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Abamectin residues in and on some horticultural crops

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ABSTRACT

Field experiments were carried out to study the residual behavior of abamectin on orange; strawberry and cucumber. The effect of household processing (peeling and squeezing for orange and washing for strawberry and cucumber) were studied. The field applied abamectin dissipated gradually and disappeared completely after 12, 14 and 9 days for orange, strawberry and cucumber, respectively. The half-life time for abamectin was calculated to be 3.93, 6.16 and 2.2 days for orange, strawberry and cucumber, respectively. The three tested crops can be harvested safely (PHI) after 9, 10 and 6 days for orange, strawberry and cucumber, respectively. The household processing was found to be effective on the dissipation of the used pesticide especially peeling and squeezing in orange, the pesticide was not detected after these two processes. Washing with running tap water was also effective in strawberry and cucumber, as it reduces abamectin gradually until the pesticide dissipated completely after 11 and 6 days respectively.

INTRODUCTION

Abamectin is a product which was developed as an insecticide and acaricide with contact and stomach action (Campbell *et al.*, 1983). It belongs to the family of avermectins which are macrocyclic lactones produced by *Streptomyces avermitilis* (Burge *et al.*, 1979). Abamectin consists of 80 % of avermectin B_{1a} and $\leq$ 20% Avermectin B_{1b} (as seen in the chemical structure).

The use of a pesticide on a crop may lead to the presence of the pesticide residue or its degradation products on the treated plants. Abamectin is notably used on orange, strawberry and cucumbers. Its use is increasing as replacement

for other acaricides. As these fruits are often consumed fresh, it is important to have a suitable method for the determination of abamectin residue levels on and in these crops. Since the presence of these residues in fruits can affect consumer health. Therefore, the regulatory authorities have established maximum residue limits (MRLs) of 0.01mg/kg for this pesticide.

The goal of this study was planned to characterize the following points:

I. To determine the dissipation rate of abamectin under field conditions.

II. To establish the safest pre-harvest interval.

III. To evaluate the impact of some home processing on the depletion of the residue level.

MATERIAL AND METHODS

Pesticide used: Abamectin

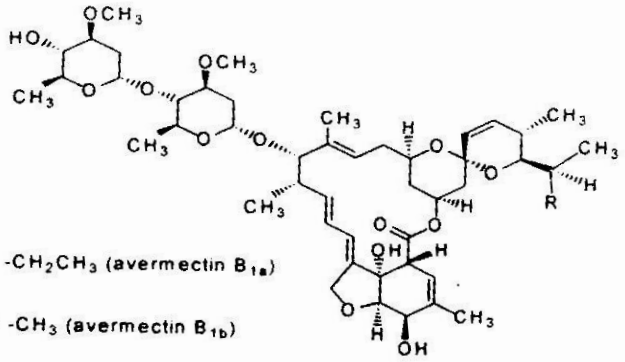
Chemical name: 5-*O*-demethylavermectin A1a (i) mixture with 5-*O*-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)avermectin A1a (ii)

Solubility: In water 7-10 g/l (20 °C). In toluene 350, acetone 100, isopropanol 70, chloroform 25, ethanol 20, methanol 19.5, n-butanol 10, cyclohexane 6 (all in g/l, 21 °C).

Vapour pressure: < 3.7 × 10-3 mPa (25 °C)

LD50: Oral LD_{50} for rats 10 mg/kg and mice 13.6 mg/kg.

Chemical structure:



(i) R = $-CH_2CH_3$ (avermectin B_{1a})

(ii) R = $-CH_3$ (avermectin B_{1b})

Tested fruits and vegetables: Orange (*Citrus sinensis*), strawberry (*Fragaria ananassa*) and cucumber (*Cucumis sativus*).

Locations and dates of the experiments: Kalubia (February 2006) for orange and Fayom (May 2007) and (July 2009) for strawberry (*Fragaria ananassa*) and cucumber (*Cucumis sativus*), respectively.

Field experiments: Abamectin was used as water emulsion using a knapsack hand sprayer fitted with one nozzle boom. The untreated control plots were left unsprayed. Three replicates were made for each treatment. The experimental area for each replicate was 8 trees for orange and 1/24 feddan for strawberry. Representative samples were randomly picked from the treated plots just post application (0-times). Additional samples were taken at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 days after treatment. Each sample was divided into three subsamples for orange and two subsamples for strawberry. The residue of abamectin was determined in whole fruit, pulp and juice for orange and unwashed and washed fruits for strawberry and cucumber under field conditions The first samples of orange were analyzed on the whole fruits and the first samples of strawberry were analyzed without washing. However, the second samples of orange were subjected to peel and the second samples of strawberry were washed with tap water for 5 min. The third one of orange in which the pulp subjected to squeeze in order to clarify the role of (peeling or squeezing) of orange and washing of strawberry on the depletion of abamectin residues. For cucumber, the experimental area for each replicate was 1/24 fed for each replicate. Representative samples were randomly picked from the treated plots just one hour after application. Subsequent-samples were taken at 1, 2, 3, 4, 6, 9, 12, 15, 18 and 21 days after treatment. Each sample was divided into two subsamples. The first was analyzed without washing and the second was washed with tap water for 5 min to study the effect of washing on loss of the abamectin residues Samples were stored at -20°C until subjected to analysis.

