

**EFFECT OF STORAGE TEMPERATURE ON THE PHYSICOCHEMICAL PROPERTIES
OF BROMUCONAZOLE FUNGICIDE FORMULATION
AND ON RESIDUES IN CUCUMBER FRUITS**

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ABSTRACT

Bromuconazole fungicide formulation recommended to be used for pest control in Egypt was subjected to storage conditions at 54 °C for 3 and 14 days and at 72 °C for 3 days. The effect of storage temperature on the chemical stability and the physical properties of Vectra (bromuconazole) was recorded. An IR analysis showed that the percentage match of bromuconazole formulation was more than 96% at different storage conditions. On the other hand, the results showed that the physical properties (Emulsification test) were not affected by all storage conditions. Cucumber plants were treated with the recommended rate of bromuconazole under the normal field conditions in the summer season of 2009. The residue analysis showed that the initial deposits were 0.5995 ppm and 0.8627 ppm for bromuconazole L- 5850646 and bromuconazole L-5850647, respectively in cucumber fruits. The percentage of loss was 53.59 and 81.92 % for bromuconazole L- 5850646 and bromuconazole L-5850647, respectively in cucumber fruits determined one day after treatment. The residual half life period in washed and unwashed cucumber fruits was 22.39 & 14.65 hours for 1, 2 bromuconazole formulation respectively. Washing process was an effective method for value 0.2103 ppm for bromuconazole L-5850646 while, bromuconazole L-5850647 was not detected as initial deposit residue on cucumber fruits. The pesticide residues were dissipated to different degrees, with the time elapsed after spraying.

Key words: *bromuconazole, physical properties, residue analysis.*

1. INTRODUCTION

Bromuconazole systemic fungicide from triazole pesticides group, is used against *Ascomycetes*, *Basidiomycetes* and Fungi including *Alternaria*, *Fusarium*, black and yellow Sigatoka, and *Pseudocercospora* spp. in cereals, fruit, vines, vegetables, tropical crops, turf and ornamentals. Pesticides may fail to comply with the FAO/WHO meeting specifications 2002 required if it is improperly stored. Chemical and physical instability usually lead to the deterioration of the active ingredient (a.i.) content and emulsion stability under the variable climatic conditions (Emara & Atalla 2006; Emara & Abdel Aziz 2007; Radwan & Atalla 2009; Sandro *et al.*, 2010). The Ministry of Agriculture in Egypt follows FAO and WHO specification. The objective of the present work was aimed to determine and evaluate the physical and chemical properties of Vectra 10% EC (bromuconazole). In addition, the purpose of this study was to demonstrate the determination of bromuconazole

in Cucumber fruits. This will reveal the rate of its persistence in the plants to determine the Pre Harvest Interval (PHI) that should pass after the last treatment and before harvesting for normal human consumption. Washing process to remove surface residues and consequently shortening the safe intervals prior to harvesting was evaluated (Nasr 2002; Hala 2003; Osman *et al.* 2004; Emara & Atalla 2006; and Radwan & Atalla 2009).

2. MATERIALS AND METHODS

2.1. Fungicide used

Vectra (10%SC.) :fungicide from (Bayer CropScience).

Chemical name: 1-[[4-bromo-2-(2,4-dichlorophenyl)tetrahydro-2-furanyl]methyl]-1*H*-1,2,4-triazole. Fig. (1).

2.2. Chemical analysis

2.2.1. Active ingredient percentage

Active ingredient percentage was determined before and after storage for tested fungicide Vectra by HPLC instrument according to Gfont *et*

al.,(1999); Pesticides Analytical Manual (2004) ; and Lidia *et al.*,(2006).

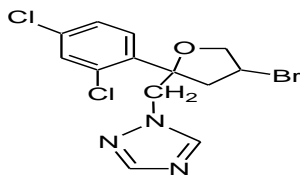


Fig.(1): The chemical structure of bromuconazole.
HPLC conditions

High performance liquid chromatography instrument (Agilent serial 1100) solvent delivery system and quaternary pump with a C18 stainless steel column (2.4X250mm) and UV detector was used under the following conditions as shown in Table (1) and Figure (2).

