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Residues and Dissipation of Imidacloprid, Oxamyl, and Emamectin Benzoate in Greenhouse Grown Tomato Fruits and Their Influence on Lycopene Content

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ABSTRACT

The dissipation of oxamyl, emamectin benzoate, and imidacloprid pesticide residues in tomato fruits and their effect on its lycopene content were evaluated. The tested pesticides were applied according to the recommendation of the Egyptian Ministry of Agriculture for emamectin benzoate (Pasha EC 1.9%, 250 cm^3/fedan), imidacloprid (Confidente SC 35%, 75 cm^3/100L), and oxamyl (Vydate SL 24%, 3L/fedan). Pesticide residues in tomato fruits were analyzed using recently developed QuEChERS method coupled with HPLC. Recovery assays confirmed the validity and efficiency of QuEChERS with 101, 93, and 110% recovery for oxamyl, emamectin benzoate, and imidacloprid, respectively. The half-life ($t_{1/2}$) values for oxamyl, emamectin benzoate, and imidacloprid in tomato fruits were 2.88, 3.12, and 3.46 days, respectively. After 12 days from application, the residues of oxamyl, emamectin benzoate, and imidacloprid in tomato fruits reached 1.0, 0.09, and 0.23 mg/kg, respectively. However, except imidacloprid, these residue values of oxamyl and emamectin benzoate were still above the maximal residue limit (MRL). In addition, the cumulative effects of imidacloprid and emamectin benzoate have significant inhibitory effects on the lycopene content compared to untreated tomato fruits.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is the second most important vegetable crop worldwide. Egypt is the main tomato producer in Africa. Lycopene is one of the most natural potent antioxidants among dietary carotenoids in tomatoes (Takeoka,*et al* 2001 and Garcia and Barrett, 2005).

The increased use of agrochemicals has resulted in many associated short- and long term human health risks (Chen *et al*., 2011). Three insecticides representing three classes of pesticides were investigated for their residues in tomato fruits. Imidacloprid [1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine] is one of the Neonicotinoids class of pesticides widely used in agriculture due to their broad-spectrum activity, low bioaccumulation potential, and relative immobility in soil (U.S.-EPA, 2004). It is used for control of sucking insects such as rice hoppers, aphids, ticks, white flies, termites, and turf insects. It is commonly used on rice, soya beans, maize, potatoes, cotton, sugar beets, and kitchen garden vegetables and fruits (Arora, 2009). Emamectin benzoate as an avermectin class insecticide developed originally produced by streptomyces microorganisms for the control of lepidopteron insects. It penetrates the leaf tissue and forms reservoir within treated leaves, which provides residual activity against pests that ingest the substance when feeding. Emamectin benzoate has been widely applied as insecticide on a variety of vegetables and fruits (Xie *et al*., 2011). Oxamyl, (N,N-dimethylcarbamoyloxyimino-2-(methylthio)acetamide) is an important carbamoyloxime insecticide and nematicide. Oxamyl has been found to act widely on different organisms. When applied as a foliar spray, oxamyl works as a contact-type and was found to be moderately persistent insecticide and miticide. Oxamyl functions in soils as a systemic miticide/insecticide as well as a broad spectrum nematicide (Gyula and McMaster, 1987).

There are different methods for pesticides residual extraction and analysis. Anastassiades *et al*.(2003) described a "quick, easy, cheap, effective, rugged, and safe" (QuEChERS) method for pesticides analysis in fruits and vegetables. QuEChERS approach was chosen

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in this study for pesticides extraction coupled with HPLC for pesticide residue analysis.

The objectives of this study are 1) to determine the residues of imidacloprid, oxamyl, and ememectin benzoate in emerging fruits of greenhouse grown tomato and 2) to evaluate the side effect of the tested pesticides on the lycopene content of tomato fruits.

