32	0.09	83.02	0.61	94.47	0.27	94.97			
15	0.16	88.81	0.06	88.68	0.08	99.27	0.09	98.32			
21	0.05	96.50	0.01	94.34	UND		0.02	99.63			
MRL		0.5	5								
T1/2		6					3				
PHI	S		1		10		6				

*Samples were taken two hours after insecticides treatments

MRL: Maximum Residue Limits

T1/2: Calculated half life values (days)
PHI: Pre-harvest interval (days)

UND: Under detectable limit

The estimated half-life values (T1/2) of the applied pesticide were 6 and 3 days for imidacloprid and pirimiphos-methyl, respectively regarding the low degradation rate of imidacloprid with respect to pirimiphos-methyl.

Naturally, such variation should be attributed to the nature of the pesticide molecule in relation to the capability of the tested plant to break down such environmental chemicals, Abo-El-Seoud et al 1995 and Fossen (2006). It was concluded that the pre-harvest intervals was 8 and 10 days for imidacloprid and pirimiphos-methyl, respectively.

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سمية مبيدى الايميداكلوبرد والبريميفوس ميثيل على الذبابة البيضاء وثباتهما على وفي أوراق نبات الكرنب

عزة اسماعيل محمد - نيفين صلاح الدين احمد الميزة المعمل المركزى للمبيدات – مركز البحوث الزراعية – الدقى - الجيزة

استهدفت هذه الدراسة إلقاء الضوء على التأثيرات الإبادية لمبيدي الايميداكلوبرد و البريميفوس ميثيل في مكافحة الذبابة البيضاء إلى جانب دراسة ثباتهما على وفي أوراق الكرنب تحت الظروف الحقلية مع تحديد فترة نصف العمر للمبيدين.

أوضحت النتائج كفاءة مبيد الإيميداكلوبرد (مبيد من مجموعة المبيدات الحديثة) عن مبيد البريميفوس ميثيل (مبيد من مجموعة المبيدات الفسفورية) حيث تميز بنشاط ابادي في مكافحة الحشرة الكاملة الذبابة البيضاء Bemisia tabaci وكانت قيمة التركيز القاتل لنصف العشيرة 43و0 و 45و1 جزء في المليون في شهر يوليو على التوالي بينما أظهرت الحشرات المجمعة في شهر أكتوبر مقاومة حيث بلغت قيمة التركيز القاتل لنصف العشيرة 15و2 و 55و جزء في المليون على التوالي . وتشير النتائج إلى وجود مقاومة أعلى الحشرة في شهر أكتوبر عنها في شهر يوليو .

طبقا للحدود المسموح بها يمكن تسويق نبات الكرنب المعاملة بمبيد الايميداكلوبرد بعد 8 أيام من المعاملة . المعاملة , بينما يمكن تسويق النباتات المعاملة بمبيد البريميفوس ميتيل بعد 10 أيام من المعاملة .