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# Degradation of the acaricides abamectin, flufenoxuron and amitraz on Saudi Arabian dates

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**Abstract**

Degradation of the acaricides abamectin, flufenoxuron and amitraz on date palms, *Phoenix dactylefera* var. *Nabout Seif,* grown in Saudi Arabia was studied during the post-harvest interval (PHI) under the local weather and soil conditions. The initial deposit of abamectin residues on dates was 0.09 mg/kg, which declined to 0.03 (66%) and 0.02 mg/kg (88%) after 7 and 14 days of spraying, respectively (PHI = 10 days, MRL = 0.03 mg/kg). The initial deposit of flufenoxuron was 0.68 mg/kg and declined to 0.25 (68%), 0.07 (90%) and 0.03 mg/kg (96%) after 16, 52 and 60 days, respectively (PHI = 50 days, MRL = 0.1 mg/kg). Finally, the initial deposit of amitraz was 0.34 mg/kg which declined to 0.02 mg/kg (95%) and was not detected (100%) after 21 and 30 days, respectively (PHI = 28 days, MRL = 0.01 mg/kg). The acceptable daily intake (ADI) for fruits and vegetables set by FAO/WHO for the three acaricides tested was based on regular and average consumption of fruit, however, in Saudi Arabia, and other neighboring countries, natives consume more date (more than 10 times) than an average person living outside this region. Such high date consumption could lead to a higher risk of exposure to pesticides, especially in children and other vulnerable individuals.


*Keywords:* Dates; *Phoenix dactylefera* var. *Nabout Seif*; Abamectin; Flufenoxuron; Amitraz; *Oligonychus afrasiaticus*; Pesticide residues; Residue decline study

## 1. Introduction

Saudi Arabia is the largest date producer in the world, with production amounting to 800,000 tonnes per year (2000 statistics), and expecting to jump to one million tonnes over the coming five years. The number of date palm trees is over 18 million, in area over 93.8 million hectares. There are about 400 different species of dates in Saudi Arabia grown in different regions of the Kingdom; the most important regions are: Riyadh, (Central), Eastern, Qassim (Central), Madina (Western), Assir (South Western) (Anon., 2004).

The search for means to improve the production of dates in Saudi Arabia is always the target of scientists, politicians and businessmen, who seek new techniques to enhance the quality and safety of this product. Recently, a number of acaricides were registered at the Ministry of Agriculture in Saudi Arabia to control the mite *Oligonychus afrasiaticus*, which infest dates and causes severe damage. Among these acaricdes, abamectin, flufenoxuron and amitraz are widely applied on dates to control the mite infestation. Due to the large amount of dates consumed by Saudi residents (an average of 10 dates daily per person), the search for safe pesticides with negligible residual deposits has always been preferred.

Abamectin belongs to the family avermectins which are macrocyclic lactones produced by the actinomycete *Strep-*

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*tomyces avemitilis*. It is a mixture of two homologues containing about 80% avermectin $B_{1a}$ and about 20% avermectin $B_{1b}$ (Pesticide Manual, 1994). Abamectin acts by stimulating the release of γ-aminobutyric acid thus causing paralysis (Turner & Shaeffer, 1989). It is used to control motile stages of mites and some other insects on fruits and vegetables and has limited plant systemic activity. Flufenoxuron is a benzoylurea pesticide and acts as an insect growth regulator and chitin synthesis inhibitor. It is used to control immature stages of insects and phytophagous mites on fruits and vegetables (Pesticide Manual, 1994). Amitraz is a member of the formamidine pesticides whose mode of action probably involves an interaction with octopamine receptors in the tick nervous system. It is used to control all stages of tetranychid and eryophyid mites on fruits and some vegetables (Pesticide Manual, 1994).

This study was carried out to determine the residues of the three acaricides on dates and to study their degradation to establish the safest post-harvest interval under the weather and soil conditions of Saudi Arabia.

