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Etoxazole Stability under Thermal Storage and Its Residues on Green Bean Rods by Quechers Method

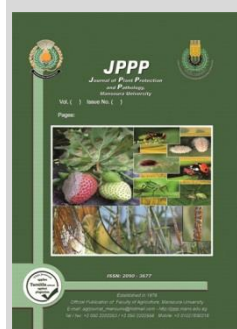
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ABSTRACT

A Commercial acaricide etoxazole suspension concentrate (SC) formulation 10% under trade name dagrozoom was purchased from the Egyptian local market to study the etoxazole behavior in the field on green bean pods to estimate the pre-harvest period (PHI) and study the stability of etoxazole active ingredient under accelerator thermal storage conditions according to the food and agriculture organization (FAO) under different temperatures 35 ± 2 °C for 90 days, 45 ± 2 °C for 21 days, 54 ± 2 °C for 14 days and 72 ± 2 °C for 5 days. Results indicated that suggested PHI for etoxazole according to the maximum residue limits (MRL) for green bean pods was 15 days from treatment and indicated also that etoxazole active ingredient was more stable and was conformity with FAO specifications when stored at 35 ± 2 °C for 90 days, 45 ± 2 °C for 21 days and 54 ± 2 °C for 14 days, on the other side the rate of thermal decomposition of etoxazole active ingredient was increased when stored at 72 ± 2 °C for 5 days and became nonconformity with FAO after 3 days of storage.

Keywords: Etoxazole, Storage, degradation, Residues.

INTRODUCTION

The organic chemical compounds of pesticides, most of them are dangerous and toxic. As a result of the decomposition of pesticides into toxic substances so that it is important to study their fate and know their behavior in terms of quantity and degradation on or in different plants (Neidert and Saschenbreker 1996). Pesticides used on agricultural for plants protection if its residue exceeds than the maximum permissible limits at harvest may cause poisoning of the people who consume these food products. This is because pesticides reach the soil through indirect and direct ways and decompose inside the soil by chemical/or microbial pathways (Ripley and Edgington 1983). Acaricide etoxazole belonging to chemical group (diphenyloxazoline) and controls mites through inhibition of chitin biosynthesis and by causing adults to lay sterile eggs. It was discovered in 1980 and used commercially to control pests in Japan in 1998 and manufactured under various commercial formulations for plant protection. Systemic acaricide etoxazole is a narrow spectrum to combat the spider mites and it working on not adult stage of most of mites in the egg, nymph stages, larvae also have insecticidal activity against aphids, diamond back moth and the green rice leafhopper. The mode of action was originally suspected to inhibit the molting process but has since been shown to inhibit chitin synthesis (Dekeyser and Mark A, 2005) and (Nauen et al., 2006). Resistance due to its high efficacy and cross resistance when used with other acaricides are both of concern similar to was seen in the fast development of cross resistance in the previous generation of acaricides. The LC50 for resistant mite strains has been observed over

100,000 times greater than that of susceptible strains. Thus resistance management strategies are important in order to limit the increase of etoxazole resistant mite strains (Nauen, Ralf et al., 2006) and (Uesugi, R et al., 2002).

MATERIALS AND METHODS

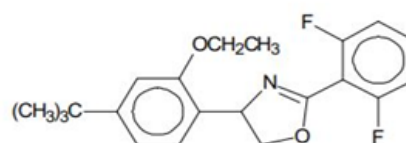
IUPAC Name: (RS)-5-tert-butyl-2-[2-(2,6-difluorophenyl)-4,5-dihydro-1,3-oxazol-4-yl] Phenetole.

Molecular formula: $C_{21}H_{23}F_2NO_2$

Molecular weight: 359.4

CAS Registry number: 153233-91-1

Chemical Formula:



Materials:

Etoxazole 99% reference standard was purchased from Dr. Ehrenstorfer, Germany. Etoxazole commercial formulations under trade name Dagrozoom 10% SC Produced by the Turkish company Deva Agro Kimya was purchased from Egyptian local market. All Organic solvents used were HPLC grade obtained from Scharlau, Spain. Quechers extraction kits with part number 5982-6669 and dispersive-kits with part number 5982-5158 for cleanup obtained from Agilent, Germany. Deionized water prepared from Evoqua water technologies.

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Thermal accelerator storage:

The storage procedure performed according to (CIPAC F, MT 46, 1995) at 35 ± 2 °C, 45 ± 2 °C, 54 ± 2 °C and 72 ± 2 °C for 90, 21, 14 and 5 days respectively. Weight about 50 grams in a bottle from commercial acaricide under study then cap it and put the bottle in the thermal oven after controlled the temperature to the required test degree for the specified days of the experiment. At the end of the storage time, take out the bottle from oven, leave it to cool, and then weigh the sample for testing.