MATERIALS AND METHODS

A greenhouse experiment has been conducted at Faculty of Agriculture, University of Alexandria, Egypt. A sandy loam soil used in the experiment was brought from the college farm in Abis and was air-dried and sieved (< 2mm). Seeds of tomato *Lycopersicon esculentum* [company: Samtrade variety: Advantage 2, 91% germination & 99% purity] were sown in black plastic germination trays. Three-week old seedlings were transferred later to plastic pots (22 cm diameter * 15 cm height) containing 8.5 Kg of soil. The moisture content of the soil was maintained at 65% of its water holding capacity. One month later, plants were foliar sprayed with tested pesticides by a hand sprayer. The commercial formulations of the tested pesticides were applied according to the recommendations of the Egyptian Ministry of Agriculture for emamectin benzoate (Pasha EC 1.9%, 250 cm^3/fedan), imidaclopried (Confidente SC 35%, 75 cm^3/100L), and oxamyl (Vydate SL 24%, 3L/fedan). Pesticides were sprayed on plants 3 times during the experimental period. the first spray was two weeks after the transfer of seedlings and the second was at the beginning of the coloring of fruits after one month from the first one and the last one was immediately before the first harvest after another month from the second application.

Sample preparation and residues extraction

Tomato fruits were collected randomly at 0, 3, 6, 9, and 12 days after spraying the tested pesticides. Thoroughly homogenized sample (10 g) was weighed into a 50 mL Falcon polypropylene centrifuge tubes. 10 mL acetonitrile was added and the tubes were shaken vigorously for 1 min with vortex mixer. Anhydrous NaCl (1g) and anhydrous $MgSO_4$ (4g) were added into the mixture and shaken for 1 min. After centrifugation at 3800 rpm for 5min, 1mL of the clarified supernatant was pipette into a 2.0 mL microcentrifuge tube containing 10 mg active charcoal, 50 mg Primary Secondary Amine (PSA) (Sigma-Aldrich, USA), and 150 mg $MgSO_4$. The mixture was shaken vigorously for 1 min and centrifuged for 5 min at 3800 rpm. Finally, 0.5 mL of the supernatant extract was taken to carry out the HPLC analysis as described below.

HPLC operating condition

HPLC analysis was carried out with special program for each pesticide. Imidacloprid was analyzed by Agilent 1260 Infinity HPLC system with HP 1260 Diode array detector at 25°C and 270 nm. The column used was eclipse plus C18 phase of 3.5 µm particles, 100 mm length, and 4.6 mm diameter. An exact 20 µl sample volume was injected with mobile phase of 70/30 HPLC grade acetonitrile and water at flow rate of 1 mL/min. Under these conditions, imidacloprid retention time has been found to be 2.43 min. The same system was used for oxamyl residue analysis at 200 nm with 80% HPLC grade methanol and 20% HPLC grade water. Oxamyl retention time was found to be 1.03 min. For emamectin benzoate, the same system was used but at 242 nm and 90/10 HPLC grade methanol and water. Retention time for emamectin benzoate was observed at 5.167 min.

Spiking and Recovery Assay

Untreated tomato fruits were used for recovery efficiency of used QuEChERS method. Samples were spiked with known amount of each pesticide, mixed, left for 2 hrs to achieve equilibrium. The tested pesticides residues were extracted and determined by HPLC as described above. Recovered amount of every pesticide was calculated as a percentage relative to the total initially spiked amount.

Spectrophotometric determination of lycopene

Tomato fruits samples were taken randomly at 7 days after the third spray to estimate the commutative effect of the tested pesticides on the lycopene content. Lycopene content in tomato was extracted with hexane/ethanol/acetone (2:1:1) and measured at 503 nm by spectrophotometer (Laxco) following Fish *et al*.(2002).

Statistical analysis

The degradation rate constant and half-life were calculated using the first order rate equation: $C_t = C_0^e\text{-}k^t$, where C_t represents the concentration of the pesticide residue at the time of t, C_0 represents the initial concentration after application, and k is the dissipation degradation rate constant ($days^{-1}$). The half-life ($t_{1/2}$) was calculated from the k value for each experiment ($t_{1/2}$ = ln2/k) according to Juraske *et al*., (2008), Hassanzadeh *et al*. (2012) and Wang *et al*.(2012).