## 2. Materials and methods

### 2.1. Pesticides and sampling

Vertimic (1.8% EC abamectin), Novartist, Cascade (10% DC flufenoxuron), Cyanamid, Mitamobeed (10% EC amitraz), Arab Industrial Company, Dammam, Saudi Arabia was used in this study. Experiments were done in the farm of ''Muhamadiya'' at Al-Kharj, Riyadh area, Saudi Arabia. Date palms, *Phoenix datylefera* var. *Nabout Seif*, were sprayed with each acaricide once at the manufacturer's label recommended levels. An area of 50 $m^2$ was sprayed and random samples from 10 palm trees at the four corners and middle sections of the area were chosen to collect about 100 g from each tree, and were combined in plastic bags. Samples were collected after 1 h (initial deposit time), and other time intervals in three replicates for each day. A control treatment was also collected without spraying.

### 2.2. Extraction and clean-up

Samples were extracted and purified following established laboratory procedures for detection of multi-residue pesticides in food products. Modifications to this procedure were made following specific clean up procedures for the three acaricides (Diserens & Henzelin, 1999; Korta, Bakkali, Berrueta, Gallo, & Vicente, 2001; Miliadis, Tsiropoulos, & Aplada-Sarlis, 1999). Collected samples were immediately transferred to the laboratory where 20 g of each sample were randomly selected and extracted with 100 ml of 80% aqueous acetonitrile in a blender for 2 min at high speed. The samples were then filtered in a Büchner funnel after rinsing the blender with an additional 20 ml of the extracting solvent. Distilled water (400 ml) was added to the filtrate to make the water/acetonitrile ratio greater than 4:1 (v/v). Three $C_{18}$ solid phase extraction (SPE) cartridges (Varian, 500 mg, 10 $cm^3$) were prepared after conditioning with acetonitrile (5 ml) and distilled water (5 ml). Sample extracts of each analyte were passed over the cartridges with a flow rate not exceeding 4 ml/min. Flufenoxuron was eluted with 5 ml 100% acetonitrile (Cartridge A). Cartridges containing amitraz and abamectin were washed with 100% acetonitrile. Abamectin (Cartridge B) was eluted with 5 ml of a solution of 2% triethylamine (TEA) in acetonitrile. Cartridge C, containing amitraz was further washed with 5 ml of 10% aqueous tetrahydrofuran (THF) and amitraz was eluted with 5 ml of 100% THF. The solvent was evaporated and exchanged with methanol prior to injection in GC/NPD and GC/MS. Further clean up on silica cartridges was needed for flufenoxuron and abamectin. The final fraction of each analyte was evaporated to dryness under a stream of nitrogen and re-dissolved in 5 ml of hexane and passed through two silica SPE cartridges (Varian, 500 mg, 10 $cm^3$) after conditioning with methylene chloride and hexane. Each cartridge was washed with 5 ml of a solution of 50% (v/v) methylene chloride in hexane. Flufenoxuron was eluted with 5 ml 100% methylene chloride. The solvent was evaporated and exchanged with 1 ml methanol prior to injection to GC/ECD and GC/MS. Finally, the cartridge containing abamectin was further washed with 10 ml of 10% acetonitrile in methylene chloride and abamectin was eluted with 5 ml of 100% acetonitrile and dried under a stream of nitrogen prior to addition of derivatization reagents.

