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PERSISTENCE OF SOME FUNGICIDE AND ACARICIDE RESIDUES ON AND IN CUCUMBER FRUITS GROWN UNDER GREEN HOUSE CONDITION

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ABSTRACT

Cucumber were planted under greenhouse conditions. Cucumber plants were sprayed after 45 days from planted with four pesticides, diniconazole (Sumi eight 5% EC) as a recommended and flusilazole (Punch 40% EC), fenarimol (Rubigan 12% EC) and hexythiazox (Maccomite 10 % WP) as non recommended pesticides. Pesticides residues were determined on and in unwashed and washed cucumber fruits at intervals of zero (one hour after spraying), 1, 3, 5, 7, 14, 21 and 28 days after spraying. Residues analysis showed that diniconazole has the lowest initial deposits 0.68 ppm than the other tested pesticide where the initial deposits were 3.006, 1.56 and 0.855 ppm for fenarimol, flusilazole and hexythiazox, respectively.

Data also indicated that the residual half lives values ($t_{1/2}$) were 5.431, 2.94, 1.98 and 1.95 days for flusilazole, diniconazole, fenarimole and hexythiazox, respectively in unwashed cucumber fruits.

On the other hand, washing process removed 18.33, 10.24, 13.83 and 50.7% of initial deposits for flusilazole, diniconazole, fenarimole and hexythiazox, respectively. This findings indicating the significant role of washing process by running tap water in reducing and elimination of hexythiazox residues from contaminated cucumber fruits than other tested pesticide.

Key words: Pesticides - Diniconazole – Flusilazole – Fenarimol – Hexythiazox – Cucumber.

INTRODUCTION

In Egypt, cucumber (*Cucumis Sativus*) is the most popular vegetables grown during the summer and winter time and considered as important fresh eaten vegetable crops.

Cucumber fruits are highly attacked by several pests, insects, mites and fungi throughout its growing season causing serious damages.

Flusilazole, diniconazole and fenarimol are demethylation inhibitor and systemic fungicide with protective and curative action (Tomlin, 2004).

Hexythiazox is a non systemic acaricide with contact and stomach action, good translaminar activity and has ovicidal, larvicidal, and nymphicidal activity uses to control of eggs and larvae of many phytophagous mites on fruit, citrus and vegetables (Tomlin, 2004)

Pesticide residues after application on vegetable plants should be followed to determine the waiting period between application and harvesting to be sure that the residues are below the tolerance levels and the edible parts become safe for human consumption. (Shoker, 1997 and Sallam, 1998).

The object of this study was to monitor residual level of flusilazole, diniconazole, fenarimol and hexythiazox in cucumber fruits grown under green houses and the effect of washing treated fruits with tap water to remove the tested pesticide residues.

MATERIAL AND METHODS

Cucumber were planted separately on May 1 th 2006 at the demonstrative green houses of agriculture, Giza governorate. Cucumber plants were sprayed after 45 days from planted with four pesticides diniconazole as a recommended fungicide for cucumber plants and flusilazole, fenarimol and hexythiazoles as non recommended pesticides.

The amount of each pesticide sprayed on targeted plants by using knapsack hand sprayer fitted with one nozzle boom.

Pesticides used and their application:

- 1- Flusilazole (Punch 40% EC): bis(4-fluorophenyl) (methyl) (1H-1,2,4-triazol-1-ylmethyl) silane; 1-[[bis(4-fluorophenyl) (methyl) silyl] methyl]-1H-1,2,4 - triazole. Flusilazole was used at the rate of 6.5 cm²/22 L/plastic green house i.e. 900m².

- 2- Diniconazole (Sumi eight 5% EC): (E)-(RS)-1-(2, 4-dichlorophenyl)-4, 4-dimethyl-2-(1H-1, 2, 4-triazol-1-yl) pent-1-en-3-ol. Diniconazole was used at the rate of 10 ml/30 L/plastic green house i.e. 1200m².
- 3- Fenarimol (Rubigan 12% EC) : (±)-2, 4-dichloro-α-(pyrimidin-5-yl) benzhydryl alcohol. Fenarimol was used at the rate of 2.1 cm³/8.5 L/ plastic green house i.e. 360 m².
- 4- Hexythiazox (Maccomite 10 % WP): (4 RS, 5RS)-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-1, 3-thiazolidine-3-carboxamide. Hexythiazox was used at the rate of 4.28 gm /22 L/plastic green house i.e 900 m².

