ALEXANDRIA
SCIENCE EXCHANGE JOURNAL
VOL. 36 OCTOBER- DECEMBER 2015

Dissipation of Penconazole and Imidacloprid Residues in Squash Fruits under The Egyptian Field Conditions

Hend M. Abdella[1], Safaa M. Halawa[2] and Dalia E. El-Hefny[1]

ABSTRACT

The dissipation and residual levels of penconazole and imidacloprid in squash fruit under field condition were determined by using GC-ECD and HPLC-DAD with QuEChERS method. The dissipation half-life time of penconazole and imidacloprid residues in squash fruits were 1.95 and 1.93 days, respectively. According to maximum residue limit (MRL). The pre harvest interval (PHI) of penconazole and imidacloprid were 10 and 7 days after application for squash fruits, respectively. This suggested that the use of squash fruits treated with these pesticides were safe for consumption. This study might be useful to prevent health problem from consumers.

Key words: Penconazole, imidacloprid, squash, dissipation, residues.

INTRODUCTION

Insect pests are major challenge to open field vegetables production all over the world. These damaging pests are important because symptoms of feeding often go unnoticed until serious damage has occurred (Daughtrey *et al.,* 1997). The controls of such insects are mainly relying on the use of chemicals pesticides (Prabhaker *et al.,* 1985). The extensive use of synthetic organic pesticides for this purpose has ineitably been followed by many problems. One of the most problems is a remaining residue in vegetables and fruits especially with highly stable and persistent insecticides. It could cause a health hazard to the ultimate consumers, particularly when the fruits freshly consumed. (AL-Eed, 2006). Penconazole was effective in controlling a broad spectrum of fungi diseases; it is a systemic triazole fungicide with protective and curative actions. It is recommended under commercial formulation topas 10% EC for controlling powdery mildew in squash vegetables.

Imidacloprid is recommended in Egypt for controlling insects belongs to the order of Hemiptera, especially aphids and white fly under commercial formulation mallet 35% SC.

The QuEChERS method covers a very wide analyte scope such as highly polar pesticides, and highly acidic and basic ones. This method involves extraction with acetonitrile and partitioning after the addition of a salt mixture. The extract in acetonitrile could be directly injected in GC and HPLC. The maximum residue limits (MRL) regulations require a pre-harvest interval (PHI) to ensure the dissipation of pesticide below the proposed MRL at harvest time (Karmakar & Kulhestha, 2009). Therefore, to ensure food safety and protecting the environment field dissipation studies on pesticide persistence in food stuffs and pesticide residue behavior in agricultural fields are needed.

The aim of this study was to investigate the dissipation rate of penconazole and imidacloprid up on application in squash fruits under field conditions so as to provide basic information for developing regulation regarding the safe use of penconazole and imidacloprid in pest management strategies and to protect the environment and public health.

MATERIALS AND METHODS

1. Materials:-

The certified reference standard of penconazole(1-[2-(2,4-Dichlorophenyl)pentyl]-1H-1,2,4-triazole) and imidacloprid(N-[1-[(6-chloropyridin-3-yl)methyl]-4,5-dihydroimidazol-2-yl]nitramide) were provided from central agricultural pesticide laboratory, Egypt, and were of ≥ 97% purity for both pesticides. All organic solvents were of HPLC grade and were purchased from Merck (Darstadt, Germany). Primary secondary amine (PSA, 40 μm Bondesil) and graphite carbon black (GCB) sorbents were purchased from Supelco (Supelco, Bellefone, USA). Anhydrous magnesium sulfate was of analytical grade and purchased from Merck Ltd. Sodium chloride is of analytical grade and was

[1]Central Agricultural Pesticides Laboratory. Agricultural Research Center,Cairo, Egypt
[2] Plant Protection Department, Faculty of Agriculture, Benha University, Cairo Egypt.
Received September 20, 2015, Accepted October 08, 2015

purchased from El-Nasr Pharmaceutical Chemical Co. (Egypt). Anhydrous magnesium sulfate and sodium chloride were activated by heating at 400ºC for 4h in the oven before use and kept in desiccators. Penconazole and imidacloprid stock standard solution (100 mg/L) were prepared in ethyl acetate and acetonitrile, respectively and stored at -20ºC. Working standard solutions were prepared daily by diluting the stock solutions.

2. Field Experiment:-

The field experiment was conducted at Qalyoubia Governorate, Egypt. Squash plants at fruiting stage were sprayed with Topas (10% EC) and Mallet (35 % SC) on September 7th 2014, at the recommended dose 25cm^3/100 L. water and 30cm^3/100 L. water from the commercial products, respectively. Using knapsack hand sprayer fitted with one nozzle. Also there some squash plants untreated was left to serve as control. There was no rainfall at any time during the experimental period.

For residue analysis, squash fruit samples (1 Kg) from each replicate were collected on one hour (zero time), 1, 3,7,10 and 15 days after application.

As soon as the fruits were picked up, they were put in polyethylene bags and transferred to the laboratory for extraction, cleaning and persistence analysis was carried out by triplicate. The samples were chopped and blended. The sub-samples (10 g) of each were then placed into a 50 ml Teflon centrifuge tube until analysis time.

