

## Residual behaviour of hexythiazox and carbendazim pesticides on and in apple fruits under field conditions

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### ABSTRACT

Apple fruit trees were sprayed in the field with an acaricide® Maccomite acaricide 10% W.P (Hexythiazox) and kemazed fungicide 50% W.P (carbendazim) at the rates of 20 and 50 g of the formulation per 100 l water, respectively. Pesticide residues were determined on and in unwashed, washed, and washed-peeled fruits at intervals of zero (one hour after spraying), 1, 3, 5, 7, 14, and 21 days after application. The obtained results indicated that the residues in the unwashed and washed apple fruits were higher (0.816 and 0.530 ppm) than the maximum residual level (MRL = 0.5 ppm) when they were detected after five days of Hexythiazox application. The washed-peeled fruits contained lower residues of 0.443 after three days of application and these residues were rapidly decreased by time. The unwashed and washed apple fruits were found to contain 0.270 and 0.120 ppm after seven days of application, respectively and those levels were below the MRL. Moreover, the unwashed apple fruits could be marketed after 7 days from application of Hexythiazox. On the other hand the washed and washed-peeled fruits could safely used after 7 and 3 days of application, respectively. In the case of carbendazim application, it was found that the unwashed, washed and washed-peeled apple fruits contained 0.510, 0.300 and 0.112 ppm, respectively after one day of application and these concentrations were below the MRL (2 ppm). Therefore, apple fruits could be marketed with a certain apparent safety margin for human consumption directly after one day of carbendazim application. On the other hand, the hexythiazox residues half – lives in the unwashed, washed, and washed-peeled apple fruits were: 1.0578, 0.9404, and 0.9909 days, while they were 1.0529, 0.9636, and 1.1468 days, for carbendazim residues, respectively.

Moreover, it is obvious that washing by running tap water and washing followed by peeling process confirm an important role in reducing and eliminating pesticide residues from contaminated apple fruits. Whereas, washing and washing followed by peeling process of the treated apple fruits reduced hexythiazox residues by 23.58 – 55.56 % and 65.53 – 100%. In the case of carbendazim the reduction was found to be 41.18 – 100% and 68.18 – 100%, respectively during the experimental periods. The difference between the amounts of pesticide residues determined on and in apple samples of washed and unwashed fruits gives an idea about the surface residue that could be removed by washing, or and washing followed by peeling.

**Key words:** pesticides, hexythiazox, carbendazim, residues, half-lives, washing and peeling process.

## INTRODUCTION

Pesticides are largely used on apple (Apple orchards) during the period of the end of May to July. These pesticides leave their residues on and in treated fruits. So, the study of pesticides persistence and residual behaviour gives an idea about the pre-harvest intervals (PHI) that should be followed after a pesticide application and before marketing in order to minimize the health hazards.

Hexythiazox as an acaricide is used for controlling eggs and larvae of many phytophagous mites on fruits. On the other hand carbendazim as a fungicide is used for controlling *Venturia inaequalis* (scab) and *Podosphaera leucotricha* in pome fruits (Tomlin 2000). In Egypt, Hexythiazox a non-systemic with contact and stomach acaricide is a new registered compound (No. 1019) and recommended on apple fruits as Maccomite® 10% W.P (Nippon soda co.) for controlling spider mites. Carbendazim a systemic with protective and curative action fungicide is recommended on apple fruits under kemazed® 50% W.P (Kafer El-Zayate for Pesticide and Chemicals Co.) for controlling apple scab.

The present investigation aims to study the degradation of the acaricide Hexythiazox and the fungicide carbendazim residues on and in unwashed, washed, and washed-peeled apple fruits under the field conditions at different time intervals after application, and the role of washing and washing followed by peeling on reducing pesticide residues. Also, pesticide rates of degradation, half-lives values, and the safe interval after application and before marketing were determined.

Several studies were carried out to evaluate the degradation of hexythiazox which used to control mites on apples (Arnaudov, 1995, Costa *et al.*, 1997 and Laffi and Bevilacqua, 1999).

In addition studies in the performance of activity and fate of carbendazim were took place in controlling apple scab pathogen *V. inaequalis* (Thakur and Malhotra, 1999, Aalbers, 2001 and Pandey and Yadav, 2002).

Sharma *et al.* (1997) found that the pre-harvest interval (PHI) of carbendazim was 1.7-3 days after application. Dipping the treated apple in distilled water reduced Carbendazim residues by 27.3% (Himonish *et al.*, 2003).