Preparation of standard solution

Weigh 10 mg (related to a purity of 100%) from both bromuconazole L- 5850646 and L-5850647 reference standard of fungicide into a 10 ml volumetric flask and dilute to the mark with acetonitrile LC grade and mixing well. This is the standard solution.

Calibration

Duplicate injections (2 μ l) of calibration solution and each sample were injected and integrated areas for each peak was recorded. Bromuconazole 10 % is a mixture of two diastereoisomers in proportion (L-5820646 5% & L-5850647 5%) content in each sample was calculated compared with an external standard.

2.3. Absorbance of Vectra formulation in infrared (IR spectra) .

The Fourier transform infrared (Avtar 330 Thermo Nicolet) was used to study the effect of storage on the absorbance of function groups and finger print of bromuconazole according to the method of Barbra (1985) with some modification. Samples were prepared by homogenizing 0.01g of sample with 0.1 g of dry (KBr) by agate mortar and pestle to a clean stainless steel slide and placed in piston to make a clear and thin film of disk sample.

2.4. Proton magnetic resonance (HNMR) of bromuconazole.

The (HNMR) spectra of the fungicide bromuconazole under present investigation have been recorded in Di Methyl Sulfoxide (DMSO) as a solvent and tetra methyl silane (TMS) as an internal reference with 90 MHz signal and 4.000 gauss magnetic field.

2.5. Separation and fragmentation of bromuconazole fungicide formulation by GC equipped with a mass spectrometric detector (GC/MS spectra)

The GC/MS analysis was used to compare the separation and fragmentation of pesticide formulations before and after storage according to the method of Saad *et al.*, (1993). GC/MS analysis was performed with an Agilent 6890 gas chromatograph equipped with a mass spectrometric detector (MSD) model Agilent 5973. A fused silica capillary column (HP-5MS), 5% phenyl polysiloxane as non polar stationary phase (30m x 0.25mm i.d) and 0.25 μ m film thickness.

Operating condition was as follows

Injector port temperature, 210 $^{\circ}$ C. The helium was used as a carrier gas at a flow rate of 1ml/min. Pulsed splitless mode. The column temperature was maintained at 80 $^{\circ}$ C, for 3min. Then, programmed at 10 $^{\circ}$ C/min to 240 $^{\circ}$ C, and held for 20min. The total analysis time was 30 min. A (1 μ l) volume was injected splitless. The mass spectrometric detector (MSD) was operated in electron impact ionization mode, scanning from m/z 50 to 550. The ion source temperature was 230 $^{\circ}$ C and the quadrupole temperature 150 $^{\circ}$ C. The electron multiplier voltages (EM voltage) was maintained 1100 V above autotun, and solvent delay of 3 min was employed. The instrument was manually turned using heptacosyl fluoro tributyl amine (HFTBA).

2.6 .Thermal stability of bromuconazole under storage conditions

Samples of bromuconazole formulation were stored in glass package, in the oven at 54 $^{\circ}$ C \pm 2 $^{\circ}$ C for 3 & 14 days and at 72 $^{\circ}$ C for 3 days .

2.6.1. Emulsion stability test

Five ml of Vectra samples before and after storage were added to a graduated 100ml cylinder filled with 95ml hard water (prepared according to CIPAC MT36) by means of pipette, and then the samples were poured onto the water directed to the center. Stopper the cylinder and invert it for 30 times, and then placed in a water bath maintained at 30 $^{\circ}$ C \pm 1 for 30min. If there is any forming of oily or creamy layer either at the top or the bottom of the cylinders must not be exceeded than 2ml according to WHO (1979 and 1985).

