PERSISTENCE OF CARBOSULFAN, CHLORPYRIFOS-METHYL AND MALATHION RESIDUES IN CUCUMBER FRUITS GROWN IN GREENHOUSE

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INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most important vegetable crop grows in commercial greenhouses under the Egyptian conditions. Cucumber plants are attacked with many insects, such as aphids, white flies, mites and other sucking pests (Anonymous, 2001). The Egyptian Ministry of Agriculture recommends certain insecticides, namely carbosulfan, chlorpyrifos-methyl and malathion for use on cucumbers to control these insects, either in the open field or in commercial greenhouses.

Many investigations have demonstrated the chemical control of aphids and white flies on cucumber plants (Abdallah *et al.*, 1991.; Abdel-Hameed *et al.* 1991.; Farrag *et al.* 1994.; El-Khawass and Khalifa, 1997.; and Badawy *et al.*, 1999) .The use of pesticides in food production has provided numerous benefits in terms of increasing production and quality. As a result, consumers are exposed to pesticides residues from consumed fruits and vegetables at the fresh state (Fan and Jackson, 1989). To protect the health of consumers, an idea about the safety period and determination of the waiting period between application and harvesting should be provided to be sure that the residues are below the tolerance levels and the edible parts become safe for human consumption. (Bates, 1979.; Ramadan *et al.* 1992.; Shokr, 1997.; and Sallam, 1998).Many studies were carried out on persistence of pesticide residues on various vegetables in greenhouse (Leidy *et al.* 1978.; Al-Samariee *et al.* 1988.; Ramadan *et al.* 1992.; Antonious and Snyder, 1994.; Liapis *et al.* 1994 and Badawy *et al.* 1999). This work aimed to study the behavior of insecticide residues in cucumber fruits grown in greenhouse. This study also aimed to throw light on the effect of these insecticides treatment on the total soluble solids of cucumber fruits.

MATERIAL AND METHODS

I- Insecticides: Three insecticides of two different pesticides groups were used in this investigation as follows:

a- Organophosphorus insecticides

1-Chlorpyrifos - methyl: o, o - dimethyl, o - (3, 5, 6 – trichloro - 2 - pyridyl) phosphorothioate. The formulation Reldan 50% E.C. was used at the rate of (0.5 liter/feddan/200 liters water).

2-Malathion: o, o - dimethyl, S (1, 2 - dicarboxy ethyl) phosphorodithioate. Malathion K.Z. formulation 57% E.C. was used at the rate of (0.5 liter/feddan/200 liters water).

b- Carbamate insecticides

Carbosulfan: 2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl [(dibutyl amino) thio] methyl carbamate. The formulation Marshal 25% W.P. was used at the rate of (300 gram/feddan/200 liters water).

II- Greenhouse conditions

Greenhouse temperatures averaged 18 and 30 °C and relative humidity averaged 60 and 65 % during February and March 2004, respectively.

III- Greenhouse experiment and sampling

The experiment was carried out in Sakha Research Station, Kafr El- Sheikh Governorate during the season of 2004. Cucumbers (*Cucumis sativum* var., *Delta stars*) were seeded on January 15/ 2004, and transplanted on February 14/ 2004. The experimental area was divided according to complete randomized block design including three replicates for each insecticide. Three plots were left untreated to serve as control. The insecticides application was carried out on March 18/ 2004 at the rates mentioned above using a knapsack sprayer.

Representative samples of cucumber fruits (1kg) were collected randomly from each block (5 fruits/replicate) after one hour of application (zero time), 1, 3, 6, 9, 12, and 15 days of spraying. Clean polyethylene bags were used for preservation of the collected samples. Composite sample of (1kg) cucumber fruits were divided into two parts, the first one was chopped well and thoroughly mixed before taking a portion of 100g in triplicates for determination of pesticide residues and the other

was used to determine total soluble solids (Tss) of the treated and untreated cucumber fruits. The samples were stored at –20 °C in a deep freezer until analysis.

