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RESIDUES OF IMIDACLOPRID AND TETRACONAZOLE ON AND IN CUCUMBER AND TOMATO FRUITS

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ABSTRACT

The residues of imidacloprid (Admire 20% SC) and tetraconazole (Domark 10% EC) on and in cucumber and tomato fruits were determined. Results confirmed that the initial deposit of tetraconazole on and in the cucumber fruits (0.125 ppm) was higher than on and in the tomato fruits (0.085 ppm). Data also indicated that the initial residue of imidacloprid on and in the tomato fruits (0.650 ppm) was higher than the initial deposit of tetraconazole on and in the tomato fruits (0.085 ppm).

In this experiment under normal field conditions, tetraconazole was more persistent on these vegetables (RL_{50} were 43.2 and 26.4 hours on cucumber and tomato fruits) than imidacloprid (RL_{50} was 16.4 hours on tomato fruits). The results also revealed that the first day following application imidacloprid is critical in the sense of sharply decrease that reach 72.3% from the initial deposit on tomato fruits, but the loss of tetraconazole at first day was 43.20% and 44.41% from the initial residues on cucumber and tomato fruits, respectively.

The estimated approximate waiting time (preharvest interval) for imidacloprid was 2 days following application on tomato plant. However, one day following application of tetraconazole on cucumber and tomato plants was enough to consum cucumber and tomato fruits safely.

Key words : Residues , Imidacloprid , Tetraconazole , Cucumber , Tomato

J.Agric.&Env.Sci.Alex.Univ.,Egypt Vol.5 (3)2006

INTRODUCTION

Cucumber plant *Cucumis sativus* and tomato plant *Lycoparsicon esculentum* are considered important vegetable crops. In Egypt, these vegetables infested by several pests causing serious quantitative and qualitative damages. Imidacloprid and tetraconazole are pesticides recommended for use on these vegetables to control the pests.

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-*N*nitroimidazolidin -2-ylidene-amine] belongs to a new chemical class of active ingredients, the chloronicotinyls, synthetic neonicotinoids. It has a new mode of action, outstanding biological efficacy, a broad spectrum of activities, low toxicity to warm-blooded animals and good plant compatibility. Because of its excellent systemic properties imidacloprid is used as seed dressing, as well as for foliar, soil and stem treatment. Since several imidacloprid metabolites have been shown to be equal or greater in toxicity than the parent compound, their presence in the environment should be studied and thus they should be included in chemical analysis of future environmental studies (Fossen, 2006). Tetraconazole [(*RS*)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl) propyl 1,1,2,2-tetrafluoroethyl ether] belongs to the azole group of chemicals and has low acute toxicity. Also is a broad-spectrum systemic fungicide with protective, curative and eradicant properties. The EPA (2005) concluded that there is not a concern for mutagenicity resulting from exposure to tetraconazole since it was not mutagenic in various mutagenic assays, but it was classified as "likely to be carcinogenic to humans" based on the occurrence of liver tumors in male and female mice. Tetraconazole is slightly toxic to mammals on an oral acute basis.

The present investigation aims to determine residues of imidacloprid on and in tomato fruits and tetraconazole on and in cucumber and tomato fruits, and to determine the preharvest intervals .

J.Agric.&Env.Sci.Alex.Univ.,Egypt Vol.5 (3)2006

MATERIALS AND METHODS

1. Experimental and pesticide treatments

The experiment work was conducted in Kalubia region at Kaha, under the normal field conditions and agricultural practice. Tomato (Jawaher) F_1-Hybrid and cucumber (Prince) F_1-Hybrid, seeding were carried out on March 3 and 24, 2003 and transplanted on April 23 and 19, 2003 on 56 and 36 rows (4 m x 25 cm) for tomato and cucumber plants, respectively.