RESULTS AND DISCUSSION

Recovery efficiency:

The recovery data of the QuEChERS for oxamyl, emamectin benzoate, and imidacloprid were found to be very comparable to those of other studies. For oxamyl, 101% recovery was obtained which is in agreement with previously reported studies (Lehotay *et al*., 2005; Glauner, 2012 and Shaderma *et al*. 2013). Glauner (2012) achieved 103.3% recovery for oxamyl from tomato fruits samples. Similarly, 92.8%- 94.3% (Shaderma *et al*., 2013) and > 90% (Lehotay *et al.,* 2005) were achieved for oxamyl residues in different commodities. For emamectin benzoate recovery, 93% recovery was achieved in this study in an agreement with the findings of Wang *et al*. (2012) for cabbage (85.7-96%) and apple (75.93- 95%). Following the same trend, using QuEChERS this study achieved 110% recovery for imidacloprid in tomato fruits samples. Rembiałkowska and Badowski (2011) achieved 70 to 110 % recoveries for more than 250 pesticides from various matrices using QuEChERS. Results of this study confirmed the validity of QuEChERS as a highly efficient approach for pesticide residues extraction and as such were used throughout the study.

Dissipation of oxamyl, imidacloprid, and emamectin benzoate

Pesticide residues in emerged tomato fruits obtained in the dissipation study of oxamyl, imidacloprid, and emamectin benzoate after foliar application are presented in Figure. The initial applied concentrations of oxamyl. imidacloprid, and emamectin benzoate were 25.0, 2.54, and 0.98 mg/kg in tomato fruits, respectively. The dissipation data of this study showed a gradual and continuous decrease of the pesticide residues. Data showed that about 60% and 56% of the initial amounts of oxamyl and imidacloprid were lost through the first three days, while only 38% of emamectin benzoate was dissipated within the same period. At 12th day of foliar application, residues of oxamyl, ememactin benzoate, and imidacloprid have decreased to 1.0, 0.09, and 0.23 mg/kg, respectively representing about 77- 96.5% dissipation. The data fitted first-order decay as presented in Table 1.

Estimation of half-lives of tested pesticides in tomato fruits

Results of this study (Table 1) showed the calculated half-lives of oxamyl, emamectin benzoate, and imidacloprid as 2.88, 3.12, and 3.46 day, respectively. The imidacloprid half-life value was found to be comparable to its experimental values reported by others. The half-life ($t_{1/2}$) values of imidacloprid was reported as8.2 days in tomato fruits (Juraske *et al*., 2007), 2.5 days in tomato fruits (Romeh *et al*., 2009), 3.5 days in cucumber (Hassanzadeh *et al*., 2012) and 2.75 for strawberry (Hendawi *et al.,* 2013).

Badawy *et al*., (2009) found that the calculated half life times of oxamyl alone or in mixture treatments were 2.79 and 2.9 days. For emamectin benzoate, Liu *et al*., (2012) reported 1 day half-life ($T_{1/2}$) of emamectin benzoate. However, Li *et al*.,(2011)found that the half-life range values of emamectin benzoate in paddy plants, water, and soil were 2.04–8.66, 2.89–4.95, and 3.65–5.78 days, respectively with a dissipation rate of 90% over 7 days after application**.** The half-life values of emamectin benzoate in cabbage, apple, and soil were found to be 1.34–1.72, 2.75–3.09, and 1.89–4.89 days, respectively. Therefore, the ($t_{1/2}$) for emamectin benzoate in tomato fruits is within others reported values. Malhat *et al*. (2013) has determined the $t_{1/2}$ of emamectin benzoate was approximately 2.5 days.

Regarding tested pesticides in this study, MRL values set by both EU pesticides database and FAO/WHO CODEX are identical for emamectin benzoate (0.02 mg/kg), oxamyl (0.02 mg/kg), and imidacloprid (0.5 mg/kg). The residue values of oxamyl and emamectin benzoate were still above the maximal residue limit (MRL). Assuming that the typical time of tomato consumption is between 7 and 12 days for imidacloprid application, it is unlikely to pose any health risk to humans. However, regarding oxamyl and Emamectin benzoate, its concentration at 12 days was still above the EU and FAO MRL value. Although this is a greenhouse study that would be helpful for the Egyptian Ministry of Agriculture, field experiments are greatly needed to establish the MRL of emamectin benzoate, oxamyl, and imidacloprid and to provide guidance on the proper and safe use of these insecticides for fresh produce.