### 2.3. Instrumentation

#### 2.3.1. Gas chromatography

Samples of flufenoxuron and amitraz were analyzed by gas chromatography using an Agilent 6890 gas chromatograph with split/splitless inlet equipped with μ-ECD and NPD detectors (Agilent Technologies, Inc., 395 Page Mill Road, Palo Alto, CA 94306, USA). Another similar gas chromatograph was equipped with a 5973 mass selective detector. A HP-5MS capillary column (30 m × 0.32 mm, 0.5 μm) (Agilent Technologies, Inc., 395 Page Mill Road, Palo Alto, CA 94306, USA) was attached to each detector using helium as carrier gas. The oven temperature was set to the initial temperature of 90 °C for 2 min., then programmed to 250 °C at 20 °C/min and held there for 15 min. Splitless injections of 1 μl were used for both instruments with 250 °C as the injector temperature. The MSD detector was set to the selected ion monitoring (SIM) mode targeting the ions at $m/z$ 293 for amitraz, and $m/z$ 331 for flufenoxuron at 100 ms. dwell time. The interface temperature was 280 °C for the GC/MS, the μ-ECD detector temperature was 300 °C, and the NPD detector temperature was 250 °C. Quantitative results were based on GC/ECD or GC/NPD specific detectors. GC/MS was used for confirmation.

#### 2.3.2. HPLC

Analysis of abamectin samples was carried out using a Shimadzu LC-10AD high performance liquid chromatograph (HPLC) (Shimadzu Corporation, 1 Nishinokyo Kuwabara-cho, Nakagyo-ku, Kyoto 604, Japan) attached to a Zorbax-ODS (0.15 m × 4.6 mm) column (Agilent Technologies, Inc., 395 Page Mill Road, Palo Alto, CA 94306, USA) which was eluted with the mobile phase acetonitrile/water (96:4, v/v) at a flow rate of 1.5 ml/min. Abamectin derivative was detected using a Shimadzu RF-10A fluorescence detector set at an excitation wavelength of 365 nm and emission wavelength of 470 nm. The injection volume was 50 μl for each run. Data processing and calculations were done by Chromatopak CR-7A software (Shimadzu Corporation, 1 Nishinokyo Kuwabara-cho, Nakagyo-ku, Kyoto 604, Japan).

#### 2.3.3. Preparation of reagents

Abamectin derivatives were prepared following the procedure of Diserens and Henzelin (1999) in which 1 volume of trifluoroacetic anhydride (TFA) is added to two volumes of $CH_3CN$ (reagent 1) and one volume of 1-methylimidazole to one volume of $CH_3CN$ (reagent 2). Reagent 1 (300 μl) and 200 μl of reagent 2 were added to the sample vial containing the dry residue of the abamectin extract or standard. Reagents were freshly prepared on a daily basis.

### 2.4. Standards and recovery

Standard pesticides were purchased from Chem Service (West Chester, PA, USA). Trifluoroacetic anhydride (TFA) and 1-methylimidazole were purchased from Sigma chemical company (St. Louis, MO, USA). All solvents were of HPLC grade. Chemicals and reagents were of analytical grade.

Analytical standards were prepared from a stock solution of 1000 μg/ml. Dilutions of this stock solution to obtain three levels of concentration containing 50, 100 and 250 ng of abamectin was carried out. The standards were then dried under a stream of nitrogen and the reagents were added as described previously and injected to the HPLC system from which a calibration curve was constructed. Concentration levels for the calibration of flufenoxuron were 1, 0.2, and 0.02 μg/ml and those for amitraz were 1, 0.5, and 0.1 μg/ml.

Recovery studies were carried out by spiking 3 replicates of untreated date samples (control) with 50, 100, and 50 μg/kg of abamectin, amitraz and flufenoxuron, respectively. Samples were analyzed using their prescribed procedure and mean values of the three replicates were calculated. Recovery percentages were satisfactory for the three acarcides and ranged from 92% to 101%. The minimum detection limits of abamectin, flufenoxuron and amitraz were 0.005, 0.001, and 0.003 mg/kg, respectively.

