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Evaluation of Degradation Kinetics for Abamectin in Cucumber

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Abstract: The degradation kinetics and residual levels of abamectin (Abalone 1.8% EC) were estimated in cucumberunder field conditions by employing a validated Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) Method followed by High Performance Liquid Chromatography with Diode Arraydetector (HPLC-DAD) for quantification. At fortification levels of 0.05, 0.1, 0.25 and 0.5 mg kg^{-1}, it was shown recovery was 92% with Coefficient Variation (CV%) was <9% in cucumber tubers. The LOQ for cucumber was found to be 0.03 μg kg^{-1}. After one single application of abamectinthe average residues levelin cucumber was observed to be 0.65 mg kg^{-1} after 1 day of application. The terminal residues of abamectin were below the maximum residue limit (MRL 0.01 mg kg^{-1}) after 10 days at a single dosage. Half-life of abamectin was observed to be 2.38 days, at the recommended dosage which considered to be safe for human beings. These data could provide guidance for the proper and safe use of this pesticide on vegetable-field ecosystems.

Key words: Abamectin, degradation, cucumber, HPLC-DAD, CV

INTRODUCTION

Due to intensive use of pesticides in vegetable farming, residues may be accumulated at levels higher than those permitted by the international MRLs. Assessment of dissipation rate of a pesticide after application is a key process for determining of the residual behavior of pesticides in agricultural crops. Additionally, residues dissipation curves can be used to estimate the time required for decreasing the residues below MRLs (Ambrus and Lantos, 2002; Castillo-Sanchez *et al*., 2000; Fenoll *et al*., 2009).

Abamectin (2,4,5,6-tetrachloroisophthalonitrile) is a non-systemic, widely used and foliar organochlorine fungicide used extensively to control of a variety of insects, fungal and bacterial diseases in many crops (Tomlin, 2000). Abamectin is a mixture of avermectins containing about 80% avermectin B1a and 20% avermectin B1b (Fig. 1). These two components, B1a and B1b have similar biological and toxicological properties. Abamectin is used as an insecticide and acaricide in many parts of the world including Jordan. It acts as an insecticide by affecting the nervous system of and paralyzing insects. Abamectin is used to control insect and mite pests of citrus, pear and nut tree crops and it is

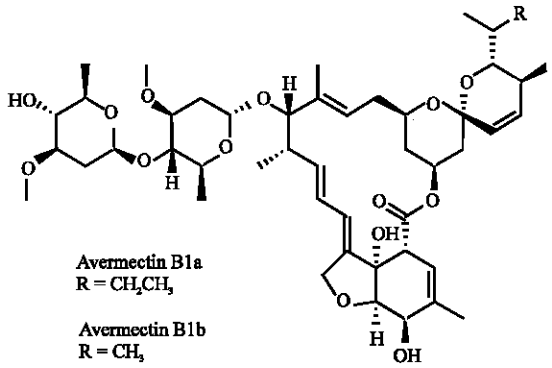

Fig. 1: Chemical structure of abamectin. A mixture containing a minimum of 80% avermectin B1a: 5-O-demethylavermectin B1a and a maximum of 20%, avermectin B1b

used by homeowners for control of fire ants. Abamectin is classified as toxicity class IV. Abamectin is highly toxic to insects and may be highly toxic to mammals (Elbetieha and Da'as, 2003; Lankas *et al*., 1989). Abamectin has been routinely analyzed by liquid chromatography after isolation via liquid liquid extraction or Solid-Phase Extraction (SPE) (Albanis *et al*., 2002; Ballee *et al*., 1976; Pea *et al*., 2010; Rouchaud *et al*., 1988;

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Dryden, 2010). More recently there has been an increased interest in the analysis of degradation products, due to an increased awareness of their potential toxic effects. There are several published studies on the environmental fate and behavior abamectin (Lankas *et al*., 1989; Kwon and Armbrust, 2006; Regitano *et al*., 2001). Recently a risk assessment performed by the US Environmental Protection Agency indicated that abamectin runoff following its application to peanuts presents ecological risk in the Southeastern United States (Elbetieha and Da'as, 2003).

Cucumber (*Cucumis sativus* L.), a herbal plant which belongs to the Cucurbitaceous (Bellucio *et al*., 2008). It is now widely planted in the temperate and tropical zones (Yu *et al*., 1986). It is one of the most important and popular vegetables in Egypt, the total cultivated area of cucumber in Egypt in 2008 was 67810 feddan and total production reached 576732 tons with an average of 8.51 tons/feddan (El-Shaikh, 2010). As active principle stand out carbohydrates, proteins and fats, salts of potassium, phosphorus, calcium, magnesium and sodium iron, vitamins A, B1, B2 and C. Cucumber has medicinal values, the stems have been used in traditional Chinese medicine for their anti-inflammatory activity also stems can expand the blood vessels and reduce blood pressure (Wu *et al*., 1988). The cucumber gives effect anti-angiogenesis and anti-tumor. In popular medicine is given as a sedative and diuretic, anti-rheumatic and somniferous has tonifying action of the liver and kidneys (Tang *et al*., 2010). However, very little is known about the antimicrobial constituents from cucumber stems, though some reports have suggested the presence of steroids and phenolics in this plant (Itoh *et al*., 1981; McNally *et al*., 2003).