Analytical method:

Extraction: The analytical methods of Vuik (1991) and Prabhu *et al.* (1992) were adopted for the extraction of abamectin. Ten gram was taken from the homogenized sample and 100 ml ethyl acetate were added and then blinded for

two minutes. The extract was filtrated through filter paper No. 1 in the presence of 30 g sodium sulphate. An aliquot (50 ml) was taken from the filtrate and evaporated using rotary evaporator to dryness at 40 °C. The residue was dissolved in 5 ml of hexane.

Clean up: The extract was cleaned up using aminopropyl solid-phase extraction cartridge previously conditioned with 5 ml n-hexane (Prabhu *et al.*, 1992) to retain avermectins. Then, the column was washed with 5 ml n-hexane, 5 ml toluene and 5 ml methylene chloride consequently and discarded. The abamectin was eluted with a mixture of 20 ml acetone: dichloromethane 1:1 (v/v) and collected in a 50 ml flask. The solvent was evaporated to dryness using rotary evaporator.

Derivatization of samples and standards:

The residue was derivatized before injection using the two following reagents:

Reagent 1: add one volume of Trifluoroacetic anhydride (TFAA) to two volumes of acetonitrile in a brown-glass flask.

Reagent 2: add one volume of 1- methylimidazole (NMIM) to one volume of acetonitrile in a brown-glass flask.

The solutions (1&2) were prepared freshly every week and kept at 4°C.

The derivatization procedure is identical to the method used by Prabhu *et al.*, (1991). The stock and standard solutions are prepared in acetonitrile to prepare 0.01, 0.02, 0.05, 0.1 and 0.2µg/ml calibration curve standards for derivatization. To each sample and standard test tube, 300µl from reagent 1, 200µl from reagent 2 and 500µl acetonitrile were added. The flask was kept at 4°C for 5 minutes, then transfer the derivatized solution to vial for injection.

Determination: The HPLC system consisted of C18 column (Thermo electron corporation, ODS hypersil, (250×4.6 mm) Particle Sz. (µ) 5) and fluorescence detector.

Chromatographic conditions: Mobile phase was 100 % acetonitrile with flow rate 2 ml/min injection volume 10 µl. The detector setting was at an excitation wavelength at 365 nm and emission wavelength at 470 nm.

Preparation of standard solution:
Stock solution: Abamectin reference standards (98% purity) from Dr. Ehrensdorfer (Augsburg, Germany).

1. 1000 µg/ml reference stock solution of abamectin was prepared in acetonitrile in a 100 ml volumetric flask. Stock solution was kept in refrigerator at 4 ± 2 °C. Dilutions were carried out to achive the suitable concentrations for the standard curve.

2. 100 µg/ml reference standard solution of abamectin was prepared by diluting 10 ml of 1000 µg/ml in 100 ml acetonitrile in a 100 ml volumetric flask. Stock solution was kept in refrigerator at 4 ± 2°C.

Intermediate solution: Individual standard of 10 µg/ml of abamectin was prepared by diluting 5 ml of stock solution in 50 ml acetonitrile. Intermediate solution was kept in refrigerator at 4 ± 2 °C.

Standard curve: Calibration standards were divided into fluorescence detector (FLD) and prepared by dissolving appropriate amounts of intermediate solutions in acetonitrile and derivatized as mentioned before.

Calculations: Analyte concentration in sample C_{sample} (mg/kg) is calculated as follows:

$$Csample = Cst \times \frac{Vtot}{Ve} \times \frac{Vf}{W} \times \frac{Hsample}{Hst}$$

Where: C_{st} = Concentration in Standard (µg/ml).
V_{tot} = Total volume of extract (ml).
V_e = Volume of aliquot taken for evaporation (ml).
V_f = Final volume (ml).
W = Sample weight (g).
H_{sample} = peak height of abamectin in sample
H_{st} = peak height of abamectin in standard

Half life calculation: Half life was calculated according to Moye *et al.* (1987) formula.

