

## **Persistence of Triadimefon Residues in Vegetable Fruits Grown in Green Houses: A Study Demonstrating Hazards of Pesticide Misuse in Saudi Arabia**

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**Abstract.** Dissipation rates and residue levels of triadimefon in fruits of tomato, squash, cucumber and pepper grown in commercial green houses were determined using HPLC. Producing Plants were thoroughly sprayed with a spray liquid of triadimefon at 250 g a.i./100 l of water using the commercially recommended formulation (Bayleton 25 WP). The average recovery of triadimefon from these fruits using the employed analytical method was found to be 83.46 to 90.32%. The detection limit of triadimefon by the utilized method was 37 ppb. Analysis of fruits collected at different intervals following the fungicide application showed that the initial deposits of triadimefon on pepper, squash, cucumber and tomato were  $191.82 \pm 21.8$ ,  $109.83 \pm 4.6$ ,  $75.63 \pm 9.3$  and  $72.46 \pm 7.8$  ppm, respectively. These levels declined by the time reaching  $79.29 \pm 4.0$ ,  $29.33 \pm 0.8$ ,  $29.03 \pm 4.0$  and  $20.76 \pm 0.1$  ppm in the four crops, respectively after 7 days. The residue half lives of triadimefon in fruits of these four crops were estimated to be 4.54, 2.7, 4.12 and 2.6 days, respectively. These results indicated that a post-treatment period of 12.2, 9.0; 11.7 and 8.9 days after triadimefone application must be allowed before harvesting pepper, squash, cucumber and tomato respectively in order to produce fruits within the maximum residue limit (MRL) recommended by FAO (0.5 ppm) for tomatoes. Practically, this cannot be achieved in these crops so the use of triadimefon at such a high level should be prohibited on these crops.

### **Introduction**

Tomato, cucumber, pepper and squash are considered important vegetable crops in Saudi Arabia. Generally, these vegetables are infested by many plant diseases [1]. Great concern has been demonstrated regarding pesticide residues and their health

and environmental impacts as a result of the heavy use of pesticides in this country during the last 10 years [2]. Human poisoning by pesticides throughout the world has increased from 500,000 cases per year in 1972 to 25,000,000 case/yr in a 1990 estimate [3]. Among 148 outbreaks (excluding Bhopal and three probable epidemics of pesticide-related suicide) reported between 1951-90, the known number of cases was 24,731 with 1065 deaths (4.3% case fatality). These figures, however, are probably under estimates [3-5].

Food was the most common vehicle of exposure in all recorded epidemic outbreaks of pesticide poisoning. However, there are only two reports recording incidents of food-poisoning due to contamination with pesticides in Saudi Arabia. The first report recorded a pesticide poisoning episode which occurred simultaneously in both Qatar and Saudi Arabia in 1967 due to consumption of flour contaminated with Endrin during international transport [6]. The second was a report on poisoning cases which occurred in Al-Qassim, Saudi Arabia due to eating desert locusts collected from Fenitrothion-treated locations [7]. There is a growing feeling, however, that the health impact of pesticides in Saudi Arabia during the last 15 years may have been underestimated and is much more likely to affect both people and their environment. Lack of legislation, non-enforcement, widespread ignorance of the hazards involved, poor labeling, inadequate supervision and not wearing protective clothing in the hot climate greatly increase the hazard both to agricultural workers and the general public.

Triadimefon is recommended as fungicide for seed treatment or foliar spray and displays marked systemic activity against several plant diseases of cereals [8-13]. In Saudi Arabia, triadimefon is being used to protect cereals and vegetables from infestation by many fungal diseases. Data on triadimefon uptake have so far been published only in respect of its absorption by barley and cucumber leaves [14,15].

Many investigators have determined the residues of triadimefon on vegetable crops at different periods after spray under field conditions [14,18]. However, few studies have been carried out to determine residues of triadimefon in and on crops grown under plastic covers [19].

Several recent reports indicate that triadimefon has neuro behavioral effects that are similar to those of psychomotor stimulants. For example, triadimefon increases overall fixed-interval (FI) response rate, disrupts FI response patterning, increases motor activity and produces stereotypes at high doses [20-23].

Triadimefon was evaluated for cytogenetic and cytotoxic effects in mammalian test systems. The frequency of chromosome aberrations and micronuclei in bone

marrow cells and the arginase enzyme profile in the liver, tend to show the genotoxicity of this fungicide in a single-exposure response study [24].