IV- Determination of insecticide residues

a- Organophosphorus insecticides (chlopyrifos- methyl and malathion)

The analytical method used is a general method suitable for organophosphorus compounds (Anonymous, 1988). According to the method, 50g of homogenized sample are mixed with 50g anhydrous sodium sulphate and 100-ml ethyl acetate. The mixture is blended for 3 min. and the extract was filtered, and evaporated just to dryness using a rotary evaporator at 40°C. The residues were dissolved in 5ml of n-hexane and cleaned up according to Mills *et al.* (1972). A chromatography column 10g. of activated florisil 60-100 mesh with 3.5% moisture covered with a layer of anhydrous sodium sulphate was used. The elution solvent system was dichloromethane: n-hexane: acetonitrite at the ratio of 50: 48.5: 1.5. The elute was than collected in a 250-ml flask and evaporated under vacuum to dryness. Residues were redissolved in the proper volume of ethyl acetate for G.C. analysis.

A pye unicam 4500 gas chromatograph equipped with FPD operated in the phosphorous mode and column PAS 1701 (30 m x 0.32 nm x 0.25 nm) a Pyrex glass column (1.5 x 4 mm i-d.) packed with 4% SE- 30 + 6% OV. 230 on gas chromosorb Q 80-100 mesh were used under the following conditions: Injector temperature 240°C, column temperature 240°C, Detector temperature 250°C, Carrier gas (N_2) flow rate 3ml/min., hydrogen and air flow rate: 75 & 100 ml/min., respectively. Chlorpyrifos- methyl and malathion retention time under these conditions were 1.820 and 2.265 minutes, respectively (Table 1).

b- Carbamate insecticides (Carbosulfan)

The insecticide was extracted and cleaned up according to the method of (FMC Agricultural Chemical Division Middleport, 1979). Twenty grams of macerated crop was blended with 100 ml 2:1 hexane/ 2-propanol (v/v) at high speed for 3 minutes and filtered through a filter paper (S& S sharkskin). The filter pad and crop were blended a second time with 100 ml of 2:1 hexane/ 2- propanol (v/v). This solution was filtered and rinsed with 50 ml of blending solution. The combined filtrate is diluted to exactly 240 ml. A 5 gram aliquot (60 ml) of the extract was placed into a 500 ml seperatory funnel. 50 ml of distilled water and 12 grams of sodium chloride were added. This mixture was shaken for 1 minute and allowed to separate. The hexane layer was decanted into a 500 ml kuderna- Danish evaporator. The remaining aqueous layer was then extracted with 2 x 100 ml of hexane.

Florisil column clean up

15 mm x 150 mm column with a 250 ml reservoir was prepared by adding a glass wool plug followed by 10 grams of florisil (3% water) and 2 grams of anhydrous sodium sulfate. The column was rinsed with 50 ml 95/5 (v/v) hexane/ ethyl acetate. Then the sample was added and rinsed with 2 ml 95/5 (v/v) hexane/ ethyl acetate. The column was eluted with 40 ml 95/5 (v/v) hexane/ ethyl acetate. After the solvent has been absorbed by the sodium sulfate layer, the eluant was discarded. The column was eluted with 50 ml 95/5 (v/v) hexane/ ethyl acetate and the eluant was collected. This is the FMC 35001 fraction. The eluant was concentrated in a kuderna- Danish evaporator on a steam both to about 5 ml. Residues of FMC (carbosulfan) were redissolved in toluene for gas chromatographic analysis (GC.).

Recovery studies

Untreated fruits were fortified by the addition of standard solutions of carbosulfan, chlorpyrifos/ methyl and malathion at levels ranged from 0.1 to 1.0 ppm. The fortified samples were processed through all steps of the analytical methods to validate the assay procedure.

Recovery percentages of carbosulfan, chlorpyrifos/ methyl and malathion were 88.42, 95.82 and 94.47%, respectively (Table 1). Residue values were corrected according to the recovery percentages obtained from fortified samples (Table 1).

TABLE (I)

Recovery percentages (R %) and retention time (Rt) min of carbosulfan, chlorpyrifos- methyl and malathion in cucumber fruits.

Added (ppm)	Carbosulfan		Chlorpyrifos- methyl		Malathion	
	R%	Rt min	R%	Rt min	R%	Rt min
1.0	90.95	4.661	98.48	1.820	96.50	2.265
0.5	88.90		95.92		94.10	
0.1	85.40		93.07		92.80	
Average	88.42		95.82		94.47	

RESULTS AND DISCUSSION

1- Recovery percentages of carbosulfan, chlorpyrifos- methyl and malathion

The procedures used for extraction and quantitation of these insecticides were reliable and provided good recoveries (Table 1). The high recovery was found to be for chlorpyrifos- methyl followed by malathion and carbosulfan. The

corresponding values were 95.82, 94.47 and 88.42%. These results agree with those obtained by Hegazy *et al.* (1997) using the same method of analysis of chlorpyrifos-methyl residues on cucumber fruits. Rate of recovery was 100%.