2.7. Persistence of bromuconazole under field conditions

2.7.1. Field experimental and sampling

Cucumber plants were planted at a selected farm at Kafr Shokr, Kalyobia Governorate on May 2009 in plots of 1/100 feddan each. The plots received the normal agronomic practices throughout the experimental period. Vectra (10%) was sprayed on May 10th 2009 at the recommended rate 100 Cm^3 /100 L. water /

feddan. A knapsack sprayer equipped with one nozzle was used. Besides another plots were left untreated as a control in both treatments. Representative samples of mature cucumber fruits (four replicates) were taken at random one hour after application. Subsequent samples were taken 1,3,5,7, and 14 days after treatment. Each sample was divided into two subsamples, the first was washed with tap water for 1 min and the second was left unwashed, the subsamples were stored in poly ethylene bags until analysis.

2.7.2. Extraction and clean up of bromuconazole fungicide

Levels of bromuconazole in cucumber fruits were determined according to the method of Nasr (2002) with some modification. Pesticide residues were extracted with 100ml acetone using the warning blender partitioned into n-hexane. The extract was carefully decanted and filtered through filter paper Watman no.1 then dried through anhydrous sodium sulphate and evaporated on water bath at 40 °C to dryness by using rotary evaporator. The resulting extract of cucumber fruits was cleared by C18 cartridge column chromatography. The fungicide extracts were evaporated at 30 °C to dryness, residues were dissolved in 1ml acetonitrile and then determined by Agilent HPLC apparatus at the same conditions as described above. Obtained results were corrected according to the rate of recovery, which were determined in fortified untreated samples. Following the techniques previously mentioned, the recovery rate of bromuconazole L-5850646 & bromuconazole L-5850647 was 97.99 & 96.09 %, respectively. Student's (t) test was used to analyze the statistical significance between the same treatments (Gad and Weil, 1989).

3. RESULTS AND DISCUSSION

3.1. Effect of storage temperature on chemical properties

3.1.1. Active ingredient percentage

The data in Table (2) show that thermal stability of the active ingredient % of bromuconazole fungicide was not affected by storage condition periods. The data indicate that bromuconazole L- 5850646 stored at 54 °C for 3 & 14 days and at 72 °C for 3 days were 4.93, 4.89, and 4.71% and for bromuconazole L- 5850647 were 4.86, 4.77 and 4.63% stored at 54 °C for 3 & 14 days and at 72 °C for 3 days, stored respectively. The bromuconazole a.i % in all storage conditions were within permissible limits for Pesticides Specification (2006) which was

declared content g/L. Above 25 up to 100 the tolerance was $\pm 10\%$ or the percentage of bromuconazole active ingredient content ($\pm 1\%$), whereas the bromuconazole active ingredient percentage was represented by 9.85% of the zero time sample. The degradation of active ingredient content in pesticide formulations under storage temperature conditions was noticed by Emara 2006; Emara & Atalla 2006; Radwan & Atalla 2009 and Radwan 2010.

3.1.2. Effect of storage temperatures on physical properties (Emulsion test)

The formulation of bromuconazole fungicide passed successfully through emulsion test in different types of storage and comply with WHO specifications (1979) except in storage at 72 °C for 3 days. The cream layer (0.5 ml) appeared but it was within permissible limits (2 ml).

3.1.3. The effect of storage temperature on the absorbance of bromuconazole formulation in infrared

The data obtained from the IR spectra of bromuconazole 10% EC samples initial, room temperature (54 °C for 3 & 14 days and at 72 °C for 3 days of application) showed no significant changes in IR spectra during the different types of storage e.g., at 54 °C for 3 & 14 days. All the IR spectra of the bromuconazole samples showed the same bands but with different intensity due to the degree of the degradation of the active ingredient during the hot storage 54 °C for 3 & 14 days. The samples showed the characteristic bands of bromuconazole as follows:

- 1- The bonds of halide substituted benzene ring C-X appeared peaks at 790-820 cm^{-1} .
- 2 - The C-H bonds aliphatic and aromatic appeared their peaks at 2843 $\pm 10 \text{ cm}^{-1}$ and 2927 $\pm 10 \text{ cm}^{-1}$, respectively.
- 3- The (N) Nitrogen atom in cyclic amid appeared its peaks at 1637 cm^{-1} .
- 4- The (O) Oxygen atom in ethoxylated ester appeared their peaks at 1105.64.

