

PERSISTANCE OF CHROMAFENOZIDE AND METHOXYFENOZIDE ON AND IN TOMATO FRUITS AND ON FILMS EXPOSED TO ULTRA - VIOLET AND SUNLIGHT

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ABSTRACT

The persistence of chromafenozide and methoxyfenozide in and on tomato fruits under field conditions during 2006 season was studied. Chromafenozide (Virtu 5% SC) and methoxyfenozide (Runner 24% SC) were sprayed on tomato fruits at their recommended rates of application, 400 ml and 150 ml/fed, respectively. The most suitable method for extracting chromafenozide and methoxyfenozide was the use of methylene chloride. The percent recoveries were 92 and 89 for chromafenozide and methoxyfenozide, respectively. The initial deposit of methoxyfenozide on the tomato fruits (0.559 ppm) was higher than that of chromafenozide (0.123 ppm).

The data show that washing tomato fruits at zero time and at different intervals after application of methoxyfenozide removed a reasonable quantity of residues, from 42.1 to 51.2% of the initial total amount. The corresponding values for chromafenozide ranged from 8.2 to 44.5%. This clearly demonstrated that after application of chromafenozide to tomato, it is either absorbed or bound to the surface or due to the solubility in waxy layer so tightly that it can not be removed by simple washing, whereas, in the case of methoxyfenozide its absorption or binding to the tomato surface was lower than chromafenozide.

Methoxyfenozide was more persistent than chromafenozide when exposed to sunlight. However, methoxyfenozide degraded more rapidly than chromafenozide during the first five hours. The half-life times of chromafenozide and methoxyfenozide were 14.3 and 26.2 hours, respectively after exposure to sunlight. The results also indicated that the photodecomposition rate of methoxyfenozide was more rapid than chromafenozide in the first 6 hours after exposure to UV-light with 14.1, 28.5 and 44.8% loss after 1, 3 and 6 hours, while the corresponding amounts for chromafenozide were 8.0, 19.4 and 44.6% loss, respectively. Statistically, the half-life of chromafenozide and methoxyfenozide as dry thin films exposed to UV light were 6.8 and 8.0 hours, respectively.

INTRODUCTION

During the past fifteen years, a new class of insect growth regulators (IGRs) has emerged from the discovery that substituted dibenzoylhydrazines act as agonists of 20- hydroxyecdysone (Wing 1988, Wing *et al.*, 1988). The sentinel member of this chemical class was discovered in 1983 (Hsu 1991). An analog tebufenozide (RH-5992) proved to be extremely potent against and selective toward larval Lepidoptera (Carlson *et al.*, 1994, Dhadialla *et al.*, 1998) and is now widely used against Lepidopteran pests on several crops throughout the world.

Methoxyfenozide is the latest compound in this class to be developed commercially and is the most potent analog to date against larval Lepidoptera (Ishaaya *et al.*, 1995; Le *et al.*, 1996; Trisyono and Chippendale 1997;

Smagghe *et al.*, 1998, 1999). The first sales of methoxyfenozide occurred in 1999, and the first full registrations in the United States were granted for cotton and pome fruits in 2000.

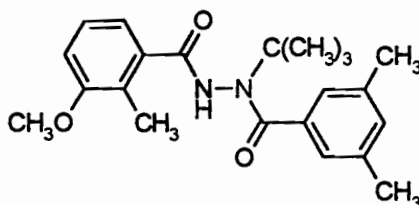
The aim of the present study was to study: 1) the persistence of chromafenozide and methoxyfenozide in tomato fruits prior to and after washing with water. 2) effect of sunlight and UV-light on the persistence of chromafenozide and methoxyfenozide.

MATERIALS AND METHODS

1. Insecticides used

Chromafenozide (Virtu 5% SC): 2'- *tert*-butyl - 5 - methyl - 2' -(3,5-xyloyl) chroman-6-carbohydrazide.

Methoxyfenozide (Runner 24% SC): N- *tert* - butyl -N'- (3-methoxy-o-toluoyl)-3,5-xylohydrazide.