Sampling for residue analysis:

Fresh fruit samples 2 kg of cucumber were collected at random from treated plants after one hour from pesticides application to determine the initial deposit and then after 1, 3, 5, 7, 14, 21 and 28 days for residue analysis. Each of the representative sample was divided into two sub samples, the fruits was left unwashed, while the second was washed thoroughly by tap water on the samples collected one hour, one and three days after treatment only to study the effect of washing on the percentage loss of pesticide residues.

The whole samples of washed and unwashed were kept in polyethylene bags. The samples were stored in a deep freezer until analysis.

Residue analysis techniques:

Extraction and clean up:

Flusilazole residues were extracted from cucumber fruit samples according to the method of Dupont de Nemours Co. (1989) and Nasr *et al.* 2003, and clean up according to the method of Johnson (1963), and diniconazole residues was extracted and clean up according to the method of El-Bouze *et al.* (1998), while fenarimole was extracted and clean up according to the method of Macnell *et al.* (1975). Also, the method of Tilting (1994) was used for extraction and cleans up of hexythiazox residues from treated cucumber fruits.

Determination of pesticides residues:

Flusilazole, diniconazole, fenarimol and hexythiazox residues were determined according to the method of Cabras *et al.* (1985) with some modification.

Jasco MD 2015 plus high pressure liquid chromatography HPLC fitted with quarter pump MD Pa-2089, multi wavelength detector, and

stainless steel column (4.6 mm x 25 cm) packed with C₁₈ were used under following conditions:

Mobile phase 55: 40: 5 ACN: MeOH: H₂O and flow rate 0.9 ml/min. At these conditions the retention time of flusilazole, diniconazole, fenarimol and hexythiazox were 3.625, 3.8, 3.714 and 3.95 respectively.

The average of recoveries were 104.2, 79.3, 105.1 and 101.1 for flusilazole, diniconazole, fenarimol and hexythiazox, respectively.

Kinetic study:

In order to study the rate of degradation of tested pesticides and the half lives period ($t_{1/2}$) for the tested pesticides on cucumber fruits were calculated according to equation (Moye *et al.*, 1987)

$$t_{1/2} = \ln 2 / K = 0.693/K.$$

$$K = 1/Tx. \ln a/bx$$

Where

K=Rate of decomposition.

Tx = Time in days.

a= Initial residue.

bx = Residue at time.

RESULTS AND DISCUSSION

Systemic and non-systemic fungicide and acaricide have been used widely for controlling wide range of economically important diseases mainly in vegetable crops. Therefore enhanced use of the fungicide and acaricide especially foliar increased the environmental pollution.

Data summarized in Table (1 and 2) represented the amount of flusilazole, diniconazole, fenarimol and hexythiazox residues in unwashed and washed cucumber fruits collected from the experiments at different intervals after pesticide spraying, respectively.

Flusilazole residues in cucumber fruits:

Results in Table (1) indicated that the initial deposits of flusilazole in unwashed cucumber fruits was 1.56 ppm after one hour of application, then gradually decreased to 1.293, 1.024, 0.838, 0.354, 0.095 and 0.019 ppm recording the rate of loss 17.11, 34.34, 46.41, 77.33, 93.88 and 98.77% at 1, 3, 5, 7, 14 and 21 days after treatment, respectively.

Data also indicated that flusilazole residue half-lives in unwashed cucumber fruits were 5.431 days.

These findings are in agreement with obtained and reported by Nevein (2005) who found that the initial deposits of flusilazole in unwashed apple fruits one hour after application were 1.39 ppm then decreased with elapse time from spraying to reach undetectable amounts, 21 days of application.

On the other hand, the effect of washing with tap water on residue level of flusilazole on cucumber fruits grown in green house condition is shown in Table (1). Washing removed 18.11, 28.77 and 30.33% of the initial residues, one and three days of flusilazole found on unwashed cucumber fruits, respectively.

The obtained results are in agreement with Nevein (2005) reported that washing process removed residues from 19.83% to 91.3% for flusilazole on apple fruits through the experimental periods. Also the obtained results are in agreement with those findings by Zidan *et al.* (1997) who reported that washing process proved to be a very effective method for decontamination of cucumbers.

Table (1): Persistence of flusilazole and diniconazole on and in unwashed and washed cucumber fruits grown under green house condition.