The IR spectrum analysis of bromuconazole fungicide characterized by the presence of peaks between 2871 cm^{-1} and 2929 cm^{-1} supported the presence of methyl group (-CH₃, -CH₂ and CH), also O-C-O group was characterized by IR between 1105 and 1154 cm^{-1} . The infrared spectrum of bromuconazole analysis and the effect of different type of storage on the absorbance are presented in Table (3). Characterizing the structure of bromuconazole appeared as bands of nitrogen (N) atom at 3442.01 cm^{-1} and disappeared after storage at 54 °C for 14 days and 72 °C for 3

Table (1): Bromuconazole calibration parameters.

Solvent system	Flow rate ml/min	Retention time (min)	Regression coefficient (R ²)	Standard deviation SD	Relatively SD	Slope		Limit of detection LOD(ng)		Limit of quantization LOQ(ng)	
Acetonitrile 90%/methanol 10%	1	(1)3.854 (2)4.020	0.9998 0.9898	6.519 5.981	0.0010 0.0011	1.66	1.59	1.243	1.438	8.1	8.6

Table (2): Active ingredient % in bromuconazole at different storage temperatures and periods.

Type of storage	Storage period (day)	Bromuconazole			
		Active ingredient%		Loss %	
		(1)	(2)	(1)	(2)
Room temperature	Initial	4.98	4.87
54° C	3 day	4.93	4.86	1.004	0.2053
54 °C	14	4.89	4.77	1.8072	2.0534
72 °C	3	4.71	4.63	5.4217	4.9281

Initial = One hour before storage. Each value is a mean of three replicates of samples.

1= Bromuconazole L- 5850646 (5%) } 10% Bromuconazole - 2= Bromuconazole L- 5850647 (5%)

Table (3) : Storage temperatures on finger print of formulated bromuconazole by using IR spectrum. Position of bands Cm-1

Bromuconazole	(54°C) for 3 days	54 °C for 14 days	72°C for 3 days
468.03	466.13	468.19	469.36
540.69	541.66	541.01	537.53
722.08	722.07	722.11	754.83
935.53	933.59	941.11	912.72
1035.85	1035.05	1031.55	1031.90
1154.04	1154.64	1156.61	(I)
1304.40	1303.43	1301.88	(I)
1376.63	1376.63	1376.63	1380.57
1456.65	1456.65	1456.61	1468.47
1658.47	1658.47	1657.43	1637.44
2286.18	2286.18	(I)	(I)
2724.13	2724.98	(I)	(I)
2843.29	2841.31	2841.66	(I)
3442.01	3442.00	(I)	(I)
Match %	100	98.86	96.13

(I) = Disappear band.

Table (4): Separation and fragmentation of bromuconazole insecticide formulation.

Type of storage	RT	Expected compound name	Formula	MW
Bromuconazole (Initial)at one hour Before and after 3 &14 days of storage at 54 °C	22.96	Benzoic acid ,2,4 dichloro- (4-methoxyphenyl)	C11H16Cl2NO2	218.00
	25.50	Benzamide, 2,4 dichloro -N-(2,5 dimethylphenyl)	C14H12Cl2O2	308.31
	26.06	1-[[4-bromo-2-(2,4-dichlorophenyl)tetrahydro-2-furanyl]methyl]-1H-1,2,4-triazole	C13H12N3OCl2Br	374.95
After 3 days of storage at 72°C	9.99	2,3,5,6-Tetrachloropyridine	C5HCl4N	215.00
	24.49	2-Amino-6-bromo-4-methoxyphenol	C7H8BrNO2	218.05
	25.83	2,2- Dichloro-1-(2,4- dichloro-phenyl)	C10H5Cl4N3O	323.00
54°C (3day)		Like completely		
54°C (14day)		Like completely		
72°C (3day)		Like completely		