2. Residue analysis of chromafenozide and methoxyfenozide on and in tomato fruits

The field experiment was carried out in the experimental Farm of the Faculty of Agric., Cairo University during the 2006 season. Virtu 5% SC (chromafenozide) and runner 24% SC (methoxyfenozide) were sprayed on tomato fruits at their recommended rates of application, 400 ml and 150 ml / fed. Random samples of the fruits were collected from the treated plants after 0, 1, 2, 3, 4, 6, 7 and 9 days after application.

For the determination of chromafenozide and methoxyfenozide total residues in tomato fruits, samples of 200 g were well macerated and blended with 200 ml methylene chloride at high speed for 2-3 min. The extract was filtered through glass wool in a porcelain Buchner funnel. The remaining filtrates were re-extracted further with methylene chloride two more times with 100 ml methylene chloride each. The solvent was dried over anhydrous sodium sulphate and the solvent was evaporated to give dry residues.

Another set of samples of 200 g each, was taken to determine the dislodgable residue in tomatoes. The tomato fruits were each washed three times with 100 ml water. The water washings were collected in a separatory funnel and extracted three times with methylene chloride 200ml. The

methylene chloride layers were received through anhydrous sodium sulphate and evaporated to dryness. For the determination of the remaining extractable residue in tomato after washing, the previous procedure for total residue was followed

3. Photodegradation studies

3.1. Effect of sunlight

One ml of the tested insecticides in acetonitrile was spread as uniformly as possible on the surface of uncovered petri dishes (7.0 cm i.d.). The acetonitrile was left to dry at room temperature. The dishes were then exposed to direct sunlight. Dominating temperature was 25 °C. Samples were taken at 0, 1, 3, 5, 6, 7, 11, 14, 18 and 24 hours.

3.2. Effect of UV-light

One ml of the tested insecticides was spread on the surface of uncovered petri-dishes (7.0 cm i.d.). After drying at room temperature the deposits in the petri-dishes were exposed to a 254 nm ultraviolet lamp at a distance of 10 cm for 0, 1, 3, 6, 9, 12 and 16 hours.

4. High-pressure liquid chromatography (HPLC) conditions

The chromatographic system consisted of a Hewlett Packard (HP series 1100), quaternary pump model (G 1311 A), with a UV variable wavelength detector model (G 1314 A) monitored at 250 nm. An ODS-Hypersil 5 µm (20 cm x 4.6 mm i.d.) was used and the column temperature was 40 °C. Chromafenozide and methoxyfenozide were eluted isocratically with acetonitrile-water (65: 35 v/v), at the rate of 1 ml/minute. Under these conditions, the retention time (R_t) for both chromafenozide and methoxyfenozide were 3.48 and 3.32 min., respectively (Anonymous, 2000).

RESULTS AND DISCUSSION

1. Persistence of chromafenozide and methoxyfenozide in tomato fruits

Different solvents were used to choose the most suitable and efficient solvent for extraction of chromafenozide and methoxyfenozide from tomato fruits. The tested solvents were acetone, acetonitrile, ethyl acetate, hexane and methylene chloride. The most suitable method for extracting chromafenozide and methoxyfenozide from tomato fruits was the use of methylene chloride where the percent recoveries were 92 and 89 for chromafenozide and methoxyfenozide, respectively.

The amounts of chromafenozide and methoxyfenozide in tomato fruits prior to and after washing with water are shown in Tables (1 and 2). As for the unwashed fruits the data demonstrate that the initial deposit of methoxyfenozide on the tomato fruits was higher than that of chromafenozide, 0.559 and 0.123 ppm, respectively. This may be due to the difference in the amount of active ingredient in the formulated products. Chromafenozide was sprayed as Virtu 5% SC with a rate of 400 ml/fed., while methoxyfenozide was used as Runner 24% SC at the rate of 150 ml/fed. The low initial amount of both pesticides may be due to the low surface area of tomato fruits.

The data showed that the amount of methoxyfenozide at zero time was 0.559 mg/kg decreased to 0.345 mg/kg one day after application. It decreased to 0.272, 0.222, 0.273 and 0.188 mg/kg 2, 3, 4 and 6 days after application. Also rapid degradation of methoxyfenozide occurred after 7 and 9 days after application to reach 0.066 and 0.037 mg/kg, respectively.