$t_{1/2} = 0.693/k$ -k = slope $\ln [A] = \ln [A]_0 - kt$

Recovery test: Recovery studies were carried out by spiking six replicates of untreated orange and cucumber samples (control samples) with 0.01, 0.02, 0.05, 0.1 and 0.2 mg/kg of abamectin. Samples were analyzed and derivatized for each concentration using the described procedure. The mean values of the six replicates were calculated.

RESULTS AND DISCUSSION

Data in Table (1) show that, the recovery percentages were satisfactory for the abamectin and ranged between 83 – 88 % in orange and cucumber samples. The HPLC/FL chromatograms for abamectin were highly selective and sensitive due to the limit of quantitation (0.01 mg/kg) and the limit of detection was 0.005 mg/kg. The retention time for abamectin was 5.53 min.

1. Analysis of abamectin residues in whole fruits:

A. Orange fruits: The residue amounts of abamectin on and in orange fruits are presented in Table (2). The data shows that the initial deposit of abamectin in orange fruits was 0.081 mg/kg. The amounts of abamectin decreased to 0.073 mg/kg at one day after application representing 9.88 % loss. The decline in residual amounts continued after 2 days to be 0.062 mg/kg with 23.46 % loss. Abamectin was gradually decline with time, and reached to 0.1mg/kg (87.65 % loss) at 9 days after application. Twelve days after application samples were free from any detectable residues. The obtained residual half-life value ($t_{1/5}$) of abamectin on and in orange fruits was 3.93 days. Hence maximum residue limits (MRL) of abamectin in orange was 0.01 mg/kg (FAO/WHO, 1997). This means from data set up in Table (2) that pre-harvest interval (PHI) was 9 days. The same trend was obtained by Maynard *et al.*, (1989) who found a decrease in the total residues of avermectin B1a on citrus fruits with time; the largest decreases occurred during the first week post application. Kamel *et al.*, (2007) found that the initial deposit of abamectin on dates of Nabout Sief type was 0.09 mg/kg and dissipated gradually with time, the residue decline reached 66 % after 7 days and 88 % after 14 days of application.

Table (1): Recovery percentages of abamectin on and in orange and cucumber fruits at different spiked levels.

Spiked level (mg/kg)	Orange				Cucumber			
	Found (mg/kg)	% Recovery	Mean Recovery	% RSD	Found (mg/kg)	% Recovery	Mean Recovery	% RSD
0.01	0.0085	85	87	4	0.0082	82	84	7
	0.0082	82			0.0076	76		
	0.009	90			0.0092	92		
	0.0093	93			0.0087	87		
	0.0086	86			0.0083	83		
	0.0087	87			0.0081	81		
0.02	0.017	85	83	5	0.015	75	84	9
	0.018	90			0.019	95		
	0.016	80			0.016	80		
	0.016	80			0.017	85		
	0.016	80			0.018	90		
	0.017	85			0.016	80		
0.05	0.045	90	88	5	0.038	76	83	7
	0.041	82			0.046	92		
	0.047	94			0.042	84		
	0.043	86			0.039	78		
	0.046	92			0.040	80		
	0.042	84			0.044	88		
0.1	0.085	85	88	4	0.090	90	84	5
	0.083	83			0.085	85		
	0.091	91			0.078	78		
	0.089	89			0.085	85		
	0.093	93			0.085	85		
	0.086	86			0.080	80		
0.2	0.185	93	86	5	0.18	90	88	4
	0.17	85			0.176	88		
	0.165	83			0.17	85		
	0.18	90			0.185	93		
	0.175	88			0.18	90		
	0.16	80			0.165	83		

RSD: Relative Standard Deviation. $RSD = \frac{Sd}{mean} \times 100$

B. Strawberry fruits: Table (2) presented the initial deposit of abamectin on and in unwashed strawberry fruits was 0.112 mg/kg one hour after application. The residue of abamectin in strawberry fruits within the first 24 hours after application decreased to 0.101 mg/kg with 9.82 % loss. The rapid degradation continued for abamectin to reach 0.092 mg/kg with 17.86 % loss after 2 days from application. The decrease of abamectin residues recovered 6 days after

Table (2): Residues of abamectin on and in orange, strawberry and cucumber fruits.