Determination of active ingredient:

Percentage of etoxazole active ingredient determined before and after storage according to method CIPAC Handbook E (1993) and the content of etoxazole after storage should not differ from the determined content before storage by more than $\pm 10\%$.

Field experimental:

In Nubaria, Beheira Governorate, Egypt, the green bean was planted in 20 April 2021 in lines inside the field and suitable volume from commercial formulation dagrozoom 10% SC was taken to prepare the treatment solution at recommended dosage (25 cm³/100 L water) to spray three plots of green beans by using knapsack sprayer having one nozzle on 5 May 2021 and one plot kept as control.

Sampling:

A randomly samples were taken from each plot and each sample was about one kilogram from green bean rods after treatment with the used acaricide in periods two hours, 1, 3, 6, 9, 12, 15, 18 and 21 days from application. Then collected samples were labeled and transferred to the laboratory. Each sample was homogenized and the sub sample was taken in three representative replicates.

Standard preparation:

100 µg/L (ppb) standard stock solution of etoxazole was prepared in acetonitrile to performing calibration curve on green bean matrix with concentrations 0.05, 1, 5, 10, 20 and 50 µg/L by serial dilution of the stock solution.

Sample preparation:

A 15 g from sample to be analyzed added to 50 mL Quechers extraction kits tube with part number (5982-6669) which containing 6 g anhydrous magnesium sulphate and 1.5 g anhydrous sodium acetate then add 15 ml acetonitrile (1% acetic acid) cap the tube and shake it vigorously by using vortex mixer at the highest speed for one min, then put it in the centrifuge for 5 min at a speed of 4000 rpm. Transfer inside dispersive SPE 15 ml tube containing 1200 mg anhydrous magnesium sulphate, 400 mg PSA and 400 mg C18 with part number (5982-5158) 8 ml of supernatant solution and shaken vigorously for 30 sec then centrifuge the tube for 5 min at 4000 rpm for. Filtrated the solution through Sterile Syringe Filter (25mm, 0.2 Micron) and use mobile phase to make dilution in ratio (1:2) and transferred the vial to LC to analysis.

Apparatus:

Etoxazole determination was performed using the Thermo Scientific LCMS/MS system consisted of HTC-PAL Autosampler and column oven controlled at 40°C having column Hypersil GOLD (100 x 2.1 mm, 1.9 µm). Liquid chromatography coupled with TSQ Quantum Access MAX triple quadrupole mass spectrometer with ion source (HESI). Conditions for samples analysis was aux gas flow

rate 15 units, spray voltage: 3500 V, capillary temp: 280°C, sheath gas flow rate was 55 units, heater Temp 295°C and cycle Time: 0.2 s. Mobile phase flow rate was 300 µL/min, consisting of two bottles, A was (water with 0.1% formic acid and 4 mM ammonium formate) and B was (methanol with 0.1% formic acid and 4 mM ammonium formate), mobile phase gradient was as described in Table (1). Etoxazole precursor 360.21 was fragmented to ion 177.10 with collision energy 22 and confirming ion 141 by collision energy 28.

Table 1. Mobile phase gradient

Gradient Time (min)	%A	%B
0.00	98	2
0.25	70	30
15.00	0	100
20.00	0	100
20.01	98	2
25.00	98	2

Recovery Assays:

Standard solution of etoxazole was spiked on an untreated green bean pods samples in levels 0.005, 0.01 and 0.04 mg/kg each one performed with five replicates and recovery results are presented in Table (2).

Table 2. Recovery and relative standard deviation for etoxazole on green bean rods.

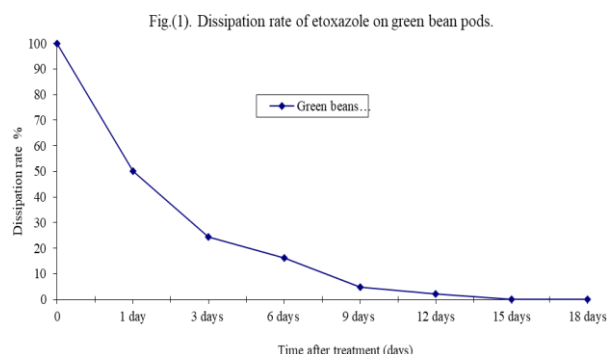
Spiked levels (mg/kg)	Recovery % (mean of 5 replicate)	RSD
0.005	90	5
0.01	95	8
0.04	97	6