The amount of methoxyfenozide residue in washed tomato fruits was lower than unwashed (Table 1 and Fig.1).

Table (1): Persistence of methoxyfenozide (Runner 24% SC) in and on tomato fruits at different intervals after application.

Time after application (days)	The amount of methoxyfenozide (mg/kg) recovered from:					
	Tomato without washing	Remaining extractable residue (a)	Dislodgable residue (b)	Total a + b	$\frac{a}{a+b} \times 100$	$\frac{b}{a+b} \times 100$
0	0.559	0.329	0.345	0.674	48.8	51.2
1	0.345	0.185	0.147	0.332	55.7	44.3
2	0.272	0.168	0.145	0.313	53.7	46.3
3	0.222	0.153	0.128	0.281	54.4	45.6
4	0.273	0.108	0.105	0.213	50.7	49.3
6	0.188	0.084	0.087	0.171	49.1	50.9
7	0.066	0.055	0.04	0.095	57.9	42.1
9	0.037	0.039	N.D.	---	---	---

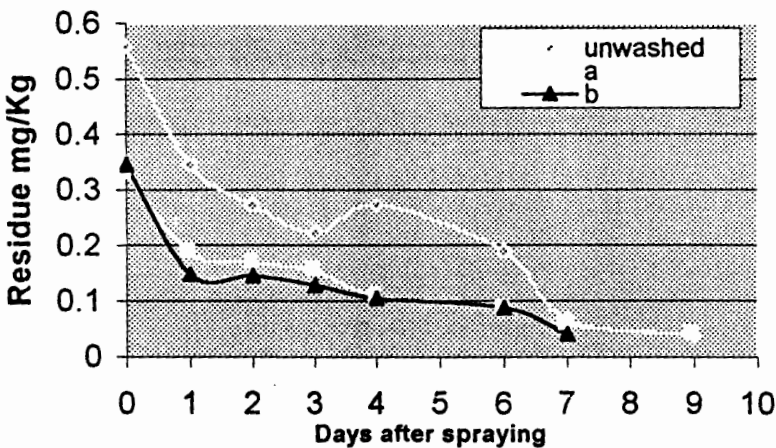


Fig.1 Methoxyfenozide residue in tomato fruits before and after washing

(a) Remaining extractible residue. (b) Dislodgable residue.

The residue in washing (dislodgable residue 'b') was 0.345 mg/kg at zero hr and decreased further with time to the level of 0.04 following the same trend as shown by the total residue. However, this decrease was most rapid one day after spray. The total residue calculated from dislodgable residue and the remaining extractable residue (a) in washed tomato closely paralleled the total residue obtained from tomato without washing with water.

The remaining extractable residue (a) of methoxyfenozide in tomato fruits was 0.329 mg/kg at zero time after application, while the dislodgable residue (b) was 0.345 mg/kg at the same time. The data show that the percent of remaining extractable residues of methoxyfenozide in tomato fruits were 55.7, 53.7, 50.7 and 57.9% after 1, 2, 4 and 7 days after application. The corresponding values for dislodgable residues (b) were 44.3, 46.3, 49.3 and 42.1%. Data in Table (1) and Fig. (1) also show that the percent losses of methoxyfenozide were 38.3, 60.3, 88.2 and 93.4% after 1, 3, 7 and 9 days, respectively.

