RATE OF DETERIORATION OF DIFENOCONAZOLE FUNGICIDE RESIDUES ON AND IN TOMATO FRUITS AND UNDER THE ENVIRONMENTAL CONDITIONS

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Abstract

The residues of difenoconazole fungicide (Score 25% EC) on and in tomato fruits and under environmental conditions were determined by high performance liquid chromatography (HPLC) equipped with UV variable wavelength detector. The initial deposit of this compound was 6.72 ppm one hour after tomato treatment. This amount decreased gradually to 5.86, 4.83, 2.25, 1.58 and 0.20 ppm 1,3,5,7 and 14 days from application respectively. These figures show 12.8, 28.13, 66.52, 76.49 and 97.02% loss in the same intervals, respectively. The half life value of difenoconazole on tomato fruits was 79 hours (about 3 days) under the environmental conditions of the experiment. As the maximum residue limit (MRL) of difenoconazole on tomato fruits was 1 ppm. Hence, the preharvest interval (safety period of consumption) is 8 days post treatment. The washing process by tap water dropped the initial deposit of difenoconazole residues from tomato fruits to 1.79 ppm recording 73.36% loss. Temperatures, UV-rays and direct sunlight affected the stability of difenoconazole when exposed on glass surface. Therefore, the time taken for 50% degradation (T1/2) of difenocoazole were 118, 34, 12 and 11 hours at 30, 35, 45 and 50 °C, respectively. Hence, the difenoconazole residue was more stable under exposure to ultraviolet rays than exposure to direct sunlight.

INTRODUCTION

Difenoconazole Cis, trans-3- chloro-4-[4-methyl-2-(1H-1,2,4- briazol-1-ylmethyl)-1,3-dioxalan-2-yl] phenyl 4- chlorophenyl ether. is a systemic fungicide with preventive and curative action. Absorbed by the leaves with acropetal and strong translaminar translocation, used in Egypt against disease complexes in fruits and various vegetable crops. In Egypt, pesticides are mainly applied during the summer season. During that period, the temperature is high because of the long sunny uncloudy days. The ultra-violt compontent which varies from 240 to 400nm is responseible for pesticide photolysis in the environment. Accordingly, both heat and light affect the efficiency of pesticides which are measured by the length of their residual effect (*Ruzo* and *Casida*, 1982, *Hegazi et al.*, 2004, *Tomlin*, 1997 and *Abdel- Razik, et al.*, 2001).

The present study was undertaken to investigate the following:

A- Persistence of difenoconazole residues on and in treated tomato fruits and the effect of washing process using tap water on their rates of loss difenoconazol residues from treated tomatoes at the initial time.

B- The effect of temperature, UV-rays and direct sunlight on the stability of difenoconazole residues.

MATERIALS AND METHODS

1-Field experiment:

Tomatoes were planted at Kafer Shoker, El-Kalyoubia Governorate, Egypt on april 28, 2007 in plots of 0.01 feddan. Mature tomato plants at fruiting stage were sprayed by difenoconazole (Score 25%EC) at the rate of 50 ml/100 litres water per feddan on june 5th 2007. Untreated plots were left as control check. All treatments were replicated three times. A knapsack hand sprayed fitted with one nozzle boom were used. Three replicates of treated and untreated tomato fruits were randomly picked up one hour and then 1,3,5,7 and 14 days after application and kept in clean polyethylene bags. One part of the samples collected at the initial deposits, (one hour after application) was washed thoroughly by tap water and kept in clean polyethylene bags. All samples were stored at -20°C in a deepfreezer until time of analysis.