Commutative effect of tested pesticides on tomato lycopene content:

For the validity of method followed, Sharma and Le Maguer (1996) and Rao *et al*. (1998) demonstrated that lycopene can be accurately estimated by either high-performance liquid chromatography or spectrophotometer method with no significant difference.

Table 1. The half-life and other statistical parameters for oxamyl, emamectin benzoate and imidacloprid in tomato fruits

Pesticide	Regression equation	Determination Coefficient(R^2)	Determination Constant (day^{-1})	Half-life (days)
Oxamyl	$y = 26.586e^{-0.24x}$	0.901	0.24	2.88
Emamectin benzoate	$y = 0.8964e^{-0.222x}$	0.803	0.222	3.12
Imidacloprid	$y = 2.2253e^{-0.194x}$	0.966	0.194	3.46

Table 2. Lycopene content (mg/kg) of tomato fruits affected by the investigated pesticides

Treatments			
Control	**Oxamyl**	**Imidacloprid**	**Emamectin benzoate**
76[a]	60[ab]	49[bc]	38[c]
± 2.3	±7.3	±6.5	±4.2

LSD 0.05 = 16.676
-Means indicated by same letters are not significantly different at 0.05 level probability.
-Average of 3 replicates ±Standard deviation (SD)
-The statistical analysis has been done by using one way complete randomized WCR by using CoStatW

Pesticides were sprayed on plants 3 times during the experimental period. The first spray was two weeks after the transfer of seedlings and the second was at the beginning of the coloring of fruits after one month from the first one and the last one was after another month from the second application. The samples has been taken after 7 days from the last pesticides application, Lycopene content data after three applications of pesticides is presented in Table (2).

The results of the present study indicated that imidacloprid and emamectin benzoate treated tomato fruits have a significantly less lycopene content than untreated tomato fruits. Emamectin benzoate negative effect on tomato lycopene content showing that it was the strongest one among tested pesticides. However, imidacloprid was more inhibitory effect on lycopene content than emamectin benzoate compared with control (Alsahaty, 2014). Data showed no significant difference between oxamyl-treated and untreated tomato. Few worldwide studies reported contradictory effects of pesticides on lycopene content in tomato fruits. It has been found that Ethephon as plant regulator significantly decreased the lycopene content of tomato (Xiao *et al*., 2008). Similarly, in a field study, Piao *et al*. (2012) reported that emamectin benzoate had reduced the carotenoid content of cotton, chinese cabbage, and radish leaves. Kandil *et al*. (2008) found that the application of tomato seedlings with Vydate (oxamyl) led to a highly significant decrease in chlorophyll and carotene contents of leaves. On the other hand, Indirani *et al.,* (2001) indicated that the tomato quality parameters such as soluble solids, titratable acidity, ascorbic acid, total sugars, and lycopene content were not affected by dazomet fungicide applied to soil. Dominguez *et al*. (2012) concluded that different fungicides may have different potential for the preservation of tomato quality, but the responses depend on the cultivar.

It was concluded that the have-lives found to be 3.46, 3.12 and 2.88 days for imidacloprid, emamectin benzoate and oxamyl on the tomatoes under greenhouse conditions.