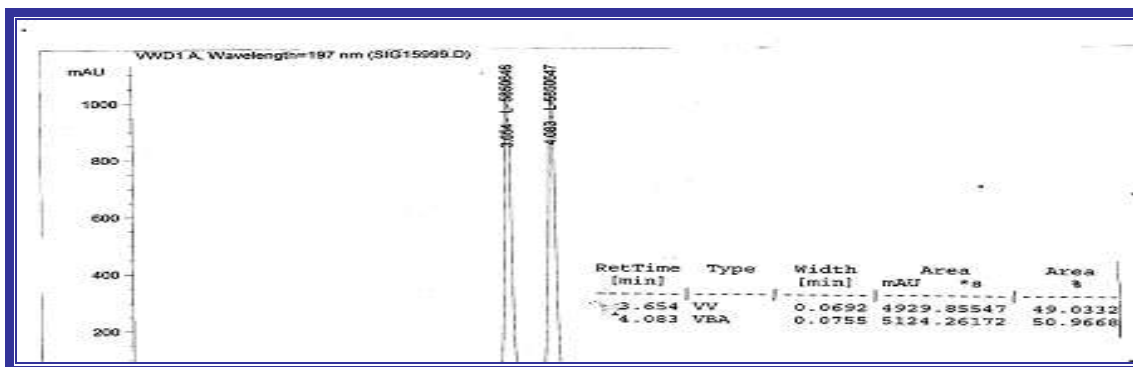


Fig. (2) : Chromatogram of bromuconazole (L- 5850646 & L- 5850647) standard

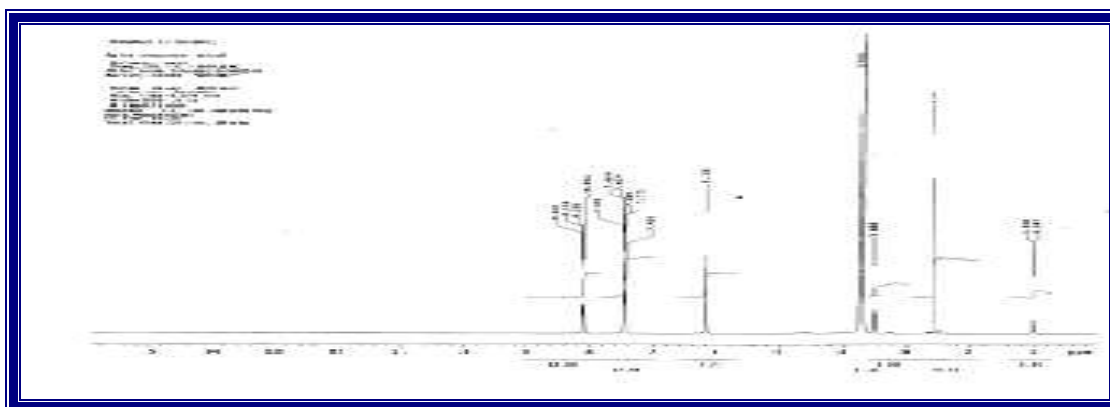


Fig.(3) : Proton NMR spectrum for bromuconazole fungicide

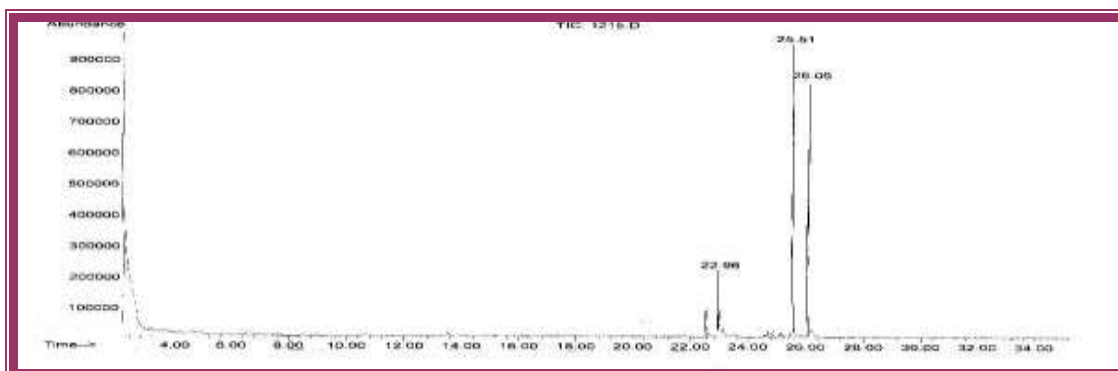


Fig (4): Bromuconazole (Initial) at one hour before and after 3 & 14 days of storage at 54 °C

