

Residue levels of preharvest-sprayed amitraz in date fruits

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ABSTRACT

The residue levels of pre-harvest-sprayed amitraz on date fruits has been investigated. Date palm tree, variety of Sukkari, was sprayed with amitraz 20 % EC at the rate of 200 ml / 100 L (49 g a.i. / 100 L). Amitraz residues in date fruits were determined at different time intervals using high performance liquid chromatography (HPLC) with UV detection at 313 nm. The method was based on the extraction with acetone followed by partitioning with a mixture of n-hexane: ethyl acetate (80 : 20, V / V). The results showed that, the dissipation of amitraz was found to follow the following equation: $Y = 79.39 - 10.11 \ln X$ with correlation coefficient (r) more than 0.96. The initial deposit of amitraz on date fruits was 0.889 mg / kg, while it decreased to 0.304 mg / kg after eight weeks of spraying. The $t_{1/2}$ was 18.9 days. The limits of detection and quantification were 0.027 and 0.01 mg / kg, respectively. The analysis was done by using spiked date fruits at the levels of 0.50, 0.75, and 1.0 mg / kg. The corresponding recoveries were 88.10, 105.50 and 94.11 %, with relative standard deviations of 7.63, 8.68, and 14.79 %, respectively.

INTRODUCTION

The modern agricultural productions in high yield and high quality significantly depend on the use of agrochemicals to control growth and pests. Generally speaking, more than 600 kinds of agrochemicals are used worldwide (Miyake *et al.*, 1999). Amitraz [N'- (2,4-dimethylphenyl)-N-(((2,4-dimethyl)imino)methyl)-N-methyl-methanimidamide] a formamidine insecticide and acaricide used primarily to control pear psylla, whiteflies and mites on cotton, pears, apples, and citrus fruits (Hornish *et al.*, 1984; EPA, 1996); livestock ticks, lice, and mange mites on beef and dairy products (Queiroz-Neto *et al.*, 1994; Yaramis *et al.*, 2000). Also, amitraz is currently employed by beekeepers in Europe to control *Varroa jacoboni* in hives (Martel and Zeggane, 2002). Amitraz is available commercially as Mitac, Taktic, Ovasyn, Byebye, Edrizar, Parsec, Racet and Sender (Tomlin, 2000) and used at rates up to 1 and 3 lb. a.i. / acre on cotton and pears,

respectively (EPA, 1996). It is unstable under moist conditions (Anonymous, 1987). The Chemical Structure of amitraz is shown in Figure (1).

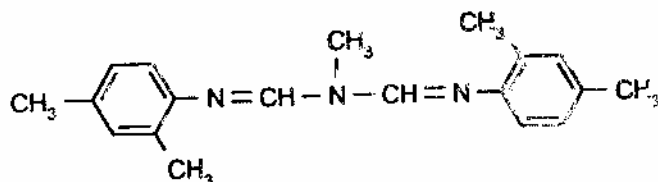


Figure (1). Chemical structure of amitraz

Pesticide residue analysis in food nowadays has a priority objective in pesticide research in order to get an extensive evaluation of quality and avoid possible risks to human health. Amitraz has been classified as a slightly hazardous (WHO, 1996), and it may produce many behavioral and physiological changes in mammals (Moser and MacPhail, 1989; Yaramis *et al.*, 2000) and all the clinical and biological effects are attributed to the parent compound itself (Hugnet *et al.* 1996). Quantitative methods for amitraz determination in agriculture products have been reported using gas chromatograph (GC) with either nitrogen-phosphorus detector (Nakamura *et al.*, 1989), electron capture detector (Taccheo *et al.*, 1988; Sánchez-Brunete *et al.*, 2002) or thermoionic detector (Iwata *et al.*, 1985) as well as high performance liquid chromatography (HPLC) (Tseng *et al.*, 1999; Martel and Zeggane, 2002). Some methods depend on the conversion of amitraz residues to 2,4-dimethylaniline (2,4-DMA) by base hydrolysis or derivatization with heptafluorobutyric anhydride (Hornish *et al.*, 1984; Hemmerling *et al.*, 1991; FDA, 1994). Amitraz is allowed to be applied to date palm in Saudi Arabia at rate the of 200 ml / 100 L to control mites. Fruits of the date palm (*Phoenix dactylifera* L.) are vital components of the diet in Saudi Arabia and there is increasing concern by consumers about both amitraz residues in date fruits and their carryover in its products. There is no information available about the residues of amitraz in date fruits.