Cano *et al.* (1987) and Sharma *et al.* (1991), reported that more than 80% of the carbendazim residues remained on peel and most residues were found on the apple peel and that amount in the pulp decreased towards the core when apple fruits were immersed in carbenazim solution.

## **MATERIALS AND METHODS**

The experiments were carried out at Kafer Shoker, El-Kalyoubia Governorate, to determine the residues of an acaricide (hexythiazox) and fungicide (carbendazim) in the unwashed, washed, and washed-peeled apple fruits at different periods after pesticides application on apple fruit trees. The experimental plot area was 13 x 13 meters (almost about  $\frac{1}{24}$  fed.). The tested pesticides were applied on May 21<sup>st</sup>, 2004. These pesticides were used as water wettable powder using a knapsack sprayer fitted with one nozzle. Complete coverage of the treated trees was attained. The untreated control plots were left unsprayed. Also, care was taken to avoid any drift among the treated trees. Three replicates were made for each treatment. Thus, at the required time, three fruit samples of  $\frac{1}{2}$  Kg each were collected at random. The representative samples were divided into three subsamples 0.5 Kg each. The first was left without washing, the second was washed for 3 min. in running tap water followed by spraying for 2 min. with tap water then dried, and the third was washed with tap water and completely peeled then dried.

The concentrations of the applied pesticides were determined at different intervals, one hour after spraying (initial), 1, 3, 5, 7, 14 and 21 days after application. Hexythiazox 10% was used at the rate of 20 g/100 l water, while carbendazim 50% was used at the rate of 50 gr/100 L water.

### **Residues determination:**

#### **A. Extraction and clean-up:**

Hexythiazox residues were extracted and cleaned up from apple fruits according to the methods of Tokieda *et al.* (1987). Carbendazim residues were extracted and cleaned-up from apple fruits according to the method of Joseph *et al.* (1973).

#### **B. Chromatography determination:**

Hexythiazox and carbendazim residues were determined by Agilent 1100 serious HPLC fitted with quartz pump G1311 A, UV detector and stainless steel column (2.6 X 250 mm) packed with C18, used under following conditions as shown in Table 1:

**Table (1). HPLC conditions for the determination of the tested pesticide residues**

Conditions	Wave length (nm.)	Mobile phase (MeOH/ Acet.)	Flow Rate (ml/min.)	Attenuation	Chart speed (cm/min)	AR Rej
Pesticides						
Hexythiazox	230	60/40	1.5	4	0.2	10
Carbenazim	197	80/20	1.0	6	0.2	100

At these conditions the retention time of hexythiazox and carbendaizm were 2.90 and 2.70 min., respectively. For recovery test, known amounts of hexythiazox and carbendaizm active ingredients were added to the untreated apple fruit samples and the recovery percentage were determined as perviously mentioned. The average of recoveries were 82.93% and 93.75% for hexythiazox and carbendaizm, respectively.

### **C. Kinetic Study:**

In order to determine the rate of degradation of the tested pesticides and the half lives period ( $t_{0.5}$ ) on apple fruits they were calculated according to the equation of Moye *et al.* (1987).

$$K = \text{Ln}_{10} \times \text{slope}$$

$$T_{0.5} = \text{Ln} 2 / k$$

## **Results and Discussion**

Tables 2 and 3 and Figures 1 and 2 show the data of the detected residues of hexythiazox and carbendaizm at different time intervals in and on unwashed, washed, and washed - peeled apple fruits after the pesticide was applied once early in the season on apple fruits trees under field conditions.

### **Hexythiazox residues:**

Data summarized in Table 2 and Fig. 1 illustrate that the initial deposits of hexythiazox residues found in unwashed, washed, and washed-peeled apple fruits as determined one hour after application were 2.68, 2.01, and 0.91 ppm, respectively. Such deposits values were degraded with time to reach 0.816 and 0.53 ppm after five days of application in unwashed and washed fruits. On the other hand, recording the dissipation and loss by washing process amounted by 69.55 and 35.05%, respectively. The determined residues was 0.69 ppm after one day of application in washed-peeled fruits recording a loss by peeling process reached 65.53%. These values were however, higher than the maximum

residual level (MRL 0.5 ppm) that have been adopted by Codex Alimentarius commission (2003). Moreover, after seven days of application the residues were found to be 0.27 and 0.12 ppm in unwashed and washed fruits, recording dissipation and loss by washing process 89.93 and 55.56% respectively. 0.443 ppm after three days of application in washed-peeled fruits, recording loss by washing followed by peeling process 72.81%. These values were below than the MRL. At the end of experiment (after 21 days of application) no detectable hexythiazox residues were shown in all apple fruit samples, recording a dissipation of 100% in unwashed fruits. The decomposition of hexythiazox residues may be due to the Egyptian prevailing environmental conditions mainly at the time of application (high temperature). These obtained results are in agreement with those of Gonzalez and Aravena (1990) and Athanasopoulos *et al.* (2004).