Tomato plant at fruiting stage was sprayed with imidacloprid (Admire 20% SC), and cucumber and tomato plants at fruiting stage were treated with tetraconazole (Domark 10% EC) on June 14, 2003, at rates 125 ml, and 50 and 50 ml from the commercial products, each per 100 L of water (recommended dose), respectively, using a Knapsak sprayer equipped with one nozzle. Replicate samples, one Kg of tomato fruits were taken at intervals of one hour after application (zero time), 1, 2, 5, 8, 11, 15, 19, and 22 days after treatment with imidacloprid, also sampling one kg, each of cucumber and tomato fruits started about 1 h after spraying and were repeated 1, 3, 5, 8, 11, and 15 days after treatments with tetraconazole.

As soon as the fruits were picked up, they were put in polyethylene bags and transferred to the laboratory. The fruits were chopped and blended. The blending was thoroughly mixed, three sub-samples 50 g of each were weighted into 100 ml beakers, capped with aluminum foil and kept deep frozen until analysis.

2. Analytical procedures

A. Extraction

A.1. Imidacloprid pesticide:- Method of Blass (1990) was used for extraction of imidacloprid from tomato fruits with slight modification of substituting methanol instead of acetonitrile in extraction. Fifty grams of tomato fruits were blended at high speed for about 3 minutes with 200 ml methanol and filtered. After evaporation of 100 ml of the methanol extract by means of a vacuum rotary evaporator, the aqueous reminder was treated with 50 ml saturated sodium chloride solution and transferred into a separatory funnel and shaken vigorously with 100 ml n-hexane. Then the organic phase was discarded and the aqueous was shaken with 100, 50, and 50 ml methylene chloride in separatory funnel. The lower methylene chloride phases were

collected and subsequently dried over anhydrous sodium sulfate. Then, it was evaporated just to dryness using a rotary evaporator at 40°C.

A.2. Tetraconazole pesticide:- The method of Mollhoff (1975) was adopted for extraction of tetraconazole from cucumber and tomato fruits, methanol was used instead of acetone. Fifty gram samples, cucumber and tomato fruits were placed in the blender cup and a constant amount of methanol (2 ml/gram fruit) were added, then blended for three minutes and filtered. Extracts were shaken successively with 100, 50 and 50 ml of methylene chloride in separatory funnel after adding 40 ml of sodium chloride solution (20%); then the water phase discarded. The combined methylene chloride phases were dired by filtration through anhydrous sodium sulphate. Then, it was evaporated just to dryness using a rotary evaporator at 40°C.

B. Clean-up of extracts.

B.1. Imidacloprid extract:- The method of Blass (1990) was followed for cleaning-up of the extracted samples. The chromatographic column was prepared by adding 20 ml of HPLC-grade ethyl acetate to topped glass column (with a plug of glass wool) followed by 4.5 g of 5% deactivated florisil (60-100 mesh) as slurry in HPLC-grade ethyl acetate. The dry extract was dissolved in 5 ml of ethyl acetate and quantitatively transferred to the top of column. The column was eluted with 20 ml HPLC-grade ethyl acetate which was discarded. The imidacloprid was eluted from the column with 25 ml of HPLC-grade acetonitrile which was evaporated just to dryness as previously described and redissolved in an appropriate volume of HPLC-grade acetronitrile. Finally, the extract was filtered through a 13 mm, 0.45 μm nylon filter into a glass stopper test tube, then the residues were ready for analysis by HPLC.

B.2. Tetraconazole extract:- The residue was dissolved in 5 ml methanol and cleaned up according to Johnson (1963) using coagulating solution (0.5 g ammonium chloride and 1 ml 85% orthophosphoric acid solution in 400 ml distilled water). The residue was thoroughly mixed with 10 ml of cooled freshly prepared coagulating solution and the contents were quantitatively transferred and filtered through a chromatography column of 2.5 cm diameter packed with a 5 cm layer of Hyflo-super cell. Transfer was repeated

three times using 5 ml methanol and 10 ml coagulating solution each. The filtrate was then collected together in 250 ml separating funnel and extracted with 3 x 50 ml methylene chloride. The extracts were collected and evaporated just to dryness as previously described and residues were ready for GC determination after redissolved in an appropriate volume of ethyl acetate.