Therefore, the present study aimed to examine the suitability of the Tseng *et al.*, (1999) method for the detection of amitraz residues in dates after different time intervals following one application using HPLC with UV detection. Although, the adoption of a method for specific commodity does not mean that it cannot be applied to other materials, it is necessary to evaluate the efficiency and suitability of the known methods when new products, such as dates, need to be examined.

MATERIALS AND METHODS

1. Chemicals. Amitraz, 20 % EC was purchased from Huppy Sananada Company, China. Acetone, n-hexane and ethyl acetate used in this study were analytical grade and obtained from Winlab Limited Company (UK). Certified HPLC-grade acetonitrile as well as water for UV, sodium chloride, sodium bicarbonate and anhydrous sodium sulfate were obtained from BDH Company. Florisil (60 - 100 mesh) and the standard amitraz (99 % purity) were purchased from Riedel-de Haën (Germany). Florisil is stored for at least 24 hr prior to use at 130 °C.

2. Application of amitraz. The variety of Sukkarī, the most widely grown variety of date grown in Al-Qassim region was selected in this study. Date palm tree cultivated in El-Soltan Farm, north of El-Meledia District, Al-Qassim area was arranged in a randomized design and sprayed at the end of May 2002 with amitraz, 20 % EC at the rate of 200 ml / 100 L using a motor sprayer calibrated to deliver 10 L / tree. Treatment was done in triplicate, 8 trees for each replicate were considered. Untreated trees were treated only with water and kept as control. Date fruits were collected after 0, 2 and 4 hr; and 1, 2, 4, 7, 14, 21, 28, 42 and 56 days from application to determine the residues. Samples were immediately transported to the laboratory and kept at -15 °C until residue analysis.

3. Extraction. Amitraz was extracted according to the method of Tseng *et al.*, 1999 with some modification. The extraction method for amitraz is summarized in Figure 2. The samples were removed from freezer and allowed to warm up to room temperature. Three of 20-grams samples from each time interval were removed, cut into small pieces, and then 2 g of sodium hydrogen carbonate and 60 ml acetone were added in a blender jar. The sample was macerated for 5 min, and the extract was vacuum-filtered. After filtration, the pellets were washed with 40 ml of acetone and then filtered. The combined filtrates were transferred into a separatory funnel containing 1 g sodium chloride and then extracted twice with 40 ml of n-hexane: ethyl acetate (8 : 2, V / V) for 2 min. The combined organic layers were passed through a funnel containing anhydrous sodium sulfate and then evaporated to just dryness at 35 - 40 °C using a rotary evaporator. The residue was dissolved in 5 ml of n-hexane: ethyl acetate (8 : 2, V / V) and then applied on a florisil column for cleanup.

4- Column cleanup. The extract was subjected to a mini glass column chromatography packed with activated florisil. The florisil was topped with 1-cm layer of anhydrous sodium sulfate. The column was washed with 5 ml of n-hexane before loading the extract and eluted with 10 ml of n-hexane: ethyl acetate (8 : 2, V / V). The eluate was evaporated to just dryness and then dissolved in 2 ml of acetonitrile. The final solution was filtered through a 0.45 μ m sterile disposable filter nylon (AcroCap, Gelman Sciences) prior to HPLC analysis.

5. Spiking. Three of 20-grams from untreated control were taken and treated as individual replicates of the entire sample and treated identically. Spiking was done at the levels of 0.50, 0.75 and 1.0 mg / kg. In addition to the spiked samples, one date fruit blank and one solvent blank were run concurrently with the extraction set. Recoveries for amitraz were calculated after HPLC analysis.