Time after application (days)	Orange		Strawberry		Cucumber	
	Detected amount (mg/kg)	% loss	Detected amount (mg/kg)	% loss	Detected amount (mg/kg)	% loss
Initial	0.081 ± 0.002	0.00	0.112 ± 0.001	0.00	0.069 ± 0.001	0.00
1	0.073 ± 001	9.88	0.101 ± 0.001	9.82	0.041 ± 0.001	40.98
2	0.062 ± 0.001	23.46	0.092 ± 0.002	17.86	0.035 ± 0.001	49.15
3	0.055 ± 0.003	32.10	0.084 ± 0.001	25.00	0.027 ± 0.001	61.11
4	0.04 ± 0.002	50.62	0.075 ± 0.001	33.04	0.016 ± 0.001	76.84
5	0.032 ± 0.001	60.49	0.066 ± 0.004	41.07	----	----
6	0.025 ± 0.001	69.14	0.057 ± 0.002	49.11	<LOQ	----
7	0.02 ± 0.001	75.31	0.043 ± 0.001	61.61	----	----
8	0.015 ± 0.001	81.48	0.035 ± 0.003	68.75	----	----
9	0.01 ± 0.001	87.65	0.027 ± 0.001	75.89	N.D.	----
10	<LOQ	----	0.02 ± 0.004	82.14	----	----
11	<LOQ	----	0.012± 0.004	89.29	----	----
12	N.D	----	<LOQ	----	N.D.	----
13	N.D	----	<LOQ	----	----	----
14	N.D	----	N.D	----	----	----
15	----	----	----	----	N.D.	----
18	----	----	----	----	N.D.	----
21	----	----	----	----	N.D.	----
$t_{1/5}$ (days)	3.93		6.16		2.2	

LOQ* Limit of Quantification

N.D.* Not detected

application continued to be 0.057 mg/kg with 49.11 % loss. After 9 days, there was a small decline to 0.027 mg/kg, which continued after 10 days with percent loss of 75.89 and 89.27 %, respectively. These results are completely agree with those obtained by Crouch and Feely (1995) who found that the total residues of emamectin benzoate in lettuce plants were declined from 365 ppb to 81 ppb after 10 days post application. At the last day of this experiment (14 day), residue of abamectin was not detected (below detection limit 0.005 mg/kg). The obtained ($t_{1/5}$) of abamectin on and in strawberry fruits was 6.16 days. The MRL of abamectin in strawberry was 0.02 mg/kg (FAO/WHO, 1997). This means from the data set up in Table (2) that PHI was 10 days.

C. Cucumber fruits: Table (2) showed the initial deposit of abamectin on and in unwashed cucumber fruits was 0.069 mg/kg one hour after application. The residue of abamectin in cucumber fruits within the first 24 hours after application decreased to 0.041 mg/kg with 40.98 % loss. The rapid degradation continued for abamectin to reach 0.035 mg/kg with 49.15 % loss after 2 days from application. The decrease in the amounts of abamectin recovered 3 days after application continued to be 0.027 mg/kg with 61.11 % loss. After 4 days, there was a small decline to 0.016 mg/kg, with 76.84 % loss. After 6 days, there was lower than quantitation limit. After 9 days of the experiment, residue of abamectin was not detected. The obtained ($t_{1/5}$) of abamectin on and in cucumber fruits was 2.2 days. The rapid dissipation of abamectin in cucumber may be due to a consistent increase in total mass of cucumber fruits with time. MRL of abamectin in cucumber was 0.01 mg/kg (FAO/WHO, 1997). This means from the data set up in Table (2) that PHI was 6 days.

2- The effect of household processing

A. Peeling and squeezing in orange fruits: The effect of peeling process using kitchen knife and squeezing process were determined upon the amount of abamectin residues left in the treated orange fruits. Data showed that 100 % of the initial deposits of abamectin on orange fruits had lost after peeling and squeezing within one hour after application. The previous results are in harmony with the results obtained by Maynard *et al.* (1989), who did not detect any residues of avermectin B1a in pulp of orange fruits.

B. Washing

B. 1- Strawberry fruits: Data in Table (3) show the effect of washing process with tap water upon the amount of abamectin residues left in the treated strawberry fruits. Data showed that 19.64 % of the initial deposits of abamectin on strawberry fruits were dislodged by washing after one hour of application. The results showed that the amounts of abamectin in washed strawberry fruits were much lower than those in the unwashed strawberry fruits. Samples taken at 1, 2, 3, 4, 5, 6, 7 and 8 days after application showed that the calculated percentages of residue removed as a result of washing process were 17.82, 18.48, 26.19, 32.00, 33.33, 45.61, 46.51 and 60.00 %, with residue amounts of

0.083, 0.075, 0.062, 0.051, 0.044, 0.031, 0.023, and 0.014 mg/kg, respectively. Ramadan *et al.*, (1988) investigated the residual behavior of methomyl and profenofos on and in eggplant and green pepper fruits. They reported that unwashed fruits of the eggplants could be safety marketed nine days after methomyl treatment while washed fruits could be safely used six days after application.