RESIDUE ANALYSIS TECHNIQUES

a- Extraction:

The freezed samples were left to reach room temperature then macerated using waring blender. Fifty grams of macerated tomato fruits was placed in glass blender and homogenized at high speed with 150 ml HPLC grade methanol for 3 minutes. The extract was filtered through a pad of clean cotton into graduated cylinder. A known volume of the filtrate was shaken successively with 100, 50 and 50 ml of methylene chloride in separatory funnel after adding 40 ml sodium chloride solution (20%). The combined methylene chloride phase were filtered through anhydrous sodium sulphate and then evaporated to dryness using rotary evaporator (*Nasr et al., 2003)*

b- Clean up:

The residues in the concentrated extract were dissolved in 5 ml methanol and cleaned up according to the technique of *(Johnson, 1963)* using Co- agulation solution (0.5 gm ammonium chloride and 1 ml 85% orthophosphoric acid solution in 400 ml distilled water).The residues in methanol were mixed with 10 ml of cooled freshly prepared co-agulating solution and the contents were quantitavely transferred and filtered through a chromatographic column of 2.5 cm diameter packed with 5 cm layer

of Hyflo-super cell. Ttransfer was repeated three times using 5 ml methanol and 10 ml co-agulating solution in each. The filtrate was then collected together in 250 ml separatory funnel and extracted three times with 50 ml HPLC grade chloroform. The extracts were collected in round bottom flask and then evaporated under vacuum to dryness. The concentrated difinoconazole cleaned residues were dissolved in known volume of HPLC grade methanol for HPLC determination.

2- Laboratory Experiment:

To study the effect of the environmental conditions i,e- temperature, UV-rays and direct sunlight on the deterioration of difenoconazole fungicide, half milliliter of 0.1% pure a.i. of difenoconazole in acetone was spreaded as a thin film as uniformly as possible on the surface of 5 cm (i.d) Petri –dish. In order to study the effect of temperature on persistence of difenoconazole, deposits in uncovered Petri dishes were exposed to 30, 35, 45 and 50 ºC in a dark electric oven for different periods of exposure from 0 to 144hours. A second group of Betri dishes contained the fungicide deposits were exposed to the short wave of ultra-violt rays (254A) at distance of 12 cm for 1,3,6,8 and 12 hours respectively. The third group of deposits were exposed to direct sunlight for 1,3,6,8 and 12 hours respectively.

The difenoconazole residues were quantitavely transferred from the aforementioned deposits in Betri dishes to glass stoppered test tubes using HPLC grade ethyl acetate solvent. The solvent was evaporated to dryness and finally dissolved in known volume of HPLC grade methanol for HPLC determination.

HPLC Determination:

Difenoconazole fungicide residues were quantitated using the chromatographic system consisted of Agilent 1100 series HPLC fitted with quaternary pump G1311A, UV variable wavelength detector monitored at 205 nm and stainless steel column (125 X 4mm) packed with C18. The fungicide residues were eluted with methanol-water (80:20v/v) at the flow rate of 1ml/minute. Under these conditions, the retention time of difenoconazole was 5 minutes.

The residues of difenoconazole on and in tomato fruits were quantitively determined by comparison with standard solution injected under the identical HPLC conditions. The reliability of the analytical method was examined by fortifying untreated tomatoes with known quantity of tested fungicide followed by the aforementioned procedure of extraction, clean up and HPLC determination. The average rate of recovery of difenoconazole fungicide on and in tomato fruits was 98.7%. The data of the tested fungicide on and in tomato fruits were adjusted by recovery percentage.

RESULTS AND DISCUSSION

A- Persistence of difenoconazole residues on tomato fruits and the effect of washing process on the percentage loss at the initial time.

Data in Table (1) indicate the residues of difenoconazole on and in tomato fruits at different intervals post treatment. The initial deposit was 6.72 ppm one hour after application. This amount was gradually decreased to 5.86, 4.83, 2.25, 1.58 and 0.20 ppm 1,3,5,7 and 14 days from applications, respectively. These figures showing 12.8, 28.13, 66.52, 76.49 and 97.02 % loss from difinoconazole residues, respectively. Therefore, the difenoconazole fungicide half life value was 79 hours (about 3 days) on and in tomato fruits. This result is in agreement with that stated by *Al-Khalaf et al (1992)* who reported that the half- life period for most OP insecticides on and in tomato fruits were less than 3 days.