C. Chromatography determination.

C.1. High pressure liquid chromatography determination:- Imidacloprid was detected and determined using an Agilent Technologies Series 1100 HPLC system equipped with workstation. UV-Photodiode array detector set at 270 nm and the analytical column Zorbax-C18, 5 μm (4.6 x 150 mm) was used. The mobile phases were acetonitrile-water at flow rate 0.8 ml/min gradient as follows:-

Time in min	Acetonitrile	Water
0	35	65
10	40	60
15	40	60
20	35	65

The injection volume was 20 μl.

C.2. Gas liquid chromatography determination:- GC-systems-HP 6890 Series equipped with an electron capture detector (ECD) and workstation was used to determine tetraconazole. The capillary column HP-PAS5, 25 m x 0.32 mm, with 0.25 μm film thickness (5% phenyl) – methyl polysiloxane. Operating conditions: nitrogen carrier gas 4 ml/min and temperature degrees were 250^{o}C for injection port, 220^{o}C for column, and 300^{o}C for detector.

Results were corrected according to the rates of recovery, which were determined in fortified untreated samples at levels ranged from 0.1 ppm to 1 ppm. Following the techniques previously mentioned, the rates of recovery for imidacloprid was 75.02% in tomato fruits and for tetraconazole were 93.74% and 92.02% in cucumber and tomato fruits, respectively.

J.Agric.&Env.Sci.Alex.Univ.,Egypt Vol.5 (3)2006

RESULTS AND DISCUSSION

Data shown in Table (1) demonstrate the initial deposit and the residual behaviour of imidacloprid on and in tomato fruits after spraying. These data demonstrated that the initial deposit found in and on tomato fruits as determined one hour after application was 0.650 ppm. The amount of residue was decreased sharply to 0.180 ppm with loss 72.31% within the first day after spraying. This figure decreased gradually until reached 0.004 ppm on and in tomato fruits with loss 99.38% of the initial residue, after 22 days of application. It was also noticed that the calculated residue half-life value (RL_{50}) of imidacloprid on tomato fruits was 16.4 hours.

The results presented in Table (2) indicated that the initial deposits of tetraconazole on and in cucumber and tomato fruits were 0.125 and 0.085 ppm, respectively one hour after application. Samples of cucumber and tomato fruits taken one day after spraying contained 0.071 and 0.043 ppm with loss 43.20% and 44.41% of the initial amounts of tetraconazole, respectively. Between days 1 and 15 after spraying a gradual decrease was observed in residues for tetraconazole which reached N.D and 0.002 ppm in cucumber and tomato fruits, 15 days after treatment, respectively. The estimated residue half-life values (RL_{50}) of tetraconazole were 43.2 and 26.4 hours on cucumber and tomato fruits, respectively.

Data summarized in Tables (1 and 2) showed that imidacloprid and tetraconazole had low initial residues in these vegetables. However, this value was higher than the Codex MRL in case imidacloprid, but were lower than the Codex MRL in case tetraconazole. The maximum residue limits (MRLs) of imidacloprid in tomato fruits was 0.2 ppm and for tetraconazole were 0.2 and 0.2 ppm in cucumber and tomato fruits (Codex Alimentarius Commission, 2003). Accordingly, the approximate waiting time value (preharvest interval) for imidacloprid was 2 days following application on tomato plant. However, one day following application of tetraconazole on

cucumber and tomato plants was enough to consum cucumber and tomato fruits safely.