As a results, unwashed fruits could be marketed with apparent safety for human consumption at least after 7 days of hexythiazox application. While washed and washed-peeled fruits could safely used after 7 and 3 days of application respectively. Also, apple fruits must be washed and peeled before use. This could help to eliminate most hexythiazox residues from contaminated apple fruits.

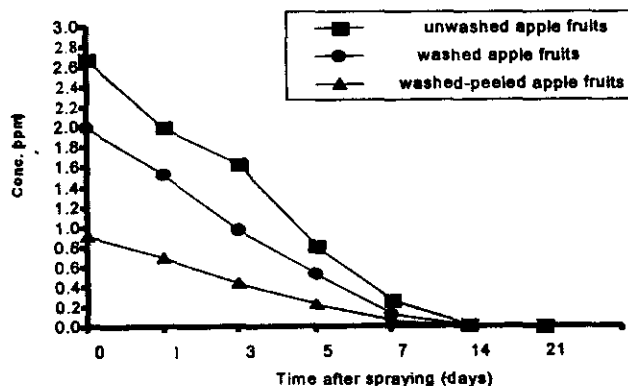
On the other hand, washing and washing followed by peeling process of the treated apple fruits reduced hexythiazox residues by 23.58 – 55.56% and 65.53 – 100% through the experiment periods, respectively. This results are in line with reported by Ong *et al.* (1996) who concluded that the acaricide formetanate hydrochloride residues on fresh apple fruits were reduced by chlorine and azone washes by 50 – 100 %, It is clearly that washing and washing followed by peeling process are significant process in reducing and eliminating of hexythiazox residues from contaminated apple fruits.

In addition, hexythiazox rates of degradation and half-lives in unwashed, washed, and washed-peeled apple fruits (days) were 0.6553 and ( $t_{0.5} = 1.0578$ ), 0.7371 and ( $t_{0.5} = 0.9404$ ), and 0.6995 and ( $t_{0.5} = 0.9909$ ), respectively. These results were similar to those obtained by Papas *et al.* (1999 and 2003) and Stuart *et al.* (1999), as they reported that half-live times of some acaricides were ranged between 3 – 8 days following the repeatedly spray of acaricides for apple fruits on trees.

**Table (2): Hexythiazox residues in unwashed, washed, and washed-peeled apple fruits at different time intervals (ppm)**

Time intervals in (days)	Unwashed		washed		Washed and peeled	
	ppm	% of Dissipation	ppm	% loss by washing process	ppm	% loss by washing and peeling process
initial <sup>(1)</sup>	2.680 <sup>(2)</sup>	0.00	2.010	25.00	0.910	66.04
1	2.002	25.30	1.530	23.58	0.690	65.53
3	1.629	39.22	0.970	40.45	0.443	72.81
5	0.816	69.55	0.530	35.05	0.220	73.04
7	0.270	89.93	0.120	55.56	0.050	81.48
14	0.010	99.63	0.006	40.00	UND	100.00
21	UND	100.00	UND	-	UND	-
Slope	0.2846		0.3201		0.3038	
K	0.6553		0.7371		0.6995	
(Mathematically)	0.6553		0.7371		0.6995	
t <sub>0.5</sub> (days)	1.0578		0.9404		0.9909	

(1) Initial = one hour after spraying  
 K. value = Rate of degradation (days)  
 t<sub>0.5</sub> = Half - life period  
 UND = undetectable  
 (2) Each value is a mean of three samples (replicates)



**Fig.(1). Residual behaviour of Hexythiazox on and in unwashed, washed, and washed peeled apple fruits (ppm).**

### **Carbendazim Residues:**

Results in Table (3) and Fig. (2) indicated that the initial deposits of carbendazim residues on and in unwashed, washed, and washed-peeled apple fruits were 0.67, 0.32, and 0.125 ppm after one hour from application, respectively. Such amounts were declined to 0.51, 0.3, and 0.112 ppm after one day from application, recording dissipation, loss by washing, and loss by washing followed by peeling calculated by 23.88, 41.18, and 78.04%, respectively. These values were below the MRL (2 ppm according to the European Union, 2004).

Therefore, unwashed apple fruits could be marketed with apparent safety for human consumption directly after one day of carbendazim application and also, washed and washed-peeled fruits could be safely used after one day of application too.