Data of pesticide residues in treated crops are required for premarket registration of pesticides and for setting maximum residue limit (toxicologically acceptable level) to protect the consumer against the possible health hazards of exposure to pesticides, *Bates (1979).*

According to the maximum residue limit, Food and Agricultural Import Ragulations and Standards, *Malysia, 2005*, the maximum residue limit (MRL) of difenoconazole on tomato fruits was 1 ppm. Hence, the preharvest interval (safety period of consumption) was 8 days post treatment under the experiment conditions, which depends on the chemistry of tested pesticide and kind of crops *(Shokr 1997 and Shady et al, 2000).*

Also, the data in Table (1) show the level of difenoconazole residues in ppm and their removal rates from treated tomato fruits by tap water washing process at the initial time. Therefore, the initial deposit of difenoconazole residues was dropped to 1.79 ppm after washing by tap water recording 73.36% loss from the initial difenoconazole residues. *Thabit (2002)* reported that the tap water washing process removed 73.40% from malathion and fenitrothion residues on tomato.

B- The effect of environmental factors

The present study investigated the effect of temperature, uv-rays (short wave, 254 nm) and direct sunlight on the deterioration of difenoconazole fungicide.

1- Effect of different temperatures:

It is clearly evident that there is a positive relationship between degree of temperature and rate of difenoconazole fungicide deterioration. The percent losses of difenconazole were 0.00, 10.2, 28.73 and 35.26 % after 6 hours of exposure at 30, 35, 45 and 50 C respectively (Table2). Results also showed that difenoconazole percent losses were 35.37, 66.72, 98.42 and 99.93 % after 48 hours of exposure to

30, 35, 45 and 50 ºC respectively. These results are in agreement with that found by *(Abdel-Razik et al. 2001).* They found The time taken for 50% degradation (T1/2) of difenoconazole were 118, 34, 12 and 11 hours at 30, 35, 45 and 50º C respectively

2- Effect of ultra –violet rays and direct sunlight:

The ultra-violet component of sunlight, which varies from 240 to 400 nm, responsible for pesticide photolysis in the environment. Both heat and light might affect the efficiency, which are measured by the duration of their residual effect. The present study was to investigate the effect of UV-rays (short wave 254 nm) and direct sunlight on the stability and degradation of difenoconazole fungicide. Generally, it is known that photodecomposition is positively correlated with exposure period to both uv-rays and direct sunlight (Table3).The data show that the difenoconazole was more stable under exposure to ultraviolet rays than exposure to direct sun light. The results showed that the percent loss of difenoconazole when exposed to UV rays were 36.60 and 68.32%, and were 97.32 and 99.06% when exposed to direct sunlight after 8 and 12 hours of exposure, respectively. Hence, the half life values (t1/2) of difenoconazole were 10 and 2 hours of exposure to UV- rays and direct sunlight, respectively. From the above results, it is clearly showed that the direct sunlight is more effective than UV rays in acceleration the photodecomposition of difenoconazole fungicide which may be due to thermal, evaporational and light intensity consideration *(Abdel-Razik et al., 2001) and Plimmer, 1972.*

Table 1. Rate of deterioration of difenoconazole fungicide residues on and in treated tomato fruits on june, 2006 at Kalyoubia Governorate.

Time after application (days)	Difenoconazole residues*				MRL**** (ppm)
	Unwashed fruits		Washed fruits		
	ppm	%loss	ppm	%loss	
Initial**	6.72	0.00	1.79	73.36	
١	5.86	12.80			
٢	4.83	28.13			
٥	2.25	66.52			
٧	1.58	76.49			
١٤	0.20	97.02			
T1/2***	79hours				

*: Each residue value represents an average of three replicates.

**: Samples were taken one hour after treatment.

***: Half life value (hours).

****: Maximum residue limit, Food and Agriculture Import Regulations and Standards, Malaysia, 2005.

Table 2. Effect of different degrees of temperatures on the stability of difenoconazole fungicide.