## 3. Results and discussion

The methods used to detect the three acaricide residues comprise an efficient extraction of each residue followed by clean up on $C_{18}$ and silica solid phase extraction (SPE) cartridges. Interferences from high sugar content of date samples were eliminated after the second SPE clean up on silica cartridges. This additional clean up step improved the recovery of abamectin and flufenoxuron residues up to 100%. The HPLC/FL, GC/μ-ECD and GC/NPD chromatograms were highly selective and sensitive for the detection of low amounts of the three acaricides as low as 0.005, 0.003, and 0.001 mg/kg for abamectin, amitraz and flufenoxuron, respectively. Other methods of sample preparation by cation-exchange cartridges (Prabhu, Wehner, Egan, & Tway, 1991), antibody mediated clean up (Li & Qian, 1996), adsorption chromatography on alumina and SPE (Doherty, Fox, & Fink, 1998), supercritical fluid extraction (Brooks & Uden, 1995), and matrix solid phase dispersion (MSPD) (Schenck, 1995; Valenzuela, Popa, Redondo, & Manes, 2001), have been employed to extract abamectin and other macrocyclic lactones from different matrices, none were specific to dates or other fruits with high sugar content. However, methods used for clean up of flufenoxuron from grapes (Miliadis et al., 1999) and amitraz from honey (Korta et al., 2001) were modified to suit dates. The analytical method for abamectin was derived from previous methods using abamectin fluorescent derivatives (Diserens & Henzelin, 1999; Valenzuela et al., 2001; Yoshii, Kaihara, Tsumura, Ishimitsu, & Tonogai, 2000). Analytical methods for flufenoxuron and amitraz followed our laboratory procedure for detection of multi-residue pesticides in food products using specific detectors.

The results in Table 1 show that the initial deposit of abamectin on dates of *Nabout Sief* type was 0.09 mg/kg and gradually with time the rate of residue decline reached 66% after 7 days and 88% after 14 days of application. This indicates that after 7 days of treatment, which is only 3 days before the PHI, the amount of abamectin was 0.03 mg/kg and this exceeded the upper limit of the maximum allowed residue set by the Codex Committee on Pesticide Residues under the Joint FAO/WHO Food Standards Program at 0.01–0.02 mg/kg for fruits (FAO/WHO, 1997). After 14 days of treatment, which is 4 days after the PHI, the amount of residual abamectin was 0.02 mg/kg which still exceeded the lower limit of the MRL set at 0.01 mg/kg, but lied within the upper limit of 0.02 mg/kg. At that time the fruit is expected to be ready for sale. Residues of flufenoxuron and amitraz did not exceed the minimum residue limit of 0.1 and 0.01 mg/kg, respectively, recommended by the Codex Committee on Pesticide Residues after the manufacturer's recommended post-harvest interval (PHI) of 50 and 28 days for flufenoxuron and amitraz, respectively.

Table 1
Initial residue deposit and residue decline of abamectin, flufenoxuron and amitraz on dates

| | Amount (mg/kg)[a] | Rate of residue decline |
|---|---|---|
| Abamectin | MRL = 0.01 | |
| PHI = 10 days | | |
| Initial deposit | 0.09 (0.017) | |
| 7 days | 0.03 (0.010) | 66 |
| 14 days | 0.02 (0.015) | 88 |
| Flufenoxuron | MRL = 0.1 | |
| PHI = 50 days | | |
| Initial deposit | 0.68 (0.14) | |
| 16 days | 0.22 (0.13) | 68 |
| 52 days | 0.07 (0.01) | 90 |
| 60 days | 0.02 (0.01) | 96 |
| Amitraz | MRL = 0.01–0.05 | |
| PHI = 28 days | | |
| Initial deposit | 0.34 (0.15) | |
| 21 days | 0.02 (0.01) | 95 |
| 30 days | Not detected | 100 |

[a] Average of three replicates, the value between parenthesis is the standard deviation.