3. Analytical procedures:

A 10 g sample was analyzed with 10 mL acetonitrile and the mixture was vortexed immediately for 1 min. Magnesium sulfate (4 g) and sodium chloride (1 g) were add to the tube, the mixture was vortexed for another 1 min, and the extract was centrifuged for 5 min. at 4000 rpm in 4ºC using a cooling centrifuge.

An aliquot of 2 ml was transferred to a 15 ml centrifuge tube containing 50 mg PSA, 300 mg $MgSO_4$ and 20 mg GCB, followed by vortexing for 1 min. and afterwards, centrifugation was carried out as mentioned above. Then aliquot of 2 mL of supernatant were taken and filtered through 0.45 µm PTFE filter (Millipore, USA). The sample was then ready for GC and HPLC analysis. (Nguyen *et al.,* 2008).

4. Determination of pesticides residue:

Penconazole residues were determined by a Hewlett-Packard series 6890 plus gas chromatograph (GLC), equipped with electron capture detector (ECD). The carrier gas was nitrogen at a flow rate of 3 ml/min .The capillary column was PAS 5, (25m x 0.32mm x 0.25 µm film thicknesses), the injection port temperature was 280 °C, the oven temperature was programmed at 200 ºC for 2 min., and raised to 280 ºC at the rate of 5ºC /min. The detector temperature was 300 °C. The retention time was 5.17 min.

Imidacloprid residues were determined by Agilent 1100 HPLC system, with photodiode array detector. The chromatographic column was C_8 zorbax SB (250mm x 4.6mm, 5 um film thickness). Flow rate of mobile phase (acetonitrile / water = 60/40, v/v) was 1 ml/min. and injection volume was 20 uL. Detection wave length for detection was set at 270 nm. The retention time (Rt) of imidacloprid was 1.3 min.

5. Recovery:

Untreated squash samples were homogenized before being spiked with standard solutions of penconazole and imidacloprid. Recovery assays were performed in the 0.05 and 0.1 mg/kg. The quantification of recovery was carried out with standard dissolved into pure solvent. The samples were processed according to the above procedure at each fortification level. Results of the recovery study for penconazole and imidacloprid were 90.21%, 88.70 % and 93.23 %, 90.01% in squash fruits, respectively.

6. Calculation of the Residues:

The dissipation dynamic of penconazole and imidacloprid was determined by the first-order kinetic reactions. The degradation rates constant and half-life were calculated using first order rate equation: $Ct = C_0e^{-kt}$ where Ct represents the concentration of the pesticide residue at the time of t, C_0 represents initial deposits after application and k is the degradation rate constant in $days^{-1}$. The half-life ($t_{1/2}$) is defined as the time required for the pesticide residual level to fall to half of the initial residue level after application and was calculated from the k value for each experiment, being $t_{1/2} = \ln 2/k$.

RESULTS AND DISCUSSION

Results in table (1) and Fig 1. Showed the concentration of initial deposits of penconazole in squash fruits one hour after application was 2.91 mg/kg. Samples of squash fruits were taken one day after application contained 1.87 mg/kg with loss 35.74 % of the initial amounts of penconazole. The residues reduced to 0.78, 0.21, 0.02 and 0.01 mg/kg after 3, 7, 10 and 15 days from treatment and the corresponding calculated rates of loss were 73.19, 92.78, 99.31 and 99.66 %, respectively. Between days 1 and 15 days after spraying a gradual decrease was observed in residues for penconazole which reached 0.01 mg/kg in squash fruits, 15 days after treatment.

The results presented in table (1) and Fig 1. indicated that the initial deposits of imidacloprid in

squash fruits was 3.98 mg/kg, one hour after application. Then gradually decreased to 2.01 mg/kg were observed within one day following application, with loss 49.49 % of the initial of amount of imidacloprid. This value declined to 1.12, 0.51, 0.07 and 0.02 mg/kg, recording the rate of loss 71.68, 87.19, 98.24 and 99.49% in squash fruits, after 3, 7, 10 and 15 days after treatment, respectively.

Data summarized in table (1) showed that penconazole and imidacloprid had low initial residues in squash fruits. The maximum residue limits (MRLs) of penconazole and imidacloprid in squash fruits were 0.1 mg/kg and 1 mg/kg (EU, 2015).

Accordingly, the approximate waiting time value pre harvest interval (PHI), for penconazol and imidacloprid were 10 days and 7 days following application on squash fruits, respectively, were enough to consume squash fruits safely. However, squash is one the vegetable that belong to cucurbitase which grow fast and the small size of squash fruits is preferable to human consumption, so the less persistent pesticides is recommended to control their pests

Data also indicated that the initial residue of imidacloprid in squash were higher than the initial residue of penconazol in squash fruits such difference could be attributed to the higher rate of application of imidacloprid 30 cm^3 (i.e 10.5 g a.i)/ 100 L. water than penconazole 25 cm^3 (i.e 2 g a.i)/100 L. water.