Exposure	30 °C		35°C		45°C		50°C	
(time hours)	ug*	%Loss	ug*	% Loss	ug*	% Loss	ug*	% Loss
0	500.00	0.00	500.00	0.00	500.00	0.00	500.00	0.00
6	500.00	0.00	448.95	10.21	356.35	28.73	323.70	35.26
24	409.40	18.12	303.30	38.54	50.30	89.94	49.60	90.08
48	323.15	35.37	166.40	66.72	7.90	98.42	0.35	99.93
96	273.90	45.22	46.25	90.75	5.00	99.79	ND	
144	223.70	55.26	5.40	98.92	ND			
T1/2 hours	118		34		12		11	

*: Actual micrograms quantitated.

**: Each residue value represents an average of three replicates.

ND: No difenoconazole fungicide trace was detected.

T1/2 : Half life values (hours).

Table 3. Effect of ultraviolet rays and direct sunlight on the stability of difenoconazole fungicide.

Exposure time	Ultra-violet rays (254nm)		Direct sun light	
(hours)	ug*	%loss	ug*	% loss
0	500.00	0.00	500.00	00.00
1	473.00	5.40	339.00	32.20
3	446.00	10.72	118.95	76.21
6	413.45	17.31	48.60	90.28
8	317.00	36.60	13.40	97.32
12	158.40	68.32	4.70	99.06
T1/2(hours)	10		2	

ug*: Actual micrograms quantitated.

*: Each residue value represents an average of three replicates.

T1/2:Half life values (hours).

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معدل تدهورالمبيد الفطري دايفينوكونازول على و في ثمار الطماطم و تحت الظروف البيئية

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اجريت دراسة لتقدير مدى ثبات المبيد الفطرى دايفيناكونازول على و فى ثمار الطماطم تحت الظروف البيئية و استخدم الكروماتوجرافى السائل عالى الاداء المتصل بكاشف الاشعة الفوق بنفسجية متغير الموجة فى تقدير متبقيات المبيد المتدهورة. و سجلت متبقيات المبيد على ثمار الطماطم بعد ساعة واحدة من الرش٦,٢٧ جزء فى المليون.

تدهورت هذة الكمية الى ٥,٨٦و ٤,٨٣و ٢,٢٥ و١,٥٨و ٠,٢٠ جزء فى المليون بعد ١ و٣ و ٥و ٧و ١٤ يوما من رش المبيد بمعدل تدهور ١٢,٨ و٢٨,١٣ و ٧٧,٥٢ و ٧٦,٤٩ و ٩٧,٠٢% على التوالى و كانت فترة نصف العمرللمبيد حوالى ٣ ايام تحت ظروف التجربة. و نظرا لان الحد المسموح بة من المبيد المذكور على الطماطم ١ جزء فى المليون فأن فترة السماح بعد الرش قدرت حوالى ٨ ايام' و من ناحيه اخرى فأن لعملية غسيل الطماطم المعاملة بماء الصنبوربعد الرش بساعة واحدة دورا اساسيا فى ازالة ٧٣,٣٦% من متبقى المبيد.كما تلعب العوامل البيئية من حرارة و اشعة شمس مباشرة و اشعة فوق بنفسجية دورا هاما و متباينا فى سرعة تدهور الدايفينوكونازول،فقد اتضح ان هناك تناسبا طرديا بين معدل تدهور متبقى المبيد و درجه الحرارة التى تعرض لها هذا المتبقى او الفترة التى تعرض لها هذا المتبقى لكل من أشعة الشمس امباشرة او الاشعة الفوق البنفسجية حيث كانت فترة نصف العمر ١١٨ و ٣٤ و ١٢ و ١١ ساعة عند تعرض متبقى المبيد لدرجات حرارة ٣٠ و ٣٥ و ٤٥ و ٥٠ م° على الترتيب كما ان تحلل المبيد ضوئيا بالتعرض لاشعة الشمس المباشرة كان اسرع كثيرا عن تحللة بالاشعة فوق البنفسجية.