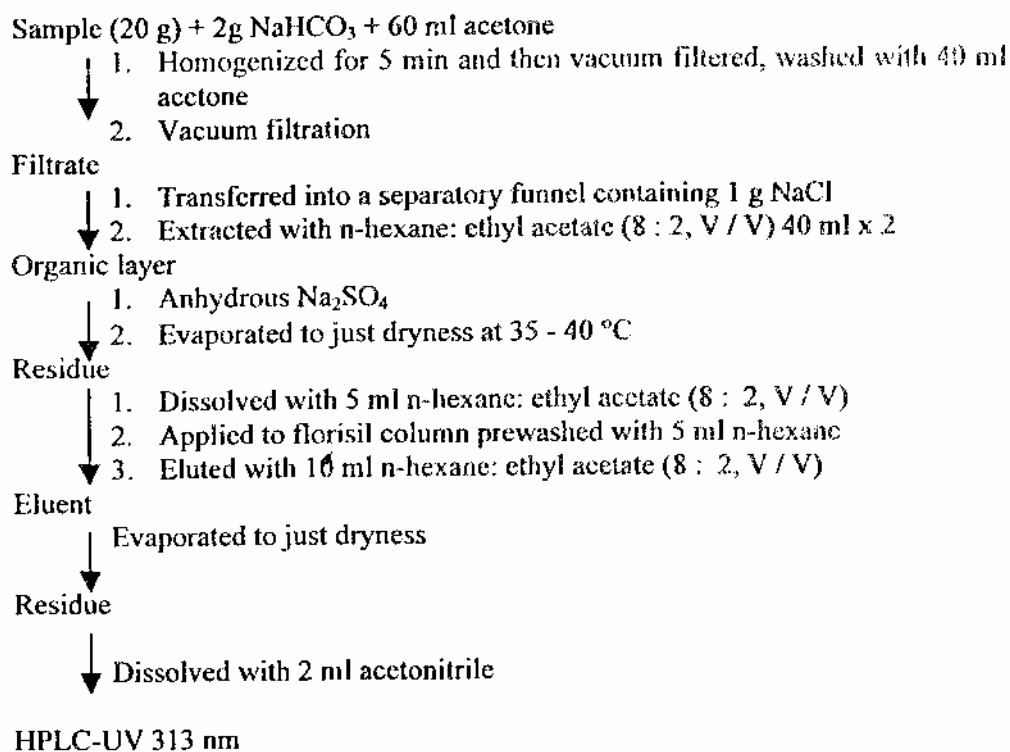


Figure (2). Analytical procedure for determining amitraz residue in date fruits.

6. Determination using HPLC. A Perkin Elmer HPLC system model 200 equipped with a degasser, quaternary LC pump model 2000Q / 410, 20 μ l loop, MicroPack MCH-NCAP5 column (25 cm x 4.6 mm i.d., 5 μ m, Varian), oven column, a LC200 UV detector, and Turbochrom Workstation Software package for instrument control, data acquisition, and data analysis were used in this study. The column temperature was kept at 25 °C. The UV detector was set at 313 nm. The mobile phase system was acetonitrile: water (80 : 20, V / V) at the flow rate of 0.5 ml / min.

7. Preparation of standard solution. Ten concentrations of amitraz ranging from 0.10 to 8 mg / kg were prepared in acetonitrile from a stock solution of 1000 mg / kg, and 20 μ l of each was injected to HPLC. The standard curve in height versus concentration were plotted and their linear equation was calculated after a regression analysis. The amount of amitraz in each sample was thus calculated based on the standard curve.

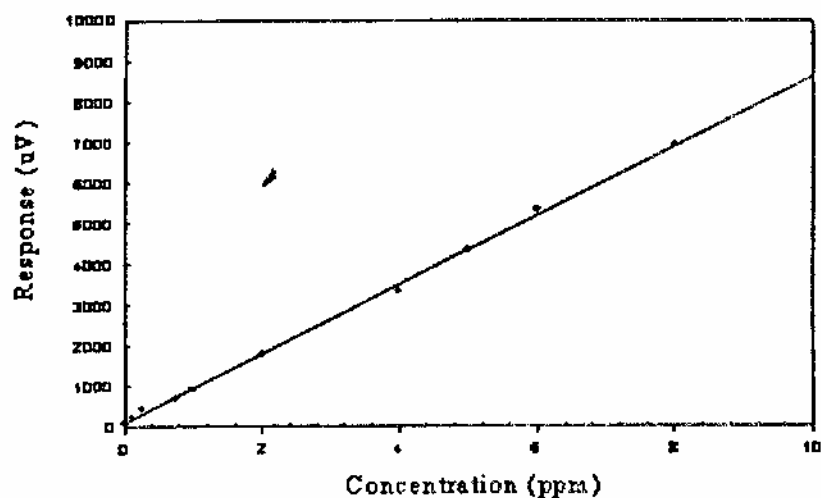
8. Detection and Quantification Limits. The limit of detection (LOD) with this procedure was defined as the concentration of the pesticide in date fruits gave three times noise ($S / N = 3$) within its retention time (t_R) window. The limit of quantification (LOQ) for the method validation was defined as the response equivalent to 10 % of full scale deflection (FSD) on the signal-processing device (FDA, 1994) and calculated by using the following formula:

$$\text{LOQ} = (\text{ng } 10 \% \text{ FSD}) / (\text{mg sample injected}).$$

RESULTS AND DISCUSSION

1. Standard curve. The HPLC response was linear ($r^2 > 0.99$) for amitraz in the range of 0.10 to 8 mg / kg (Figure 3). Under the experimental conditions, the HPLC retention time (t_R) was 8 min. The equation of the curve in standard solutions at 313 nm was $Y = 14.08 + 857.41X$.