On the other hand , the different levels of initial deposits of both tested pesticides in fruits of squash mainly due to many factors; evaporation of the surface residue which is dependent on temperature condition, chemical or biochemical decomposition, metabolism and photolysis. (Shokr and Nasr, 2006).

The above results seem to show that the half-life value of penconazole and imidacloprid ($t_{1/2}$) were 1.95 and 1.93 days in squash fruit.

Table 1. Behavior of penconazole and imidacloprid residues in squash fruits

Time after treatment (days)	Penconazole		Imidacloprid	
	Residues (mg/kg)	% loss	Residues (mg/kg)	% loss
Initial*	2.91	0.00	3.98	0.00
1	1.87	35.74	2.01	49.49
3	0.78	73.19	1.12	71.68
7	0.21	92.78	0.51	87.19
10	0.02	99.31	0.07	98.24
15	0.01	99.66	0.02	99.49
$t_{1/2}$ (days)	1.95		1.93	
MRL	0.1		1	
PHI	10 days		7 days	

* Samples were taken after one hour of application
$t_{1/2}$: Have life value
PHI: Pre-harvest intervals

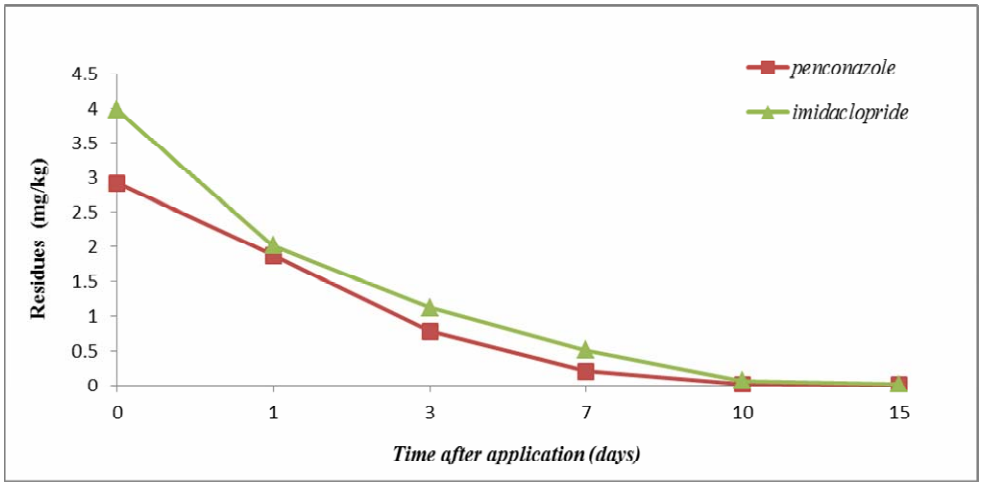

Fig. 1. Behavior of penconazole and imidacloprid residues in squash fruits

These results are quite comparable with those reported by many investigators regardless of experiments were carried out under different climate conditions, geographical locations, time of application, application technique, concentrations, type of formulation and plant species. (Barakat *et al.,* 2006) who studied the residues of tetraconazole and penconazole on and in some vegetable crops grown in greenhouse and found that the half -life values of these fungicides were 1.78 and 1.5 day in cucumber fruits. Similar results were reported by (Mahmoud, 2013) who found that the half-life value of acetamiprid was 14.4 hours in tomato fruits. (Shams EL Din *et al.,* 2012) reported that the half-life values of acetamiprid in tomato fruits was 1.04 day. Also the results were agree with (Mahmoud and Eissa, 2007) who calculated half-life values of diniconazole residues in cucumber fruits were 73.79 hours. (Hegazy *et al*., 1999) studied the residues of diniconazole on and in grape leaves and found that the safety period that should be waited before marketing grape leaves is at least three weeks. (Romeh 2001) showed that diniconazole initial deposit and half-life value in the whole green pods of peas were 1.165 ppm and 72 hours, respectively. (Amer *et al.,* 2007) determined the half-life of tetraconazole and diniconazole fungicides in tomatoes and green beans around 3 days for diniconazole in both vegetables and from 4.5 to 6.5 days for tetraconazole in tomatoes and green beans, respectively. Also with the findings of (Ahmed, *et al.,* 2004; Sanyal, *et al.,* 2008 and Abd EL-Zaher, *et al.* 2011).

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الملخص العربي

اختفاء ومتبقيات البنكونازول والايميداكلوبديد في ثمار الكوسه تحت الظروف البيئية المصرية

هند عبد الله، صفاء حلاوه، داليا الحفني

تم تقدير متبقيات ومعدل اختفاء كلا من مبيدي البنكونازول والايميداكلوبريد في ثمار الكوسة باستخدام جهاز الكروماتوجرافي الغازي والكروماتوجرافي بالسائل عالي الاداء مستخدما طريقة كوشر وجد ان زمن نصف العمر لكل من البنكونازول والايميداكلوبريد هو١,٩٥ و١,٩٣ يوم علي الترتيب.

تبعاً للحدود القصوي للمتبقيات والمده اللازمه قبل الحصاد للمبيدات المستخدمه فإن استهلاك الكوسه المعامله بتلك المبيدات تعتبر آمنه للادواجن والحيوانات.