Time after application (days)	Flusilazole				Diniconazole			
	Unwashed		Washed		Unwashed		Washed	
	ppm	% Loss	ppm	% Loss	ppm	% Loss	ppm	% Loss
Initial*	1.560	0.000	1.274	18.33	0.680	0.000	0.610	10.24
1	1.293	17.11	0.908	28.77	0.580	14.76	0.315	48.28
3	1.024	34.34	0.633	30.33	0.425	35.96	0.150	52.39
5	0.838	46.41			0.291	57.16		
7	0.354	77.33			0.077	88.70		
14	0.095	93.88			0.066	90.30		
21	0.019	98.77			0.003	99.5		
28	UND**	-			UND	-		
t1/2	5.431				2.94			

* One hours after treatment. ** Undetectable

Diniconazole residues in cucumber fruits:

Data summarized in Table (1) represented that the initial deposits of diniconazole residues in unwashed cucumber fruits as determined

one hour after spraying were 0.68 ppm. The amounts of diniconazole residues were decreased with time to reach undetectable amounts 28 days of application.

Diniconazole half- life periods ($t_{1/2}$) of the unwashed cucumber fruits were 2.94 days.

These results are in the line with those reported by Attalla (2005), Nevein *et al.* (2004) and Nasr *et al.* (2003).

Data also indicated that washing process of the treated cucumber fruits with running tap water reduced diniconazole residues by 10.24, 48.28 and 52.39 % of the initial residues, one and three days after application, respectively. These above results are in agreement with El-Boze (2001) who reported that washing by running tap water removed 75.53% to 88.89 % of diniconazole residues from contaminated cucumber samples.

The maximum residual limit (MRL) was 0.5 mg/kg for diniconazole in cucumber fruits by CAC (1996 and 1997). Comparing this levels with the amounts of diniconazole residues found in cucumber fruits, it is clear that cucumber fruits could be safely used for human consumption after 3 days in case of unwashed fruits. While washed cucumber fruits could be used after 1 days of diniconazole application.

The results in agreement with these of Sole *et al.* (1973) who reported that small but definite percentage of systemic fungicide applied to leaves is transport to roots and shoots, via the phloem. Also, El-Bouze (2001) obtained that the degradation of diniconazole systemic fungicide was assumed to occur enzymically within the symplast of cucumber fruits.

Fenarimol residues in cucumber fruits:

Data shown in Table (2) demonstrate that the initial deposits on unwashed cucumber fruits one hour after application were 3.006 ppm, and then gradually decreased to 1.249 ppm one days of application revealing 58.42% loss. This value declined to 1.026, 0.365, 0.150 and 0.028 ppm recording the rate of loss 65.86, 87.87, 95.0 and 99.11 % at 3, 5, 7 and 14 days after treatment, respectively.

The calculated half-life value of fenarimol was 1.98 days on unwashed cucumber fruits.

The residues level of fenarimol in cucumber samples analyzed during this study were higher than MRL (0.5 mg/kg) established by

the Codex (2003) at the beginning of the study, and only five days after treatment values were below the MRL. This indicates that cucumber fruits could be safely used for human consumption after 5 days incase of unwashed fruits. The aforementioned results agree with the results of Nasr and Shereen (2005) who found that the rate of fenarimol residues gradually decreased as a function of time in cucumber fruits. Also, these obtained results are in agreement with those of Cabras *et al.* (1985) as they reported that fenarimol fungicide completely degraded in three weeks.

The effect of washing with tap water residue levels of fenarimol on cucumber fruits is shown in Table (2). Washing the contaminated cucumber fruits removed 13.83 % of the initial residues of fenarimol found on unwashed cucumber fruits. Washing of treated fruits collected after one and three days removed 30.47 and 33.43 %. The difference between the amounts of residues determined on and in cucumber samples of washed and unwashed fruits give an idea about the surface residues that could be removed by washing. These results are in agreement with those obtained and reported by El-Bouze (2001) and Nasr and Shereen (2005) who found that washing process removed residues from 40.62 to 73.68 % for fenarimol in cucumber fruits.

Table (2): Persistence of fenarimol and hexythiazox on and in unwashed and washed cucumber fruits grown under green house condition.