Such fast dissipation of carbendazim residues is due to the rapid degradation by high temperature under field conditions. These results are in agreement with Sharma *et al.* (1997) and Gupta (1988) as they reported that carbendazim residues degraded more rapidly at higher temperature (20 and 25°C) and it was concluded that apple was safe for consumption at a range between 1.7 – 3 days after treatment, when carbendazim was sprayed twice (20 days interval) on apple trees close to harvest.

It was noticed that carbendazim residues were decreased with time to reach 0.04, 0.008, and 0.002 ppm after seven days of application in unwashed, washed, and washed-peeled fruits, recording dissipation, loss by washing, and loss by washing followed by peeling of 94.03, 80, and 95% respectively. At the end of experiment after 21 days of application carbendazim residues became undetectable in all apple fruit samples. These results are in agreement with those of Sharma *et al.* (1997) and Knapik *et al.* (1984) as they reported that initial deposits of carbendazim residues were lower in apple fruits than in leaves, rate of degradation was highest during the first 5 days after application but decreased with time, and residues were detected on apple fruits up to 10 days.

On the other hand, washing process of treated apple fruits reduced carbendazim residues by 41.18 – 100 % through the experiment periods, these result is in line with Hwang *et al.* (2002) and Himanish *et al.* (2003), and as they found that the decontamination percentage of the carbendazim residue by washing from apple fruits was 27.3%. Sato and Maki (1989) reported that more than 50% of guazatine fungicide residues in apple was washed out with 0.1%, detergent solution.

In the present work it was found that washing followed by peeling process of the treated apple fruits reduced carbendazim residues by 68.18 – 100 % through the experiment periods, these result is in agreement with that of Cano *et al.* (1987), Sharma *et al.* (1991) and Hwang *et al.* (2002) as they reported that more than 80% of the carbendazim residues remained on the apple peel, and the amount in the pulp decreased towards the core. Therefore, peeling play an important role in reducing carbendazim residues level in processed apple.

In addition, carbendazim rates of degradation and half lives in unwashed, washed, and washed-peeled apple fruits (days) were: 0.6583 and ( $t_{0.5} = 1.05291$ ), 0.7193 and ( $t_{0.5} = 0.9636$ ) and 0.6044 ( $t_{0.5} = 1.1468$ ), respectively. These results are in agreement with those of Sharma *et al.* (1997) who reported that half- life time of carbendazim lie in a range of 2 – 3.9 days when carbendazim was sprayed twice on apple trees.

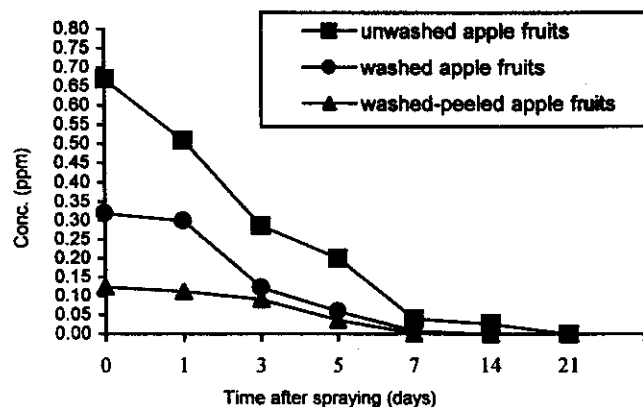
**Table (3). Carbendazim residues in unwashed, washed, and washed-peeled apple fruits (ppm) at different time intervals**

Time intervals (days)	Unwashed		washed		Washed and Peeled	
	ppm	% of Dissipation	ppm	% loss by washing process	ppm	% loss by washing and peeling process
initial <sup>(1)</sup>	0.670 <sup>(2)</sup>	0.00	0.320	52.24	0.125	81.34
1	0.510	23.88	0.300	41.18	0.112	78.04
3	0.286	57.31	0.122	57.34	0.091	68.18
5	0.201	70.00	0.060	70.15	0.037	81.59
7	0.040	94.03	0.008	80.00	0.002	95.00
14	0.027	95.97	UND	100.00	UND	100.00
21	UND	100.00	UND	-	UND	-
Slop K	0.2859		0.3124		0.2625	
Mathematically	0.6583		0.7193		0.6044	
$T_{0.5}$ (days)	1.0529		0.9636		1.1468	

<sup>(1)</sup> Initial = one hour after spraying  
 K. value = Rate of degradation (days)  
 $t_{0.5}$  = Half - life period  
 UND = undetectable