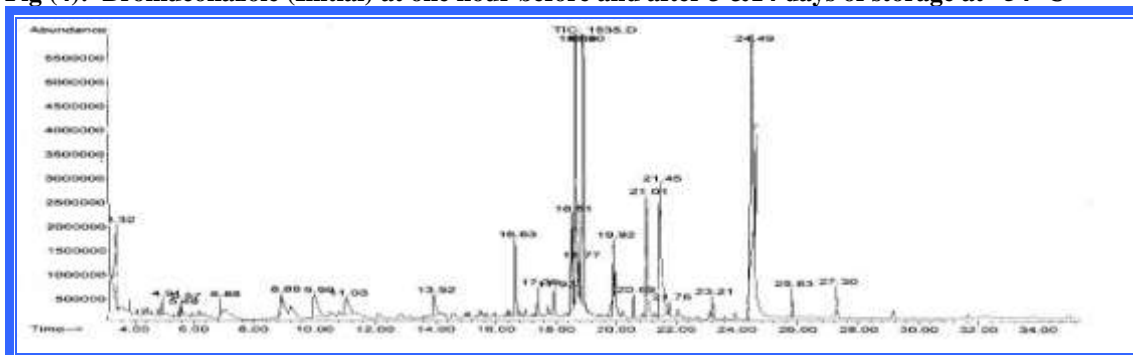


Fig (5):GC/MS chromatogram of Bromuconazole after 3 days of storage at 72°C

Table (5) : Residues of bromuconazole (ppm) on and in unwashed and washed cucumber fruits under field conditions at different time intervals.

Days after treatment	Bromuconazole							
	unwashed				washed			
	ppm (1)	Loss% (2)	(1)	ppm (2)	Loss% (2)	(1)	(2)	
Initial	0.5995	0.8627	0.4734	0.3622	21.03	58.02
1	0.2782	0.1614	53.59	81.92	0.2103	ND	64.92	100
3	0.1648	ND	72.51	100	0.1117	ND	81.37	100
5	0.1547	ND	74.19	100	0.0901	ND	84.97	100
7	0.1333	ND	77.76	100	0.0064	ND	98.93	100
14	0.0743	ND	87.60	100	0.0007	ND	99.88	100
RI₅₀	(1)22.39 hour		(2)14.65 h.		(1)18.48 h.		(2)12.00h.	

1= Bromuconazole L- 5850646 (5%) } 10% Bromuconazole - 2= Bromuconazole L- 5850647 (5%)

days . Also , the results showed that the percentages of match were 100, 98.68 and 96.13 % , after storage at 54 °C for 3 & 14 days and at 72 °C for 3 days, respectively (Table , 3) characteristic IR absorbance obtained by(CRC hand book 1994).

3.1.4. HNMR spectra of bromuconazole

HNMR spectra of bromuconazole revealed the following signals δ ppm 2.571 (H of (CH₂)) ; δ ppm at 3.7-3.4 (H of -CH₂-O-CH₂) ' δ ppm at 8.09 (H of aromatic protons)

(Fig 3).

3.1.5.Effect of storage temperatures on separation and fragmentation of bromuconazole fungicide formulation by GC/MS

The results in Table (4) and Fig (4) show that the bromuconazole formulation gave the same separation compound before and after storage at 54 °C for 3&14 days storage . The results in Table (4) and Fig.(5) show the different separation compounds with many degradation after storage at 72 °C for 3 days .