2- Residues of carbosulfan, chlorpyrifos- methyl and malathion on and in cucumber fruits

Data presented in Table (2) show that the chemical structure of the used insecticides and the rates of application did affect the amounts of the initial deposits as well as the subsequent amounts of residues on and in cucumber fruits. The initial deposits of carbosulfan, chlorpyrifos- methyl and malathion were 3.339, 1.550 and 0.920 ppm, respectively. The amount of residues decreased to 1.071, 0.523 and 0.275 ppm, respectively within the first 24 hours after spraying. The residues of these insecticides dropped to non-detectable, 0.002 and 0.005 ppm after 12 days of spraying. The results indicated also that the loss percentage of carbosulfan, chlorypyifos- methyl and malathion deposits one day after spraying were 67.92, 66.26 and 70.11%. The figures of loss show that loss progressively increased up to the 6th day post treatment reaching more than 95% of the initial deposits.

TABLE (II)

Residues* (mg/ kg) of carbosulfan, chlorpyrifos- methyl and malathion on and in cucumber fruits grown in a greenhouse.

Days after spraying	Carbosulfan		Chlorpyrifos- methyl		Malathion	
	Amount	% less	Amount	% less	Amount	% less
One hour**	3.339	0.00	1.550	0.00	0.920	0.00
1	1.071	67.92	0.523	66.26	0.275	70.11
3	0.377	88.71	0.251	83.81	0.184	80.00
6	0.113	96.62	0.032	97.94	0.031	96.63
9	0.024	99.28	0.008	99.48	0.016	98.26
12	N.D***	100.00	0.002	99.87	0.005	99.46
15	N.D	100.00	N.D	100.00	0.002	99.78

* The reported values are means of three replicate analysis.
** Initial deposits of the insecticide.
*** Non detectable.

The previously mentioned results clearly show that the rate of persistence of the three tested insecticides were influenced by many factors, *i.e.*; chemical structure, formulation as well as the rate of the used insecticide, vapor pressure, and the climatic conditions; especially the ambient temperature during pesticides application. In

general, increasing temperature degrees increased the rate of residues degradation. The present results are in agreement with those of Badawy *et al.* (1999) who found that carbosulfan residues in cucumber fruits decreased rapidly after 24 hrs. after application and then gradually decreased from 1 day to 3 days after application followed by slow decrease until not detected. It was found also by those authors that carbosulfan and its major metabolite carbofuran have a short persistence time in cucumber fruits. Also Abdel- Aal *et al.* (2002) found that the loss percentage of carbosulfan residues in tomato fruits were 96.69 after 12 days of spraying. Kashyap and Walia (1986) observed the initial deposit of malathion on Okra leaves by 15.02 ppm. Dissipation of deposits was quick in the 1st day and the deposits finally decreased to a level of 0.03 ppm on 6th day. No residues were present on 10 and 14 days. Malathion degradation was very fast during the first three days, where 83.4% residues degraded.

The values of half- life were obtained from calculation according to Moye *et al.* (1987). The half- life periods of carbosulfan, chlorpyrifos- methyl and malathion residues in cucumber fruits were 22.42, 23.97 and 27.84 hours, respectively. These results are in agreement with those obtained by Badawy *et al.* (1999) who found that the half- life value (RL_{50}) of carbosulfan residues was 12 hours on cucumber fruits growing in green house, while Abdel- Aal *et al.* (2002) showed another picture differed in open field and type of crop. The half- life value of carbosulfan on tomato fruits was 2.8 days. Hegazy *et al.* (1997) found that half-life value of chlorpyrifos- methyl on cucumber fruits was 17 hours. Shokr (1997) found that the half- life value of malathion in cucumber fruits was 14.7 hours.