The acceptable daily intake (ADI) for abamectin and amitraz in fruits and vegetables was set by FAO/WHO at 0.01 mg/kg (body weight) daily is based on regular consumption of fruit. In Saudi Arabia, and other neighboring countries, natives consume more date than an average person living outside this region. An average person in Saudi Arabia will consume, not less than 100 g daily of dates (about 10 dates), which is the highest in the world. Such high date consumption could lead to a higher risk of exposure to pesticides, especially in children and other vulnerable individuals. International agencies usually base the estimated ADI, and MRL values on average consumption in the world. Saudi Arabia and its surrounding nations will exceed the average date consumption by at least 10-folds. Therefore, the value of ADI for dates in Saudi Arabia should be set at a much lower level than the value set for other fruits. Accordingly, the PHI should be revised for abamectin and the MRL of pesticides used on dates in Saudi Arabia should be set to at least 10-folds less than the value set for other commodities.

## References


Anon. (2004). The fruit of the desert. Saudi Date Market, Al-Butain Agricultural Cooperative Association (BACA), issue June 7, 2004. Available from: http://www.dates.com.sa/SaudiDates/SaudiDates.htm.

Brooks, M. W., & Uden, P. (1995). The determination of abamectin from soil and animal tissue by supercritical fluid extraction and fluorescence detection. *Pesticide Science, 43*, 141–146.

Diserens, H., & Henzelin, M. (1999). Determination of abamectin residues in fruits and vegetables by high-performance liquid chromatography. *Journal of Chromatography, A833*, 13–18.

Doherty, S. J., Fox, A., & Fink, D. W. (1998). Liquid chromatographic determination of ivermectin in feed. *Journal of AOAC International, 81*, 869–872.

FAO/WHO. (1997). Joint FAO/WHO Food Standards Program, Codex Committee on Pesticide Residues, CX/PR 97/9, February 1997, p. 159.

Korta, E., Bakkali, A., Berrueta, L., Gallo, B., & Vicente, F. (2001). Study of semi automated solid phase extraction for the determination of acaricide residues in honey by liquid chromatography. *Journal of Chromatography A, 930*, 21–29.

Li, J., & Qian, C. (1996). Determination of avermectin B1 in biological samples by immunoaffinity column cleanup and liquid chromatography with UV detection. *Journal of AOAC International, 79*, 1062–1067.

Miliadis, G. E., Tsiropoulos, N. G., & Aplada-Sarlis, P. G. (1999). High-performance liquid chromatographic determination of benzoylurea insecticides residues in grapes and wine using liquid and solid-phase extraction. *Journal of Chromatography A, 835*, 113–120.

Pesticide Manual (1994). (10th ed., pp. 3–4). Hampshire, UK: Crop Protection Publications, British Crop Protection Council.

Prabhu, S. V., Wehner, T. A., Egan, R. S., & Tway, P. C. (1991). Determination of 4″-deoxy-4″-(epimethylamino)avermectin b1 benzoate (MK-0244) and its delta 8,9-isomer in celery and lettuce by HPLC with fluorescence detection. *Journal of Agricultural and Food Chemistry, 39*, 2226–2230.

Schenck, F. J. (1995). Isolation and quantification of ivermectin in bovine milk by matrix solid phase dispersion (MSPD) extraction and liquid chromatographic determination. *Journal of Liquid Chromatography, 18*, 349–362.

Turner, M. J., & Shaeffer, J. M. (1989). Biochemistry. In W. C. Campbell (Ed.), *Ivermectin and abamectin* (pp. 73–88). New York, NY: Springer.

Valenzuela, A. I., Popa, D. S., Redondo, M. J., & Manes, J. (2001). Comparison of various liquid chromatographic methods for the analysis of avermectin residues in citrus fruits. *Journal of Chromatography A, 918*, 59–65.

Yoshii, K., Kaihara, A., Tsumura, Y., Ishimitsu, S., & Tonogai, Y. (2000). Liquid chromatographic determination of emamectin, milbemectin, ivermectin and abamectin in crops and confirmation by liquid chromatography–mass spectrometry. *Journal of Chromatography A, 896*(1/2), 75–85.