Eight pesticides including triadimefon were tested in a medium-term (6 weeks of exposure) bioassay based upon the induction of preneoplastic lesions in the rat liver. Hepatocarcinogenic potential was assessed by comparing the number and area of glutathione S-transferase placental form positive foci in the liver with those of controls given diethylnitrosamine alone. Positive results were seen with triadimefon indicating its possible carcinogenic activity [25].

Growing crops under plastic houses in Saudi Arabia is of great economic importance. Therefore, it has been increased drastically in the past few years. We have demonstrated that pesticides are misused in most cases by end users in this country specially with regard to selection, method and rate of application and timing of both application and harvesting specially in green houses where harvesting in most cases does not take residues into consideration. Therefore, this study was designed to monitor the persistence of triadimefon residues on fruits of pepper, tomato, cucumber and squash grown under plastic houses and to find out the safety period required before harvesting these vegetables in Saudi Arabia.

## Materials and Methods

### Chemicals

A purified sample of triadimefon [1-(4-chlorophenoxy)-3,3-dimethyl-1H-1,2,4-triazol-1-yl] butanone], (99.0% purity) was purchased from Chem. Service Co., USA (lot # 30-107A). A formulated sample of triadimefon (Bayleton, 25% wp) was obtained from Al-Amar group, Riyadh, Saudi Arabia, the local distributor of this Bayer product. All other chemicals used in this study were of the highest purity grade available from either Sigma or Aldrich Chemical Companies. Stock solution of pure triadimefon (0.1 g/100 ml) in methanol was prepared. Working standards were made by suitable dilutions of the stock solution with methanol and used for chromatographic analysis.

### Treatment and sample preparation

Vegetables (tomato, squash, pepper and cucumber) producing plants grown in a commercial green house farm located 10 km north of Bureidah were sprayed with triadimefon formulation (aqueous dispersion) using a hand sprayer fitted with one nozzle boom in the same manner as that adopted by users in this area and at the same rate they usually use (1 kg formulated triadimefon per 100L of water). Homogeneous

coverage of the treated plants was attained. Untreated plots of each crop were left unsprayed as check plots. Caution was taken to avoid any drift among the treatments. Sprayed samples, (one fruit from each replicate), were collected for residue analysis at the following time intervals: zero, 1, 24, 48, 72 hrs and 7 days after treatment. Fruits were collected in plastic bags, transported to the lab and stored at  $-18^{\circ}\text{C}$  until they were used for analysis.

### **Recovery experiments**

Aliquots of the standard solution of triadimefon were added to 50 g portions of untreated tomato, squash, pepper or cucumber fruits. The fortification levels were carried out at three levels of triadimefon (10, 50, and 100 ppm). Each sample was mixed thoroughly, with 50 ml of distilled water and then blended in the presence of methylene chloride (100 ml). The mixtures were filtered through a Büchner funnel fitted with a glass sinter. The residue of each sample was washed with sufficient amount of methylene chloride. The combined filtrate and wash of each sample was partitioned in a separatory funnel. The organic layer was taken and the aqueous layer was saturated with sodium chloride and re-extracted with methylene chloride ( $2 \times 50$  ml). The combined organic layers were dried over anhydrous sodium sulfate and then rotary evaporated at  $40^{\circ}\text{C}$  under vacuum to dryness. The residue was dissolved in 5 ml of methylene chloride and subjected to clean up on a silica gel-charcoal column (1:1) using methanol as the eluting solvent. The eluate was concentrated by rotary evaporation to 5 ml and kept in a freezer until analysis.

### **Residue determination**

Stored fruit samples of each crop at each time interval after spraying with triadimefon along with the corresponding untreated controls were taken from the freezer and left to thaw. After complete thawing, fruits were weighed and used for triadimefon residue analysis. The residue analysis was carried out as described under recovery experiments.

### **Liquid chromatographic analysis and quantification**

This was made by a Varian-VISTA 5500 High performance liquid chromatograph (HPLC), equipped with an ultra-violet detector (UVD) and an analytical stainless steel column ( $30 \times 0.5$  cm id),  $\text{MCH}_{10}$ . The HPLC analysis was conducted using an isocratic elution system with methanol at a wavelength of 240 nm. Identification was accomplished by retention time and compared with triadimefon known standard at the same conditions. The peak areas corresponding to each injected sample were compared with that of standard solutions of the fungicide and used for quantification.

### Construction of calibration graph

Solutions of the pesticide standard prepared at six concentration levels 1,5,25,50,75 and 100 ng/ml were chromatographed on the HPLC system at the same conditions. A calibration graph was constructed by plotting the peak area on the Y-ordinate and the corresponding concentrations on the X-ordinate.