<sup>(2)</sup> Each value is mean of three samples replicates





**Fig. (2). Residual behaviour of carberdazin on and in unwashed, washed, and washed peeled apple fruits.**

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## الملخص العربي

### دراسة سلوك متبقيات ميبيدات هيكسيثيازوكس وكاربندازيم على وفي ثمار التفاح تحت الظروف الحقلية

محمود فهمي رفاعي البوز

قسم بحوث تحليل المبيدات - المعمل المركزي للمبيدات - مركز البحوث الزراعية - الدقي -  
الجيزة - مصر

تم رش أشجار ثمار التفاح في محافظة القليوبية بمبيد أكاروسي ماكوميت 10% مسحوق قابل للبلل (هيكسيثيازوكس) ومبيد فطري كيمازد 50% مسحوق قابل للبلل (كاربندازيم) وذلك بمعدلات 20، 50 جرام لكل 100 لتر ماء على الترتيب ثم قدرت متبقيات هذه المبيدات على وفي ثمار التفاح الغير مغسولة والمغسولة بماء الصنبور جيدا وكذلك في الثمار التي تم غسلها ثم تقشيرها وذلك على فترات زمنية صفر (1 ساعة بعد الرش)، 1، 3، 5، 7، 14، 21 يوم بعد الرش وكانت النتائج المتحصل عليها من التجربة ما يلي:

وجد في ثمار التفاح الغير مغسولة والمغسولة أنها تحتوي على متبقيات مبيد هيكسيثيازوكس بتركيزات 0.816، 0.530 جزء في المليون بعد 5 أيام من الرش على الترتيب وهي أعلى من الحد المسموح به دولياً (0.5 جزء في المليون) بينما احتوت تلك الثمار التي تم غسلها ثم تقشيرها على تركيزات 0.443 جزء في المليون بعد 3 أيام من الرش وهي أقل من الحد المسموح به دولياً وأن هذه المتبقيات تقل بمرور وقت للتجربة حتى وصلت إلى 0.270، 0.120 جزء في المليون بعد 7 أيام من الرش في الثمار الغير مغسولة والمغسولة على الترتيب وهي أقل من الحد المسموح به دولياً وبناء على ذلك يمكن تسويق ثمار التفاح الغير مغسولة والمعاملة بمبيد هيكسيثيازوكس بأمان للاستهلاك الآدمي بعد 7 أيام من الرش بينما الثمار المغسولة والثمار التي تم غسلها ثم تقشيرها يمكن استعمالها بأمان بعد 7، 3 يوم من الرش على الترتيب.

لما بالنسبة لمتبقيات مبيد كاربندازيم أظهرت للنتائج من التجربة أن متبقيات هذا المبيد على وفي ثمار التفاح الغير مغسولة والمغسولة والتي تم غسلها ثم تقشيرها كانت أقل من الحد المسموح به دولياً (2 جزء في المليون) في جميع فترات التجربة حيث كانت محتوى الثمار من المتبقيات هي 0.51، 0.3، 0.112 جزء في المليون بعد يوم واحد من الرش على الترتيب ولذلك يمكن تسويق ثمار التفاح بأمان للاستهلاك الآدمي بعد يوم واحد فقط من رشها بمبيد كاربندازيم . وعلى هذا فإن النتائج المتحصل عليها تفيد في تحديد الفترات التي يسمح فيها للمستهلك بتناول التفاح بعد رش تلك المبيدات المستخدمة . وبتقدير نصف العمر لمتبقيات مبيد هيكسيثيازوكس على ثمار التفاح الغير مغسولة والمغسولة والتي تم غسلها ثم تقشيرها كانت : 1.0578، 0.9404، 0.9909 يوم بينما كانت تلك الفترات لمتبقيات مبيد الكاربندازيم هي 1.0529، 0.9636، 1.1468 يوم على الترتيب.

من ناحية أخرى أثبتت عمليات الغسيل بماء الصنبور وعمليات التقشير لثمار التفاح المعاملة فاعليتها في تقليل وإزالة متبقيات المبيدات المستخدمة خلال فترات التجربة، حيث تراوح متوسط انخفاض متبقيات مبيد هيكسيثيازوكس لثمار التفاح المعاملة بواسطة عملية الغسيل بماء الصنبور من 23.58 إلى 55.56% وبواسطة عمليات الغسيل ثم التقشير تراوح هذا الانخفاض بين 65.53 إلى 100%. بينما كانت لمتبقيات مبيد كاربندازيم من 41.18 إلى 100% بواسطة عمليات الغسيل بماء الصنبور ، 68.18 إلى 100% بواسطة عمليات الغسيل ثم التقشير وذلك خلال فترات التجربة (21 يوم) .