Table (3): Residues of abamectin on and in unwashed and washed strawberry fruits.

Time after application (days)	Unwashed	Washed	
	Detected amount (mg/kg)	Detected amount (mg/kg)	% dislodgable amount by washing
Initial	0.112 ± 0.001	0.09 ± 0.003	19.64
1	0.101 ± 0.001	0.083 ± 0.001	17.82
2	0.092 ± 0.002	0.075 ± 0.001	18.48
3	0.084 ± 0.001	0.062 ± 0.002	26.19
4	0.075 ± 0.001	0.051 ± 0.001	32.00
5	0.066 ± 0.004	0.044 ± 0.001	33.33
6	0.057 ± 0.002	0.031 ± 0.001	45.61
7	0.043 ± 0.001	0.023 ± 0.001	46.51
8	0.035 ± 0.003	0.014 ± 0.001	60.00
9	0.027 ± 0.001	<LOQ	----
10	0.02 ± 0.004	<LOQ	----
11	0.012 ± 0.004	N.D	----
12	<LOQ	N.D	----
13	<LOQ	N.D	----
14	N.D	N.D	----

LOQ* Limit of Quantification
N.D.* Not detected

B. 2- Cucumber fruits: Data in Table (4) illustrates the effect of washing process with tap water upon the amount of abamectin residues left in the treated cucumber fruits. Data showed that 49.42 % of the initial deposits of abamectin on cucumber fruits were dislodged by washing after one hour of application. The results showed that the amounts of abamectin in washed cucumber fruits were much lower than those in the unwashed cucumber fruits. Samples taken at

1, 2, and 3 days after application showed th: the calculated percentages of residue removed as a result of washing process were 62.95, 59.96 and 57.39 %, with residue amounts of 0.015, 0.014 and 0.011 mg/kg, respectively. According to the data and remarks of El-Kins (1989) and Tag El-Din (1993), the removal of pesticide residues by washing depends on several factors: character of the surface of the plant food (smooth or rough, waxy or non-waxy), surface to volume ratio (washing is more effective for bigger fruits) reference point of residue levels (higher levels easier to remove); chemical and physical properties of the applied pesticides, the length of time that the pesticide has been in contact with the plant foods, rate and number of applications and permeability of pesticide into fruit tissues.

Table (4) Residues of abamectin on and in unwashed and washed cucumber fruits.

Time after application (days)	Unwashed	Washed	
	Detected amount (mg/kg)	Detected amount (mg/kg)	% dislodgable amount by washing
Initial	0.069 ± 0.001	0.035 ± 0.001	49.42
1	0.041 ± 0.001	0.015 ± 0.001	62.95
2	0.035 ± 0.001	0.014 ± 0.001	59.96
3	0.027 ± 0.001	0.011 ± 0.001	57.39
4	0.016 ± 0.001	<LOQ	---
6	<LOQ	N.D.	---
9	N.D.	N.D.	---
12	N.D.	N.D.	---
15	N.D.	N.D	---
18	N.D.	N.D	---
21	N.D.	N.D	---

LOQ* Limit of Quantification
N.D.* Not detected

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متبقيات الأباميكتين في و على بعض المحاصيل البستانية

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أجريت تجارب حقليه لدراسة سلوك متبقيات مبيد الاباميكتين على ثمار البرتقال والفراوله والخيار. كما تم دراسة المعاملات المنزليه (تقشير وعصير ثمار البرتقال و الغسيل لثمار الفراوله والخيار). وقد وجد ان مبيد الاباميكتين تدهور تدريجيا ثم إختفى تماما بعد 12 و14 و9 أيام لكل من البرتقال والفراوله والخيار على التوالي. كما وجد ان فترة نصف العمر له كانت 3.93 ، 6.16 ، 2.2 ايام لكل من البرتقال والفراوله والخيار على الترتيب. ووجد ان فترة ما قبل الحصاد كانت 9 و10 و6 أيام لكل من البرتقال والفراوله والخيار على التوالي. المعاملات المنزليه كانت لها تأثير فعال على التخلص من بقايا مبيد الاباميكتين خاصة التقشير والعصير في البرتقال حيث لم يتم تحديد أي من كميات متبقيات المبيد طبقا لحساسية طريقة التحليل المستخدمه بعد هاتين المعاملتين. بينما كان للغسيل بواسطة ماء الصنبور تأثير فعال في الفراوله والخيار حيث خفضت من كمية متبقيات المبيد تدريجيا حتى إختفى المبيد تماما تحت ظروف التجربه بعد 11 و6 أيام في الفراوله و الخيار على التوالي.