### Quantification limit

Detection limit for triadimefon using the employed method was determined according to the analyte concentration that produces a chromatographic peak equal to three times of baseline noise. The detection limit was found to be 37 ppb.

### Kinetic analysis

The elimination rate constant, and slopes as parameters for the dissipation of triadimefon in vegetables were calculated using the following equation:

$$t_{1/2} = \ln 2/K = 0.693/K$$

where:  $t_{1/2}$  is the half-life value in days.

K is the elimination rate constant in  $\text{day}^{-1}$

The half lives and the time required to bring the residue limits to 0.5 ppm on each of the tested fruits were estimated by regression analysis of the obtained data using least square method [Statistical Analysis System Program (SAS)].

## Results and Discussion

The recoveries of triadimefon from the tested vegetable fruits at various spiking levels are presented in Table 1. The recovery values ranged from 89.57 to 90.98%, 76.38 to 99.63%; 82.82 to 97.39% and 70.70 to 98.64% in the case of tomato, pepper, cucumber and squash, respectively. These values demonstrate the applicability of the employed procedure and are similar to that recently reported for yellow pepper and some other vegetable fruits [26].

The residues of triadimefon in squash, pepper, tomato and cucumber fruits grown under plastic houses at different intervals after spraying are presented in Table 2. The results showed that the initial deposits of triadimefon in fruits of pepper, squash, cucumber and tomato determined directly after spraying were 191.82, 109.83, 75.63 and 72.46  $\mu\text{g/g}$ . The value of initial deposit in pepper was higher than

**Table 1. Percent recovery of triadimefon from tomato, pepper, cucumber and squash fruits after fortification at three levels**

Conc. in ug/g	Tomato	Pepper	Cucumber	Squash
100	89.57 ± 06.8	83.44 ± 7.7	97.39 ± 01.2	80.05 ± 4.4
50	90.41 ± 06.8	76.38 ± 5.7	82.82 ± 01.9	98.64 ± 5.8
10	90.98 ± 10.0	99.63 ± 4.4	91.66 ± 11.6	70.70 ± 5.8
Average	90.32 ± 04.0	86.48 ± 4.6	09.62 ± 04.0	83.46 ± 4.0

Each value represents the mean ± S.E. of four samples.

**Table 2. Concentration of triadimefon in squash, pepper, tomato, and cucumber fruits following pesticide application**

Time	Squash	Pepper	Tomato	Cucumber
Zero	109.83 ± 4.6	191.82 ± 21.8	72.46 ± 7.8	75.63 ± 9.3
1 hr	086.42 ± 1.5	150.15 ± 22.4	53.83 ± 1.6	66.25 ± 6.1
24 hr	58.33 ± 4.8	135.02 ± 12.4	43.47 ± 0.9	41.99 ± 9.1
48 hr	44.19 ± 2.8	120.43 ± 7.90	24.16 ± 0.8	39.31 ± 4.2
72 hr	37.06 ± 4.5	087.80 ± 7.00	22.50 ± 2.5	37.08 ± 4.1
7 days	29.33 ± 0.8	079.29 ± 4.00	20.76 ± 0.1	29.03 ± 4.0

Concentrations are expressed as µg/g fruit.

Each value represents the mean ± S.E. of four samples.

that in the other tested vegetables. This can be attributed to the variation in the specific plant structure of these vegetable crops specially the surface area to weight ratio and the water contents of the fruits. After 1 hr the remaining amounts of triadimefon were reduced to 86.42, 150.15, 53.82 and 66.25 µg/g in squash, pepper, tomato and cucumber, respectively.

The corresponding values for these vegetables decreased by the time. These values were found to be 29.33, 79.29, 20.76 and 29.03 µg/g, respectively at 7 days after treatment. In general, it seems that the disappearance rate of triadimefon is very slow and the compound needs more time to reach the permissible dose in squash, pepper, tomato or cucumber. These findings may be due to the condition of planting in plastic houses where there is no direct contact between the compound and climate conditions such as light, moisture and/or heat and due to the huge rate that is being applied.

The kinetic parameters for the degradation of triadimefon were calculated and illustrated in Table 3. The data revealed that the loss of the pesticide in the examined vegetables can be described by the first order kinetics throughout the entire tested intervals. The disappearance phase of triadimefon for these vegetables had rate constants (K) of 0.267, 0.153, 0.168, and 0.257 day<sup>-1</sup> for tomato, pepper, cucumber and squash, respectively. These values lead to half-life ( $t_{1/2}$ ) values of 2.60, 4.54, 4.12, and 2.7 day, respectively. It seems logic to conclude that triadimefon dissipation was slow and its persistence was very high under plastic houses conditions when used at the adopted high rate of application.