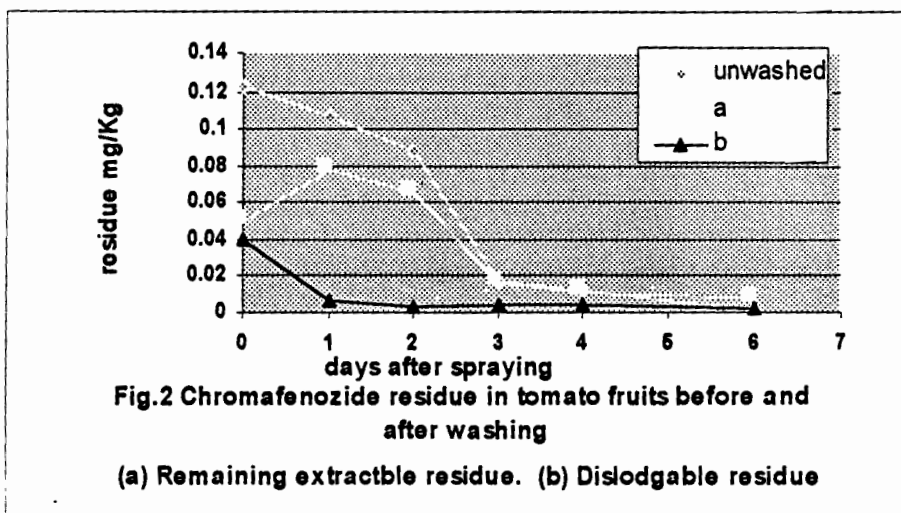
The residue of chromafenozide was also determined in washed and unwashed tomato fruits. The initial amount of chromafenozide recovered was 0.123 mg/kg in unwashed tomato fruits. It decreased further with time to 0.088, 0.012 and 0.0014 mg/kg at 2 and 6 days after application. The amount of chromafenozide residue in washed tomato fruits was lower than unwashed (Table 2 and Fig. 2). The residue in the washing water (dislodgable residue 'b') was 0.04 mg/kg at zero hour and decreased further with time to 0.003, 0.004 and 0.002 after 2,4 and 6 days after application. The percent losses of chromafenozide were 13.0, 86.2, 90.2 and 98.9% after 1, 3, 4 and 6 days, respectively.

It can be noticed from data in Table (2) and Fig. (2) that the remaining extractable residue (a) of chromafenozide in tomato fruits at zero time was 0.05 which increased to 0.078 mg / kg at 1-day after application. From the 2nd day the residues of chromafenozide in the unwashed fruits decreased.

At the zero time there was no enough time for the adsorbed residue to be absorbed. But one day after application the remaining residue was able to penetrate with high amounts through the fruits.

Table (2): Persistence of chromafenozide (Virtu 5% SC) in and on tomato fruits at different intervals after application.

Time after application (days)	The amount of chromafenozide (mg/kg) recovered from:					
	Tomato without washing	Remaining extractable residue (a)	Dislodgable residue (b)	Total a + b	$\frac{a}{a + b} \times 100$	$\frac{b}{a + b} \times 100$
0	0.123	0.05	0.04	0.09	55.5	44.5
1	0.107	0.078	0.007	0.085	91.8	8.2
2	0.088	0.066	0.003	0.069	95.7	4.3
3	0.017	0.016	0.004	0.020	80.0	20.0
4	0.012	0.011	0.004	0.015	73.3	26.7
6	0.0014	0.008	0.002	0.01	80.0	20.0
7	N.D.	N.D.	N.D.	----	----	----



The average percent contribution of methoxyfenozide dislodgable residue to the total residue was approximately equal to the remaining extractable residue.

On the other hand, the average percent contribution of chromafenozide dislodgable residue to the total residue was low, while the remaining extractable residue was high and ranged from 55.5 to 95.7%.

This showed that washing tomato fruits at zero time and at the different intervals after application of methoxyfenozide removed a reasonable quantity of residues equal to the half of the total amounts and ranged from 42.1 to 51.2%.

Washing tomatoes at 0 time after application of chromafenozide removed a reasonable quantity of initial residues 44.5 %, while to 6 days later these values were low and ranged from 8.2 to 26.7 %.

This clearly demonstrates that after application of chromafenozide to tomato, it is either absorbed or bound to the surface so tightly that it can not be removed by simple washing, whereas, in the case of methoxyfenozide its absorption or binding to the tomato surface was lower than chromafenozide. This may be due to the presence of the chromono heterocyclic group which has high electronegativity to the chromafenozide structure instead of the methoxy group in the methoxyfenozide structure. Also, it may be due to the greater solubility of methoxyfenozide in water (3.3 g/l) than chromafenozide (1.12 g/l). Thompson *et al.* (1979) observed, in a study on methidathion application to citrus leaves, that the dislodgable residue comprised about 20% of the total residue, 80% being either absorbed or bound to the leaf surface. Barakat *et al.* (1985) found that the residue in grapes after washing with water was still high and ranged from 47.9 to 92.1% of the total residue.