The present study confirmed that the initial deposit of tetraconazole on the cucumber fruits was higher than on the tomato fruits, this variation probably relates to their different surface to weight ratios and perhaps, different surface properties. Data also indicated that the initial residue of imidacloprid on the tomato fruits was higher than the initial deposit of tetraconazole on the tomato fruits, such difference could be attributed to the higher rate of application of imidacloprid 125 ml (i.e. 25 g a.i.)/100 L water than tetraconazole 50 ml (i.e. 5 g a.i.)/100 L water. In this respect, El-Sayed *et al*. (1976) stated that the amounts of deposits depended on the rate of application, the nature of the treated surface and the relation between the surface treated and its weight.

On the other hand, the different levels of initial deposits of both tested pesticides on fruits of cucumber and tomato mainly due to many factors; the ratio of surface to mass area and character of treated surface (smooth or rough and waxy or non-waxy) (Abo El-Ghar and Ramadan, 1962); systemic and non-systemic character of both compounds, high wax content of fruit surface and hydrophilic-lipophilic balance of investigated pesticides controlled the penetrability of applied agrochemicals into fruit tissues (Polen, 1971 and Cabras *et al*., 1988).

Degradation and dissipation residues of imidacloprid and tetraconazole from these vegetable fruits happened because the initial deposits and residues at different intervals of these pesticides are influenced by different factors: evaporation of the surface residue which is dependent on temperature condition, biological dilution which is dependent on the increase mass of fruits, chemical or biochemical decomposition, metabolism and photolysis. Great interest to note that the same factors were studied by several investigators, Christensen (2004) reported that the decline of pesticides may due to biological, chemical or physical processes, or if still in the field, due to dilution by growth of the crop. Plant growth, particularly for fruits is also responsible to a great extent for decreasing the pesticide residue concentrations due to growth dilution effects (Walgenbach *et al*., 1991). In addition, the rapid dissipation of originally applied pesticide are dependent on a variety of environmental factors such as sunlight

and temperature (Lichtenstenin, 1972). However, high temperature is reported to the major factor in reducing the pesticides from plant surfaces (Awad *et al*., 1967). Light plays an important role in the behaviour of pesticide in the environment (Zepp and Cline, 1977).

The above results seem to show that tetraconazole has high persistence on these vegetables (RL_{50} were 43.2 and 26.4 hours on cucumber and tomato fruits) than imidacloprid (RL_{50} was 16.4 hours on tomato fruits). The data also revealed that the first day following application is critical in the sense of sharply decrease that reach 72.31% from the initial deposit of imidacloprid on tomato fruits, but the loss of tetraconazole at first day were 43.20% and 44.41% from the initial residues on cucumber and tomato fruits, respectively. In this respect, several investigation have been carried out to examine the residual behaviour of these pesticides on treated plants. Fossen (2006) reported that imidacloprid is rapidly moved through plant tissues after application, and can be present in detectable concentrations in tissues such as leaves, vascular fluids, and pollen. Imidacloprid is a systemic pesticide with physical/chemical properties that allow residues to move into treated plants and then throughout the plant via xylem transport and translamilar (between leaf surfaces) movement (Buchholz and Nauen, 2002). Miles, Inc. (1993) found that the main breakdown products of imidacloprid in plants are a monohydroxy metabolite, imidacloprid guanidine, imidacloprid olefin and monoglucoside of 6-chloropicolyl alcohol. Khalfallah *et al*. (1998) studied dissipation of tetraconazole on greenhouse-grown cucumber, and pointed out that its dissipation could mainly be attributed to degradation by chemical and physical properties and less by growth dilution effects when the cucumber plants were almost mature. Also, Federal Register (1999) treated sugar beet plants in an outdoor field with tetraconazole labeled twice more at 21-day intervals, and reported that the total radioactive residue (TRR) in the root was always < 0.01 ppm, but in the leaf were 1.6, 1.9, 3.1 and 1.3 ppm, at days 0, 20, 41 and 76 after first treatment. As a group, the triazoles have short residual periods, under a 20 days application schedule they may be dissipated from the plant tissue, unless high rates are used (Miles *et al*., 2004).