**Table 3. Kinetic parameters for the dissipation of triadimefon in vegetable fruits under plastic houses condition**

Vegetable fruits	Elimination rate constant, K (day <sup>-1</sup> )	Half-life value $t_{1/2}$ (days)
Pepper	0.153	4.54
Cucumber	0.168	4.12
Squash	0.257	2.70
Tomato	0.267	2.60

In conclusion, the application of triadimefon on these vegetable crops to protect the fruit from plant diseases renders the vegetable fruits hazardous to the consumer if harvested during the first week after the last spray. The Joint Meeting of the FAO and WHO panel of experts on pesticide residues has suggested a maximum residue limit of 0.5 mg/kg for triadimefon in tomato [27]. The time required to decrease triadimefon residues in the tested vegetable fruits to this limit in our study was estimated by computing the obtained results and regression analysis. If we adopt this limit, it would need at least 8.9, 9.0, 11.7, and 12.2 days to bring triadimefon residue in tomato, squash, cucumber, and pepper, respectively, to this limit. Clearly this is not practical, so the use of this fungicide in controlling plant diseases in producing vegetables at such high concentration should not be considered specially when knowing the fact that the treated fruits are harvested daily or every other day for marketing.

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متبقيات مبيد الفطريات ترايادايميوفون بعد الرش  
على ثمار بعض الخضروات المزروعة في البيوت المحمية  
دراسة توضيحية للأضرار الناجمة عن سوء استخدام المبيدات في  
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ملخص البحث. تم تقدير معدلات اختفاء متبقيات مبيد ترايادايميوفون في ثمار الطماطم والكوسة والخيار والفلفل الرومي المزروعة في البيوت المحمية وذلك باستخدام التحليل الكروماتوجرافي السائلي عالي الكفاءة.

وقمت هذه الدراسة بمعاملة هذه النباتات في طور الإثمار بمستحضر رش يحتوي على ٢٥٠ جم من المادة الفعالة للمبيد في كل ١٠٠ لتر ماء باستخدام التجهيزة التجارية الموصى باستخدامها حقلياً (بايليتون ٢٥٪ مسحوق قابل للبلل). وقد تم تقويم طريقة التحليل المستخدمة في استخلاص وتقدير متبقيات المبيد على الثمار فكانت نسبة الاسترجاع للمبيد من الثمار تتراوح من ٤٦، ٨٣ إلى ٣٢، ٩٠٪، وكانت القيمة الدنيا لتقدير هذا المبيد باستخدام الطريقة المتبعة هي ٣٧ جزءاً في البليون.

وقد أوضحت النتائج أن التركيز الابتدائي المتخلف من المبيد على الثمار عقب الرش مباشرة كان ٨٢، ١٩١، ٨٣، ١٠٩، ٦٣، ٧٥، ٤٦، ٧٢ جزء في المليون في كل من الفلفل الرومي والكوسة والخيار والطماطم على الترتيب. وقد تناقصت هذه الكميات مع مرور الوقت حتى وصلت إلى ٢٩، ٧٩، ٣٣، ٢٩، ٠٣، ٢٩، ٧٦، ٢٠ جزء في المليون على الترتيب نفسه، وذلك بعد سبعة أيام من الرش، وقد تم حساب الزمن الذي تختفي عنده نصف كمية المبيد منذ بداية الرش فكان ٥٤، ٤، ٧، ٢، ١٢، ٤، ٦، ٢ يوم وذلك في كل من الفلفل الرومي والكوسة والخيار والطماطم على الترتيب.

وتوضح هذه النتائج أنه بعد رش هذه الخضروات بمبيد ترايادايميوفون يجب عدم جمع الثمار إلا بعد انقضاء ٢، ١٢، ٩، ٧، ١١، ٩، ٨، ٩ يوم بعد المعاملة بالمبيد في حالة كل من الفلفل الرومي والكوسة والخيار والطماطم على التوالي، وهي الفترة اللازم مرورها حتى تنخفض مستويات هذا المبيد إلى الحد الأقصى المسموح به (٥، ٠ جزء في المليون) والذي تقترحه منظمة الأغذية والزراعة العالمية في حالة الطماطم. ومن الناحية العملية لا يمكن أن يتوافر ذلك في حالة هذه المحاصيل الأربعة ولهذا فإن استخدام ترايادايميوفون بهذا المعدل المرتفع في إنتاج الخضروات يجب أن يتوقف.