Effect of Sunlight

The data in Table (3) show that the decomposition percentage of chromafenozide and methoxyfenozide increased gradually after exposure to sunlight. Photodecomposition was positively correlated with the exposure

period. The residues of both tested insecticides deteriorated when exposed to direct sunlight especially for long periods. Methoxyfenozide was more persistent than chromafenozide. However, methoxyfenozide degraded more rapidly than chromafenozide during the first five hours. Then the decrease in the amounts of methoxyfenozide became slower and more gradual. The half-life times of chromafenozide and methoxyfenozide were 14.3 and 26.2 hours, respectively.

The amounts of chromafenozide and methoxyfenozide decreased from zero to one hour at the rate of 2.4 and 9.0%/ hour, respectively. The decrease in the amounts of chromafenozide and methoxyfenozide continued after one hour and reached 13.3 and 15.8% loss after five hours, respectively. After, five hours the decrease in the amounts of methoxyfenozide became slower with 25.8, 48.0 and 48.0% loss after 14, 18 and 24 hours, respectively. The amounts of chromafenozide decreased more rapidly than methoxyfenozide after 5 hours. After six hours of exposure to sunlight the percent amount recovered for chromafenozide was 75.4% with 24.6% loss. The decline in the residue of chromafenozide continued after six hours to 47.5, 65.2 and 70.9% loss after 14, 18 and 24 hours, respectively.

Sensitivity to sunlight limits the use of some potential pesticides in agriculture. Classical approaches to overcome this obstacle have involved chemical modifications of the molecular structure of the pesticide or the use of UV-absorbing materials in pesticide formulations. However, both methods suffer serious drawbacks since chemical modification may affect pesticidal activity or biodegradability may introduce ecological problems related to soil and water pollution (Rozen and Margulies, 1991). The radiation energy of sun might be absorbed by a pesticide molecule principally at a given wavelength. The energy might increase the transitional, rotational, vibrational or electronic energy of the molecule. If enough energy was absorbed to interact with the electrons of molecules an electronically excited molecule may result.

Table (3): Effect of sunlight on stability of chromafenozide and methoxyfenozide.

Exposure time (hours)	Chromafenozide		Methoxyfenozide	
	% Amount recovered	% Loss	% Amount recovered	% Loss
Zero	100	0.0	100	0.0
1	97.6	2.4	91.0	9.0
3	91.5	8.5	86.7	13.3
5	86.7	13.3	84.2	15.8
6	75.4	24.6	84.0	16.0
7	63.0	37.0	76.8	23.2
11	55.5	44.5	76.4	23.6
14	52.5	47.5	74.2	25.8
18	34.8	65.2	52.0	48.0
24	29.1	70.9	52.0	48.0
DT₅₀ hours	14.3		26.2	

Effect of UV-light

It has been demonstrated that UV rays produces chemical changes in a large number of pesticides. Several types of photodecomposition such as hydrolysis, oxidation and isomerization may occur. If similar reactions occur under field conditions, such investigations will be of great importance in questions concerning environmental contamination, pesticide residues in agricultural products and practical use of pesticides.

Generally, photodecomposition is positively correlated with exposure period. The data in Table (4) show that the decomposition percentages of chromafenozide and methoxyfenozide on glass surfaces increased gradually after exposure to UV-light.

Table (4): Effect of UV light on stability of chromafenozide and methoxyfenozide.

Exposure time (hours)	Chromafenozide		Methoxyfenozide	
	% Amount recovered	% Loss	% Amount recovered	% Loss
Zero	100	0.0	100	0.0
1	92.0	8.0	85.9	14.1
3	80.6	19.4	71.5	28.5
6	55.4	44.6	55.2	44.08
9	36.3	63.7	47.3	52.7
12	22.5	77.5	36.9	63.1
16	8.8	91.2	22.2	77.8
DT₅₀ hours	6.8		8.0	

Statistically, the half-life of chromafenozide and methoxyfenozide as dry thin films were 6.8 and 8.0 hours, respectively.

The results also indicated that the photodecomposition rate of methoxyfenozide was more rapid than chromafenozide in the first 6 hours after exposure to UV-light with 14.1, 28.5 and 44.8% loss after 1,3 and 6 hours, while the corresponding amount for chromafenozide were 8.0, 19.4 and 44.6% loss, respectively.