The aim of this study was to investigate the dissipation rate of abamectin upon application on cucumber to provide basic information for developing regulations regarding the safe use of abamectin in pest management strategies and to protect the environment and public health.

For this purpose, the QuEChERS Method has been applied Prior to Liquid Chromatography connected with Diode Array Detector (HPLC-DAD).

MATERIALS AND METHODS

Materials: An Emulsifiable Concentration (EC) formulation, Abalone 1.8% EC containing 1.8% (w/v) abamectin was obtained from the Syngenta Agro. Egypt. Cucumber were used as test crop in this study.

Chemicals: The abamectin reference standard (purity >93%) was obtained from the Central Agricultural Pesticide Laboratory (CAPL). The chemical structure of abamectin is presented in Fig. 1. All HPLC-grade organic solvents, methanol and acetonitrile were purchased from Sigma. Glacial acetic acid was purchased from Merck. Primary secondary amine (PSA, 40 μm bondesil) sorbent was purchased from Supelco. Sodium acetate and anhydrous magnesium sulfate were of analytical reagent grade and were purchased from Merck Ltd. These were activated by heating at 150°C for one night before use and kept in desiccators.

Field experiment: For the field experiment, a random block scheme was used with three replications for each test. Abamectin were applied with a backpack motorized sprayers with an adjustable nozzle size of 1 mm using the commercial formulation Abalone 1.8% EC. The pesticide application was carried out in cucumber at the dose recommended by the manufacturers 40 cm^3/100 L water. Samples 1 kg were collected 1 h after application and then after 1, 3, 5, 7 and 10 days. A control sample was also taken at each sampling time. Immediately after collecting the samples, each individual sample were put into plastic bags and transported to the laboratory, the samples were homogenized using a food processor (Thermomix, Vorwerk). The homogenate of each sample was then placed into polyethylene 50 mL centrifuge tube and stored frozen at -20±2°C until further analysis.

Standard calibration curves: The stock solution of abamectinwas prepared by dissolving 50 mg of the analyte (accurate weight) in 50 mL n-hexane to obtain solution concentration 1 mg mL^{-1}. Working standard solutions of 0.05, 0.1, 0.25 and 0.5 μg mL^{-1} were prepared by appropriately diluting the stock solution with n-hexane. Stock solution was stored at -20±2°C and working standard solutions were stored in the dark ≤4°C when not in use. Calibration curves were generated by plotting peak area versus concentration. Standard calibration curves were presented good linearity with regression coefficient r^2>0.976 with good separation and repeatability. The calibration curve and recovery validation study were all repeated three times (n = 3).

Sample preparation: Cucumber samples (1 kg) was chopped and homogenized for 5 min at high speed in a laboratory homogenizer and extracted according to the procedure described and modified by Lehotay *et al*. (2010) and validated by Abd-Alrahman (2013), Abd-Alrahman and Ahmed (2012), Abd-Alrahman and Almaz (2012) and Abd-Alrahman *et al*. (2012). Briefly, 10 g of the homogenized sample was weighed into a 50 mL centrifuge tube. The 10 mL of 1.0% acidified acetonitrile with acetic acid was added; closed and vigorously shaken for 1 min using a vortex mixer at maximum speed. Afterwards, 4 g of anhydrous $MgSO_4$, 1 g of NaCl, 1 g

sodium citrate dihydrate and 0.5 g disodium hydrogen citrate sesquihydrate were added and then extracted by shaking vigorously using vortex for 2 min following centrifugation for 10 min at 5,000 rpm. An aliquot of 3 mL was transferred from the supernatant to a new clean 5 mL centrifuge tube and cleaned by dispersive solid-phase extraction with 75 mg of PSA and 500 mg of magnesium sulfate. Afterwards, centrifugation was carried out at 6,000 rpm for 5 min. An aliquot (2 mL) from the supernatant was filtered through a 0.2 μm PTFE filter (Millipore, USA) and then analyzed by Agilent 1100 HPLC-DAD.

Chromatographic analysis: Agilent 1100 HPLC (USA) high performance liquid chromatography coupled with diode array detector HPLC-DAD was used for determination of abamectinresidues using analytical column C18 (150×4.6 mm ×5 μm). Mobile phase was methanol: water (90:10 v/v) with flow rate was 0.6 mL min^{-1}. UV detection at 254 nm. The injection volume was 20 μL for all standard and samples.