RESULTS AND DISCUSSION

The obtained results listed in Table (3) explaining levels of etoxazole residual (mg/kg) after two hours of treatment (initial residue level) until 18 days from treatment illustrating degradation rate of etoxazole on green bean rods. Average of three samples replicates from initial residue level was 1.68 ± 0.06 mg/kg at recommended dosage and decreased to 0.84 ± 0.07 mg/kg recording loss 50% after one day of treatment. Dissipation rates of etoxazole as indicated in figure (1) was increased rapidly with extending days to reached 0.41 ± 0.04 , 0.27 ± 0.03 , 0.08 ± 0.02 , 0.05 ± 0.04 and 0.01 ± 0.01 mg/kg recording loss 75.60 %, 83.93 %, 95.24 %, 97.02 % and 99.40 % after 3, 6, 9, 12, 15 days respectively, while after 18 days of treatment the residues of etoxazole not detected. Results are in harmony with these obtained by (Dali Sun et al., 2021) who reported that final residual of etoxazole in pulp, peel, and whole citrus were ranged at 0.010-0.011, 0.012-0.174 and 0.010-0.637 mg kg⁻¹, respectively. The half-lives ($t_{0.5}$) of etoxazole dissipation at recommended dosage on green beans was 3.17 days calculated according to (Moye et al., 1987). (Saber. A. N et al., 2013) reported that half-life ($t_{1/2}$) and dissipation of etoxazole in field for strawberry fruits in Egypt were 2.8 days and a significant degradation 97.7% after 14 days of application. According to European commission database (EU. 2017) which reported that maximum residue levels (MRL) for etoxazole on beans with rods was 0.01mg/kg the results suggested that pre-harvest period for green beans rods 15 days after treatment with recommended dosage.

Table 3. Etoxazole residues on green bean pods (mg/kg ± SD).

Time (days)	Residue level (mg/kg) ± SD	Dissipation %
Initial	1.68 ± 0.06	0.00
1	0.84 ± 0.07	50.00
3	0.41 ± 0.04	75.60
6	0.27 ± 0.03	83.93
9	0.08 ± 0.02	95.24
12	0.05 ± 0.04	97.02
15	0.01 ± 0.01	99.40
18	ND*	
MRL	0.01	
t _{1/2} (days)	3.17	



Effect of different thermal storage conditions on etoxazole formulation 10% SC.

Data listed in Table (4) and (5) describe the effect of accelerator thermal storage on the commercial etoxazole formulation under four different temperatures according to the food and agriculture organization on 35±2 °C, 45±2 °C, 54±2 °C and 72±2 °C for 90, 21, 14 and 5 days respectively showed that active ingredient of etoxazole was more stable against thermal storage at 35±2 °C and 45±2 °C where the initial percentage before storage was 9.96% and after 1 day of storage was 9.96% and 9.95% respectively. Extended days of storage indicated slowly increasing in rate of degradation as mentioned in figure (2) also the active ingredient percentage reached 9.76 % with loss 2.01% and reached 9.96 % with loss 2.71% after 90 and 21 day at 35±2 and 45±2 respectively.

Results in Table (5) indicated the resistance of etoxazole active ingredient against thermal storage at 54±2 °C for 14 days, where the percentage of etoxazole active ingredient was 9.91% with loss 0.5% after 1 day of storage showing a slightly increasing in the degradation rate with extending storage time to reached 9.25 with loss 7.13% after 14 days of storage.

Table 4. Effect of thermal storage at 35 oC and 45 oC on etoxazole formulation 10 % SC.

Storage at 35±2 °C			Storage at 45±2 °C		
days	a.i %	Loss %	days	a.i %	Loss %
Initial	9.96	0.00	Initial	9.96	0.00
1	9.96	0.00	1	9.95	0.10
7	9.94	0.20	3	9.92	0.40
15	9.93	0.30	7	9.9	0.60
30	9.9	0.60	10	9.85	1.10
45	9.88	0.80	13	9.81	1.51
60	9.84	1.20	16	9.76	2.01
75	9.81	1.51	19	9.72	2.41
90	9.76	2.01	21	9.69	2.71

On the contrary from Table (5) and figure (3) showed that etoxazole active ingredient influenced with thermal accelerator storage at 72 ± 2 °C and it was less stable from the other storage conditions at 35, 45, 54 ± 2 °C with recording loss of 3.71%, 9.94% and 12.25% after 1, 3, 5 days of storage. From the previously data observed that degradation rate of etoxazole formulation was influenced by thermal storage temperature and with long time of storage at 72 ± 2 °C.

Fig.(2). Effect of thermal storage at 35 °C and 45 °C on etoxazole formulation 10 % SC.