2. Recovery. Data in Table (1) illustrate the recoveries of spiked samples at the levels of 0.50, 0.75, and 1.0 mg / kg. The corresponding recoveries of amitraz from control date fruits were 88.10, 105.50 and 94.11 % with relative standard deviations of 7.63, 8.68 and 14.79 %, respectively. The



Figure(3) Standard curve for amitraz

over-all mean of recovery was 95.90 ± 8.84 %. The recoveries of amitraz at the levels of 0.25 - 0.75 mg / kg for apples and 0.10 - 0.30 for grapefruits were 88.80 - 92.10 % and 87 - 90.90 %, respectively (Tseng *et al.*, 1999). The percentage of recoveries for the present study can be considered satisfactory according to the guidelines of Schuller *et al.*, (1976) who stated that any method that showed recovery of 70 % or more could be eligible for future official adoption. Also, the low RSDs indicate a high level of repeatability for the tested method.

Table (1): Mean recoveries of amitraz from spiked date fruits.

Amount spiked (mg / kg)	No of determination	% of Recovery	% of Relative standard deviation (RSD)
0.50	3	88.10	7.63
0.75	3	105.50	8.68
1.00	3	94.11	14.79

3. Fate of amitraz in date fruits. Data in Table 2 illustrate the levels of amitraz in date fruits following one application at the rate of 200 ml / 100 L (49 g a.i. / 100 L.) at different time intervals up to 56 days. During the experiment course, the amitraz decreased from 0.889 mg / kg at zero time to 0.512, 0.487, 0.428, 0.398, 0.364 and 0.304 mg / kg after 1, 2, 3, 4, 6, and 8 weeks, respectively, from amitraz application with an over-all mean of 0.60 ± 0.21 mg / kg. The present results show that during the first week, amitraz residues decreased more rapidly than the following periods, where about 42 % of residues dissipated. No maximal limit of amitraz residues was established on date fruits. The residue level after 14 days of spraying was 0.487 mg / kg which is less than the maximum residue limits (MRLs) for either pome fruits or oranges i.e. 0.5 mg / kg (FAO / WHO, 1985).

Table (2): Amitraz residues in pre-harvest-sprayed date fruits after different time intervals.

Interval (day)	Amitraz level (mg / kg) Mean $\pm$ S.D	% of Initial deposit	% of RSD
0	0.889 $\pm$ 0.100	100.0	11.25
2 / 24	0.884 $\pm$ 0.070	99.43	13.59
4 / 24	0.833 $\pm$ 0.120	93.70	8.29
1	0.782 $\pm$ 0.044	87.96	5.63
2	0.673 $\pm$ 0.100	75.70	14.86
4	0.601 $\pm$ 0.090	67.60	14.98
7	0.512 $\pm$ 0.130	57.59	25.39
14	0.487 $\pm$ 0.060	54.78	12.32
21	0.428 $\pm$ 0.082	48.14	19.16
28	0.398 $\pm$ 0.090	44.97	22.61
42	0.364 $\pm$ 0.072	40.94	19.78
56	0.304 $\pm$ 0.050	34.20	16.45

Residue-dissipation curve for amitraz in date fruits was plotted on natural log paper (Figure 4) and tabulated in Table (3). The rate of amitraz loss was found to follow the following formula: $Y = 79.39 - 10.11\text{Ln}X$ with

correlation coefficient (r) higher than 0.96. The $t_{1/2}$ (the time required to decrease the initial deposit of amitraz to 50 %) of amitraz was 18.9 days.

In terms of sensitivity, this method, with detection limit of 0.027 mg / kg can satisfy the requirements set by the international regulations for the limits of maximum residues which are usually at the mg / kg level for the majority of pesticides and ppb for some others. The present results are in agreement with the results of Tseng *et al.*, (1999) who found that the LOD of amitraz was 0.02 mg / kg in fruits of apples and grapefruits. Also, LOQ for the present method validation was found to be 0.01 mg / kg. Interferences from co-extractives raise the LOQ of a method by masking the detector response to the residue or by preventing injection of the specified sample equivalent without undesirable damage to the system (FDA, 1994). Additional procedures to cleanup the sample extract prior to determination may improve the LOQ by removing these interferences.