The steep decline in the residues of chromafenozide continued to 63.7, 77.5 and 91.2% loss after 9, 12 and 16 hours of exposure to UV-rays. While the corresponding amounts for methoxyfenozide were 52.7, 63.1 and 77.8% loss, respectively.

Based on the above results, it could be stated that UV-rays is more effective than sunlight in accelerating the photodecomposition of chromafenozide and methoxyfenozide.

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ثبات مبيدي الكرومافينوزايد والميثوكسي فينوزايد على وفي ثمار الطماطم وكذلك عند تعرضها للأشعة فوق البنفسجية وأشعة الشمس
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تم دراسة مدى ثبات مبيدي الكرومافينوزايد والميثوكسي فينوزايد على وفي ثمار الطماطم تحت الظروف الحقلية وذلك في موسم ٢٠٠٦. وقد عوملت نباتات الطماطم بمبيدي الكرومافينوزايد (الفيرتو SC 5%) ومركب الميثوكسي فينوزايد (الراتر SC 24%) بالمعدلات الموصى بها وهي ٤٠٠ ، ١٥٠ مل/فدان على الترتيب. وأوضحت النتائج أن الكمية الأولية لمبيد الميثوكسي فينوزايد على ثمار الطماطم كانت أعلى من كمية مركب الكرومافينوزايد حيث كانت ٠,٥٥٩ ، ٠,١٢٣ جزء في المليون على الترتيب. كما أوضحت النتائج أيضا أن غسيل ثمار الطماطم ابتداء من الرش مباشرة (عينة zero time) وخلال جميع الفترات المأخوذة بعد المعاملة بمركب الميثوكسي فينوزايد تؤدي إلى غسل كميات مقبولة من المتبقيات وكانت مساوية في كل مرة لنصف الكمية الكلية الموجودة على الثمار وتراوح ما بين ٤٢,١ - ٥١,٢% بينما في حالة مركب الكرومافينوزايد فكانت هذه القيمة تتراوح ما بين ٨,٢ - ٤٤,٥%.

وتوضح هذه النتائج أنه عقب المعاملة بمركب الكرومافينوزايد لنباتات الطماطم فإنه من السهل أن يحدث له امتصاص أو ارتباط مع سطح الثمار أو ذوبان في الطبقة الشمعية بحيث أنه لا يمكن إزالته من على سطح الثمار بواسطة عملية الغسيل العادية، بينما في حالة مركب الميثوكسي فينوزايد فإن امتصاصه وارتباطه على سطح الثمرة يكون أقل من مركب الكرومافينوزايد.

كذلك أوضحت الدراسة أيضا أن مركب الميثوكسي فينوزايد يكون أكثر ثباتا من مركب الكرومافينوزايد عقب تعرضه لأشعة الشمس المباشرة وعلى الرغم من ذلك فإن ثبات مركب الميثوكسي فينوزايد يكون أقل من مركب الكرومافينوزايد خلال الساعات الخمس الأولى من التعرض لأشعة الشمس وأن فترة نصف العمر لمركب الكرومافينوزايد و الميثوكسي فينوزايد هي ١٤,٣ ، ٢٦,٢ ساعة على الترتيب عقب التعرض لأشعة الشمس المباشرة.

كذلك أوضحت النتائج أن معدل التحطيم الضوئي لمركب الميثوكسي فينوزايد يكون أعلى من مركب الكرومافينوزايد خلال الساعات الستة الأولى من التعرض للأشعة فوق البنفسجية حيث أعطى نسبة فقد وصلت إلى ١٤,١ ، ٢٨,٥ ، ٤٤,٨ بعد ١ ، ٣ ، ٦ ساعات من التعرض. وكانت هذه القيم في حالة مركب الكرومافينوزايد هي ٨ ، ١٩,٤ ، ٤٤,٦% على الترتيب. وكانت فترة نصف العمر لمركب الكرومافينوزايد و الميثوكسي فينوزايد هي ٦,٨ ، ٨ ساعات عقب تعرضها للأشعة فوق البنفسجية على الترتيب.