3.2.Total residues in cucumber fruits

The data in Table (5) demonstrate the initial deposits and the residual behavior of bromuconazole on cucumber fruits after treatment. The initial deposits revealed that the amounts of deposits depended on the nature of the treated surface and the relation between the treated surface and its weight (El-Sayed *et al.*,1976). The results indicated that the initial deposits of bromuconazole residues in washed and unwashed cucumber fruits as determined one hour after application were 0.5995 &0.8627 ppm for bromuconazole L- 5850646 & L- 5850647, respectively. The residue level of initial deposits with bromuconazole is due to many factors , the ratio of surface to mass area and character of treated surface (smooth or rough and waxy or non waxy) (Abo El- Ghar and Ramadan 1962 ; Juan *et*

al., 2007 ;and Mazur *et al.*, 2008) .Moreover , some pesticides are rapidly degraded in open field by sunlight and its stability in some crops which with waxy layer of fruit surface that absorbs or reflects the rays,(Cabras *et al.*, 1990). One day after treatment, the residue were 0.2782 & 0.1614 ppm for bromuconazole L- 5850646 & L- 5850647 , respectively.

From the previous results, it is of a great interest to note the following remarks. Storage at high temperature conditions had not significant effect on the physical properties of bromuconazole formulations while the active ingredients in bromuconazole were degraded but within the permissible limits for FAO Pesticides Specification (2006) . In conclusion, it is clear that the Vectra (bromuconazole) was more stable and Tolerate to be stored at different temperature conditions.

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تأثير درجة حرارة التخزين على ثبات الخواص الكيميائية و الطبيعية و دراسة المتبقي
من مستحضر مبيد بروموكونازول الفطري في ثمار الخيار

الفت عبد اللطيف سيد رضوان

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

ملخص

تتأثر الخواص الكيميائية و الطبيعية لمستحضرات المبيدات بالعديد من العوامل ومن أهمها ظروف التخزين الحرارية . اهتمت هذه الدراسة بمعرفة تأثير التخزين على درجة حرارة 54 درجة مئوية لمدة 14، 3 أيام و لمدة 3 أيام على 72 درجة مئوية على كل من الخواص الكيميائية و الطبيعية لأحد المبيدات الفطرية بروموكونازول 10% بصورة مركز قابل للاستحلاب. بينت الدراسة أن مستحضر البروموكونازول كان ثابتا تحت كل ظروف التخزين . كما أظهرت النتائج نسبة تطابق للمسح بالأشعة تحت الحمراء حيث كانت أعلى من 96% لكل العينات المخزنة تحت ظروف التخزين المختلفة. أيضا لم يحدث تأثير في الخواص الطبيعية للمستحضر تحت نفس ظروف التخزين وهذا ما أظهره التحليل باستخدام جهاز التحليل الكروماتوجرافي الغازي المتصل بمطياف الكتلة حيث أن نواتج التكسير في الجهاز كانت مختلفة فقط في حالة التخزين على 72 درجة مئوية لمدة 3 أيام . كما اهتمت هذه الدراسة بمعرفة مدى ثبات المستحضر بعد الرش حقليا بمعدلات الرش الموصى بها وهي 100 سنتيمتر مكعب لكل 100 لتر ماء للقدان على ثمار الخيار في الموسم الصيفي لسنة 2009 وكان المتبقي بعد الرش بيوم واحد 1.3653 جزء / للمليون بمعدل تكسير 53.59 و 81.92 % لكل من بروموكونازول & L- 5850646 (L- 5850647) .

كما أوضحت النتائج أن فترة نصف الحياة على الثمار بعد الحصاد بدون غسيل بلغت 22.39 , 14.65 ساعة كما أدت عملية غسيل الثمار إلى خفض واضح في فترة نصف الحياة لمستحضر البروموكونازول حيث وصلت إلى 12.00 و 18.48 ساعة لكل من (L- 5850647 & L- 5850646) .

المجلة العلمية لكلية الزراعة – جامعة القاهرة (المجلد 62) العدد الثاني (أبريل 2011):245-252.