According to the maximum residue limits (MRLs) of carbosulfan (0.1 ppm), chlorpyrifos- methyl (0.5 ppm) and malathion (0.2 ppm) in cucumber fruits or similar vegetable, presented in Anonymous (2003). Data presented in Table (3) show that the periods (days) after which cucumber fruits sprayed with carbosulfan, chlorpyrifos- methyl and malathion can be picked up for human consumption are 6,1 and 1 day, respectively. This short waiting period post treatment for both chlorpyrifos- methyl and malathion might be due to the low residue level as achieved at the initial deposit (zero time). The results of this study are quite comparable with those reported by Abdel Hameed *et al.* (19991).; Hegazy *et al.* (1997).; Shoker, (1997) and Badawy *et al.* (1999).

Abdel- Hameed *et al.* (1991) found that waiting period of less than 7 days intervals is suggested for the use of recommended rate of carbosulfan on cucumber fruits. While Badawy *et al.* (1999) concluded that cucumber fruits could be used safely for human consumption after three days of spraying with carbosulfan. Shoker (1997)

found that the safe period was one day for malathion on cucumber fruits. Hegazy *et al.* (1997) indicated that only three days period was enough for the chlorpyrifos- methyl residues in cucumber to reach a safe level less than the (MRLs) (0.5 ppm).

TABLE (III)

Application rates and legal limits of pesticides on cucumber fruits.

Pesticides	Rate of application/ 100 litter water	(MRLs)* (ppm)	(PHI)** (day)	RL_{50}*** (hour)
Carbosulfan	250 cm^3	0.1	6	22.42
Chlorpyrifos- methyl	250 cm^3	0.5	1	23.79
Malathion	150 g	0.2	1	27.84

* Maximum residue limits according to Codex Alimentarius Commission.
** Pre harvest interval days after application.
*** Half- life period (hour).

3- Effect of carbosulfan, chlorpyrifos- methyl and malathion residues on total soluble solids (TSS) in cucumber fruit

The results of the effects of carbosulfan, chlorpyrifos- methyl and malathion residues on total soluble solids (TSS) of cucumber fruits are shown in Table (4).

TABLE (III)

Effect of carbosulfan, chlorpyrifos- methyl and malathion residues on total soluble solids in cucumber fruits.

Time from treatment (day)	Total soluble solids (% Tss)			
	Control	Carbosulfan-treated cucumber	Chlorpyrifos-methyl treated cucumber	Malathion treated cucumber
Zero time	6.4	6.4	6.4	6.4
1	5.8	6.2	7.0	5.4
3	6.4	5.4	5.8	5.8
6	5.6	5.4	6.0	6.4
9	6.2	6.0	7.0	6.0
12	5.6	5.0	7.0	6.0
15	5.6	4.6	6.2	5.8
Mean	5.9	5.6	6.5	6.0

Results in Table 4 indicated that, total soluble solids content of cucumber fruits increased with organophorous (chlorpyrifos- methyl and malathion) while with carbamaite insecticides, (carbosulfan) it was less than the control. The same phenomenon took place with Ismail *et al.* (1993), who found that TSS and acidity contents of profenofos- treated tomatoes were increased during the test period. The percentage of TSS increase was gradually reduced after pesticide application.

SUMMARY

Experiments were carried out on greenhouse cucumbers for studding the degradation of three insecticides *i.e.* carbosulfan (Marshal 25% W.P.), chlorpyrifos-methyl (Reldan 50% E.C.) and malathion (Agrothion 57% E.C.) and for estimating the residues left on fruits harvested at commercial ripening. The effects of these insecticide treatments on the total soluble solids of cucumber fruits were also studied. Analysis of fruits at intervals following application showed that the initial residue of carbosulfan (3.339 ppm) was higher than those of chlorpyrifos-methyl (1.550 ppm) and malathion (0.920 ppm). These figures were decreased to undetectable amounts (UN), 0.002 and 0.005 ppm after 12 days of spraying. The dissipation of carbosulfan was faster than those of chlorpyrifos-methyl and malathion. (The half-live values were 22.42, 23.79 and 27.84 hours, respectively).

The maximum residue levels (MRLs) of carboulfan (0.1 ppm), chlorpyrifos-methyl (0.5 ppm) and malathion (0.2 ppm) were reached after 6, 1 and 1 days of application, respectively.

Data also indicated that the pesticide treatment increased the total soluble solids of cucumber fruits.

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