Table (1): Residues of imidacloprid on and in tomato fruits.

Time after application (days)	Residues	
	ppm	% Loss
Zero time*	0.650	00.00
1	0.180	72.31
3	0.160	75.38
5	0.120	81.54
8	0.080	87.69
11	0.050	92.31
15	0.030	95.38
19	0.020	96.92
22	0.004	99.38
RL_{50} in hours**	16.4	
MRL (ppm)***	0.2	
Approximate waiting time (days)	2	

* One hour after application.

** Calculated from persistence curve

*** According to Codex Alimentarius Commission (2003)

Table (2): Residues of tetraconazole on and in cucumber and tomato fruits.

Time after application (days)	Cucumber fruits		Tomato fruits	
	Residues		Residues	
	ppm	% Loss	ppm	% Loss
Zero time*	0.125	00.00	0.085	00.00
1	0.071	43.20	0.043	44.41
3	0.048	61.60	0.028	67.05
5	0.012	90.40	0.008	90.58
8	0.004	96.80	0.007	91.76
11	0.001	99.20	0.004	95.29
15	ND**	–	0.002	97.64
RL_{50} in hours***	43.2		26.4	
MRL (ppm)****	0.2		0.2	
Approximate waiting time (days)	1		1	

* One hour after application.
** Not detectable.
***Calculated from persistence curve
**** According to Codex Alimentarius Commission (2003)

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الملخص العربي

متبقيات مبيدي الايميدا كلوبريد والتتراكونازول على وفي ثمار الخيار والطماطم

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تم معامله نباتات الطماطم (صنف جواهر) بمبيدي الايميداكلوبريد (أدمير) والتتراكونازول (دومارك) ونباتات الخيار (صنف برنس) بمبيد التتراكونازول . حيث أكدت الدراسة أن كمية المتبقيات الأولية لمبيد التتراكونازول على وفي ثمار الخيار كانت 0.125 جزء في المليون حيث كانت أعلى من كمية المتبقيات الأولية لتلك المبيد على ثمار الطماطم التي كانت 0.085 جزء في المليون . وأكدت النتائج أيضاً أن المتبقيات الأولية لمبيد الأيميداكلوبريد على وفي ثمار الطماطم كانت 0.650 جزء في المليون وكانت أيضاً أعلى من المتبقيات الأولية لمبيد التتراكونازول على وفي ثمار الطماطم التي كانت 0.085 جزء في المليون .

في هذه التجربة تحت الظروف الحقلية العادية أظهر مبيد التتراكونازول ثباتاً اعلى نسبياً من مبيد الأيميداكلوبريد ، حيث كانت فترات نصف العمر لمبيد التتراكونازول 43.2 ساعة و 26.4 ساعة على ثمار الخيار والطماطم على التوالي ، وكانت فترة نصف العمر لمبيد الأيميداكلوبريد على ثمار الطماطم 16.4 ساعة .

أظهرت النتائج أيضا حدوثً فقد وتدهور متبقيات هذين المبيدين عند كل الفترات التي أخذت عندها العينات ، حيث وصل هذا التدهور بعد يوم واحد من المعامله بمبيد الأيميداكلوبريد على ثمار الطماطم إلى72.31 % وأيضاً كان الفاقد بعد يوم واحد من المعامله بمبيد التتراكونازول على ثمار الخيار والطماطم 43.20% و 44.41% على التوالي .

طبقاً للحد المسموح به من قبل لجنة دستور الأغذية والزراعه (الكودكس) لمبيدي الأيميداكلوبريد والتتراكونازول على ثمار الخيار والطماطم ، فيمكن أعتبار فترة الأمان لمبيد الأيميداكلوبريد على ثمار الطماطم يومان من المعامله ، وأيضاً يوم واحد بعد المعامله بمبيد التتراكونازول كان كافياً لاستهلاك ثمار الخيار والطماطم بأمان .