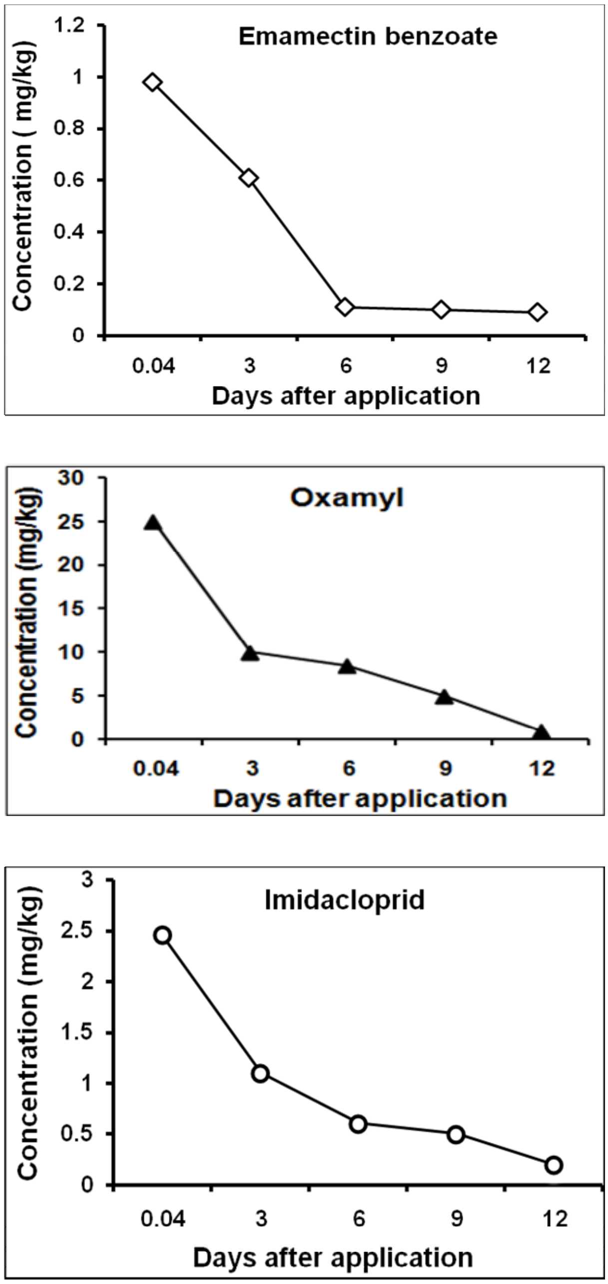

Figure 1. Dissipation curves of imidacloprid, emamectin benzoate and oxamyl in tomato fruits

At 12th day after treatment, the residue of imidacloprid reached below its MRLs. The commutative effects of imidaclprid and emamectin benzoate have significant while that of oxamyl has non-significant inhibitory effect on the lycopene content compared to the untreated tomatoes.

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الملخص العربي

متبقيات واختفاء كل من الاوكساميل والايميداكلوبريد والايمامكتين بنزوات على ثمار الطماطم النامية فى صوبه زراعية وتأثير هذه المبيدات على محتوى الثمار من الليكوبين

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تم دراسه اختفاء كل من الاوكساميل، ايماميكتين بنزوات والايميداكلوبريد على ثمار الطماطم والتى تم معاملتها بهذه المبيدات سالفه الذكر حسب الجرعات الموصى بها من قبل وزارة الزراعة المصرية وقد تم تقدير معدل الإختفاء وفتره نصف العمر لكل مركب وكذلك التأثيرات الجانبية لهذه المبيدات على تركيز الليكوبين فى ثمار الطماطم. تم استخدام طريقة QuECHERS للاستخلاص والتنقيه وتم التقديربواسطه HPLC. ولتوثيق هذه الطريقه تم حساب نسبه الاسترجاع والتى كانت تتراوح بين ٩٣-١١٠% لثلاث مبيدات. اتضح من الدراسة أن قيمه نصف العمر للاوكساميل والايماميكتين بنزوات والايميداكلوبريد كانت ٢,٨٨ و٣,١٢ و٣,٤٦ يوم على التوالى. وبعد ١٢ يوم من المعامله بالمبيدات السابقه، كان تركيز المبيدات ١ و ٠,٠٩ و٠,٢٣ ملجرام/كجم على التوالى. وقد لوحظ ان متبقيات كل من الاوكساميل وايمابكتين بنزوات المتواجده بعد اليوم الثانى عشر من المعامله مازالت أعلى من الحدود المسموح بها تبعا للمنظمات العالمية. ولقد اوضحت هذه الدراسه ايضا التأثير السلبى لكلا من الايميداكلوبريد والايمامكتين بنزوات على محتوى الليكوبين فى ثمار الطماطم بينما لم يكن للاوكساميل تأثير معنوى.