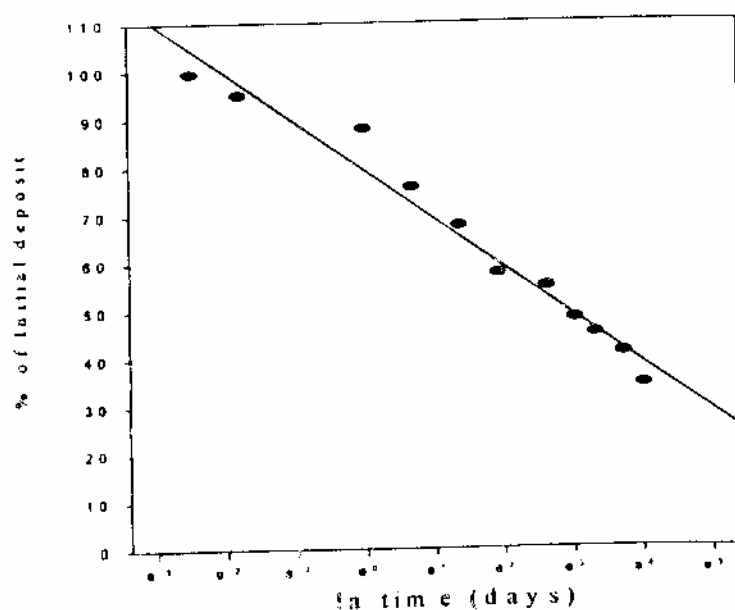


Figure (4). Dissipation curve of amitraz in date fruits

Table (3): Half-life value, limit of detection (LOD), and limit of quantification (LOQ)

λ	Regression Equation	Correlation Coefficient (r)	Half-life ($t_{1/2}$)	LOD	LOQ
313 nm	$Y = 79.39 - 10.11 \ln X$	0.9699	18.9 days	0.027 mg / kg	0.01 mg / kg

In conclusion, HPLC method has been applied for the determination of amitraz in date fruits using reversed-phase chromatography with UV detection. The method is rapid and highly sensitive, with satisfactory recoveries. Moreover, this method permits the determination of amitraz residues at ppb levels. The maximum residue limits for amitraz residue was 0.5 mg / kg for various commodities (FAO / WHO, 1985). The list, however, lacks MRLs for this insecticide on date palm. So, dates fruits could be marketed with apparent safety for human consumption when these fruits were free from any residues of amitraz.

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مستوي متبقيات مبيد الأميتراز في ثمار نخيل البلح

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السعودية

تم تقدير مستوى متبقيات مبيد الأميتراز علي ثمار نخيل البلح من الصنف السكري وذلك بعد رش المبيد علي الأشجار بمعدل ٢٠٠ مل / ١٠٠ لتر. وقد تم تقدير متبقيات المبيد بعد الرش علي ثمار النخيل بعد فترات مختلفة باستخدام جهاز التحليل الكروماتوجرافي عالي الأداء باستخدام كشاف الأشعة فوق البنفسجية وعلي طول موجة ٣١٣ نانوميتر. وتعتمد طريقة التحليل المستخدمة علي استخلاص المبيد بواسطة الأسيتون ثم توزيعه مع مخلوط من الهكسان العادي : خلاص الإيثانول (٨٠ : ٢٠ حجم / حجم). هذا وقد تم استخدام عمود كروماتوجرافي ذو وجه معكوس ومخلوط من الأسيتون/هكسان : ماء (٨٠ : ٢٠ حجم / حجم) كوجه متحرك وبمعدل سريان قدره ٠,٥ مل / دقيقة.

أوضحت النتائج أن اختفاء مبيد الأميتراز يتبع المعادلة التالية $Y = 79.39 - 10.11 \ln X$ وكانت قيمة معامل الارتباط أكبر من ٠,٩٦ وتوضح النتائج أن التركيز الابتدائي لمبيد الأميتراز يساوي ٠,٨٨٩ جزء في المليون قد تناقص ليصبح ٠,٣٠٤ جزء في المليون بعد ٨ أسابيع من رش أشجار النخيل وأن فترة نصف عمر المبيد ($t_{1/2}$) علي أشجار النخيل تساوي ١٨,٩ يوم. أيضا أظهرت النتائج أن قيم حدود التعرف وصلاحية الطريقة المستخدمة تساوي ٠,٠٢٧ ، ٠,٠١ جزء في المليون علي التوالي. وقد تم حساب نسبة استرجاع المبيد بعد عمل تقوية لعينات ثمار النخيل غير المعامل بمستويات قدرها ٠,٥ ، ٠,٧٥ ، ١ جزء في المليون وكانت نسبة الاسترجاع المتحصل عليها ٨٨,١٠ ، ١٠٥,٥٠ ، ٩٤,١١ % وانحراف قياسي قدره ٧,٦٣ ، ٨,٦٨ ، ١٤,٧٩ % علي التوالي.