Statistical analysis: Data were statistically evaluated by one-way Analysis of Variance (ANOVA). Determination the differences among means were carried out by using the Least Significant Differences (LSD) test. All statistical analyses were done using the Statistical Package for Social Sciences (SPSS 16.0) Program.

RESULTS AND DISCUSSION

Control cucumber samples (without pesticide application) were used for the evaluation of selectivity. The absence of any signal at the retention time of abamectin indicated that no matrix compounds are present which could give false positive signal.

The calibration curve of abamectin showed a good linearity and strong correlation between concentrations and area in the studied range (0-200 ng mL^{-1}) ($r^2 \geq 0.976$). Recoveries for abamectin from spiked cucumber samples was 89.0%. Precision was studied by performing repeatability studies, expressed as RSD. Satisfactory precision was obtained for abamectin. Repeatability was lower than 5% for all three levels assayed. Similarly with the examination of the matrix effect, a general tendency was observed towards higher values of RSDs at low spiking concentrations. The LOD and LOQ were 0.01 and 0.03 μg kg^{-1}, respectively.

Abamectin mean residue levels during the sampling period for each application derived from three sub samples are shown in Fig. 2. Residue levels of abamectin were found to be below the MRLs established by the FAO/WHO Codex Committee 0.01 mg kg^{-1}, after the application of recommended dose which was 40 cm^3 100/L water for cucumber throughout the experimental period 10 days. The highest mean residue level 0.65 mg kg^{-1} was found in samples taken after 1 day of pesticide application. Residue levels of abamectin had been decreasing in the following period was reached 0.05 mg kg^{-1} in 7 days after single application.

The results showed that the persistence of abamectin, the dissipation of abamectin was tested for the reaction order and it was indicated that this degradation kinetics followed the first-order kinetics (r^2 = 0.9534) (Fig. 3) with half-life (t1/2) and PHI of abamectin on cucumber were 2.38 and 10 days, respectively. These

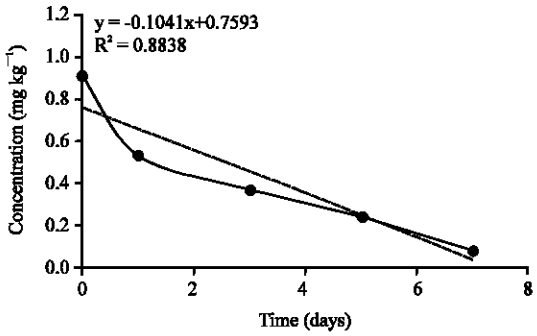

Fig. 2: Degradation kinetics of abamectin in cucumber

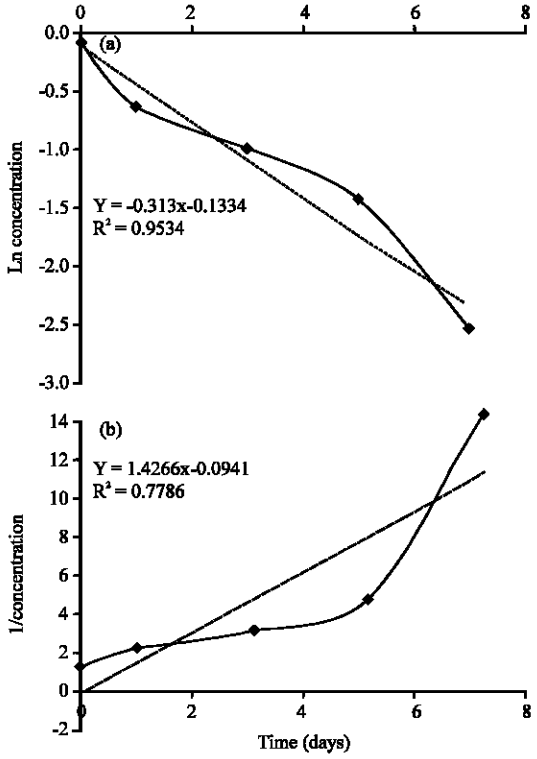

Fig. 3: Degradation kinetics of abamectin: a) Followed the first-order kinetics; b) Second order kinetics

results are very similar to those reported in the literature for the HPLC-FL analysis of abamectin in other fruits or vegetable samples (Diserens and Henzelin, 1999; Hernandez-Borges *et al*., 2007; Valenzuela *et al*., 2001, 2000). As it has been previously indicated there were no interfering peaks from the sample matrix and a quantitative and feasible extraction can be developed with the proposed method. They have been reported recovery was 92% with RSD values lower than 9%.

CONCLUSION

In this study, the dissipation rate of abamectin after a single application at recommended doses on cucumber were evaluated. Researchers used an improved method (QuEChERS) for sample preparation. The half-life and PHI were determined. The long stability of abamectin might lead to a higher risk of exposure to abamectin residues. Further studies are required to assess the residual behavior, exposure risk and the environmental fate of these pesticide abamectin.

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