Time after application (days)	Fenarimol				Hexythiazox			
	Unwashed		Washed		Unwashed		Washed	
	ppm	% Loss	ppm	% Loss	ppm	% Loss	ppm	% Loss
Initial*	3.006	0.000	2.59	13.83	0.855	0.000	0.421	50.70
1	1.249	58.42	0.869	30.74	0.325	61.98	0.124	61.98
3	1.026	65.86	0.683	33.43	0.248	70.84	0.096	88.73
5	0.365	87.87			0.155	81.87		
7	0.150	95.0			0.055	93.56		
14	0.028	99.11			0.0379	95.56		
21	UND**	-			UND**	-		
28	UND**	-			UND**	-		
t1/2	1.981				1.951			

* One hour after treatment. ** Undetectable

Hexythiazox residues in cucumber fruits:

Results in Table (2) demonstrate the initial deposits and the residual behavior of hexythiazox on and in cucumber fruits after treatment.

The initial deposits revealed that the amounts of deposits depended on the nature of the treated surface and the relation between the surface treated and its weight (El-Sayed *et al.*, 1976).

The data indicated that the initial deposits of hexythiazox residues in and on cucumber fruits as determined one hour after application were 0.855 ppm. These values rapidly declined to 0.325, 0.248, 0.155, 0.055 and 0.0379 ppm recording the rate of loss 61.98, 70.84, 81.87, 93.56 and 95.56 % after 1, 3, 5, 7 and 14 days after treatment, respectively.

Also, the results showed that the calculated half lives value of hexythiazox was 1.951 days on unwashed cucumber fruits.

On the other hand, the washing process of the contaminated cucumber fruits with running tap water reduced hexythiazox residues by 50.7, 61.98 and 88.73 % of the initial residues, one and three days after application, respectively.

According to data of Table (1 and 2) and remarks of El-Kins (1989), and Tag El-din (1993) removal of pesticide residues by washing depends on several factors: character of the surface of plant food (smooth or youth, waxy or non waxy) surface to volume ratio washing is effective for bigger fruits; reference point of residue levels (higher levels easier to remove); chemical and physical properties of the applied pesticide; the length of time that the application and penetrability of pesticide into fruit tissues.

Generally, great interest to note the following remarks:

- 1- The residue amount in cucumber fruits sprayed with diniconazole was the lowest than that with flusilazole, fenarimol, and hexythiazox.
- 2- The effect of washing with tap water was more effective in case of hexythiazox treatment than other tested pesticide. This could be attributed to that hexythiazox is non systemic acaricide.

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ثبات متبقيات بعض المبيدات الفطرية والآكاروسية على وفي ثمار الخيار تحت ظروف الصوب الزراعيه

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في هذه الدراسة تم زراعة نبات الخيار تحت ظروف الصوب الزراعيه وقد تم رش النباتات بعد ٤٥ يوم من الزراعة بالمبيدات التالية :-
الداينكونازول (سومى ايت ٥% مركز قابل للاستحلاب) وذلك كمبيد موصى به للاستعمال على نبات الخيار.

والفلوسيلازول (بانث ٤٠% مركز قابل للاستحلاب) والفيناريمول (روبيجان ١٢% مركز قابل للاستحلاب) وهيكسيثيازوكس (ماكومايت ١٠% مسحوق قابل للبلل) وذلك كمبيدات غير موصى بها للاستعمال على نبات الخيار.

وقد وجدت المقارنة بين نتائج تحليل متبقيات المبيدات أن الداينكونازول كان اقلهم من حيث المتبقيات الأولية (ساعة واحدة بعد الرش) حيث كانت ٠,٦٨ جزء في المليون بينما كانت ٣,٠٠٦ ، ١,٥٦ و ٠,٨٥٥ جزء في المليون لكل من الفيناريمول والفلوسيلازول والهيكسيثيازوكس على الترتيب.

كما دلت النتائج أيضا أن فترة نصف العمر كانت ٥,٤٣١ و ٢,٩٤ و ١,٩٨ و ١,٩٥ يوم لكل من الفلوسيلازول و الداينكونازول والفيناريمول والهيكسيثيازوكس على الترتيب في ثمار الخيار الغير مغسولة.

ومن جهة اخرى فأن عملية الغسيل ادت الى ازالة متبقيات المبيدات بعد ساعة واحدة من الرش بمقدار ١٨,٣٣ و ١٠,٢٤ و ١٣,٨٣ و ٥٠,٧% لكل من الفلوسيلازول و الداينكونازول والفيناريمول والهيكسيثيازوكس على الترتيب وهذه النتائج تدل على أن عملية الغسيل بماء الصنبور كانت أكثر معنوية في نقص واختزال متبقيات المبيدات وكان مبيد الهيكسيثيازوكس اكثرهم اختزالا في ثمار الخيار المعاملة.