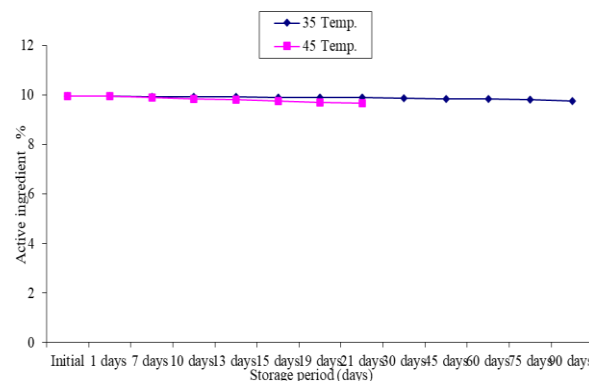
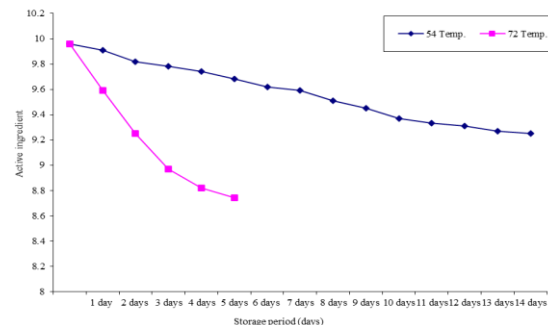


Table 5. Effect of thermal storage at 54 °C and 72 °C on etoxazole formulation 10 % SC.

Storage at 54 C			Storage at 72 C		
days	a.i %	Loss %	days	a.i %	Loss %
Initial	9.96	0.00	Initial	9.96	0.00
1	9.91	0.50	1	9.59	3.71
3	9.78	1.81	2	9.25	7.13
7	9.59	3.71	3	8.97	9.94
10	9.34	6.22	4	8.82	11.45
14	9.25	7.13	5	8.74	12.25

Fig (3) Effect of thermal storage at 54 oC and 72 oC on etoxazole formulation 10 % SC



According to the tolerances (±10%) for pesticide formulated in (FAO/WHO specifications 2006), the used etoxazole acaricide formulation passed successfully through thermal storage and was conformity with this specifications when stored at 35±2 °C, 45±2 °C and 54±2 °C for 90, 21 and 14 days respectively but become nonconformity when stored at 72±2 °C after 3 days. Results are in line with (Ramadan 2009, Kamal El-Din and Ola. M. Emara 2007, Khozimy et al., 2017) reported that the rate of pesticides degradation increased with long time of thermal storage and increasing storage temperature.

CONCLUSION

Suggested PHI for etoxazole according to the maximum residue limits for green bean pods was 15 days from treatment at recommended dose. Etoxazole active

ingredient was more stable when stored at 35±2 °C for 90 days, 45±2 °C for 21 days and 54±2 °C for 14 days but it influenced by thermal storage and degradation rate increased after 3 days of storage at 72 ±2 °C.

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ثبات الإيتوكزازول تحت التخزين الحراري ومتبقيات في الفاصوليا الخضراء بطريقة كويتشرز Quechers

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تمت الدراسة على المبيد الأكاروسى داجروزوم 10% SC المحتوى على الإيتوكزازول 10% حيث تم زراعة الفاصوليا الخضراء في النوبارية بالبحيرة ومعاملتها بالمبيد حسب المعدل الموصى به لتحديد فترة ما قبل الحصاد كما تم تخزين المبيد على درجات حرارة مختلفة وهي 35 ، 45 ، 54 و 72 درجة مئوية لمدة 90 و 21 و 14 و 5 يوم التوالى لدراسة تأثير التخزين الحراري على ثبات المادة الفعالة للمبيد تحت الدراسة وكانت النتائج كالتالي: أوضحت الدراسة أن بقايا الإيتوكزازول على الفاصوليا الخضراء أصبح أقل من الحدود المسموح بها (0.01 ملليجرام/كجم) بعد 15 يوم من المعاملة بالمعدل الموصى به حيث كان المتبقي من المبيد على الفاصوليا الخضراء بعد المعاملة بساعتان 1,68 ملليجرام/كجم وبعد 15 يوم أصبحت 0,01 ملليجرام/كجم والفترة 15 يوم هيا التي نوصى بها لتكون آمنة على المستهلك. كما أوضحت الدراسة أن المادة الفعالة للإيتوكزازول لم تتأثر بالتخزين الحراري على درجات الحرارة 35 لمدة 90 يوم و 45 لمدة 21 يوم ولكنها تأثرت قليلا بالتخزين الحراري على درجة 54 لمدة 14 يوم ولكنها تطابقت مع منظمة الأغذية والزراعة أما التخزين الحراري على درجة 72 درجة مئوية أثر على المادة الفعالة وزاد معدل التحطم للمادة الفعالة بزيادة أيام التخزين وأصبحت غير مطابقة لمنظمة الأغذية والزراعة عند التخزين بعد ثلاثة أيام.