



Evaluate the Equivalency of Some Insecticides Pesticides Containing Diazinon and Diazinonox, with Emphasis to Their Content of Impurities and Degradation

Nahed M. M. Selim^{1*} and Hala M. Ibrahim¹

¹*Pesticide Analysis Research Department, Central Pesticides Laboratory, Agricultural Research Center, Dokki, Giza, Egypt.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAERI/2020/v21i1130125

Editor(s):

(1) Dr. Daniele De Wrachien, State University of Milan, Italy.

Reviewers:

(1) Cristina Hegedüs, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, România.

(2) Aba-Toumnou Lucie, University of Bangui, Central African Republic.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/54856>

Received 11 December 2019

Accepted 21 February 2020

Published 06 March 2020

Original Research Article

ABSTRACT

The aim of this study has investigated the degradation of diazinonox 5% and five different concentration of diazinon (diazinon 95%, diazinon 15%, diazole 50%, diazot 60% and octadat 60%) before and after storage at 54 +2°C for 0, 7, 14 days. Besides, the effects of storage on the formation of its toxic impurities (sulfotep, monothiono-tepp, and water) were studied. The data showed that diazinonox 5%, was less stable than different concentrations of diazinon, whereas the percentage loss 38.5% while, diazinon 95% was more stable for storage which has percentage loss is 1.14% after 14 days of storage at 54+2°C. For results, diazinon 15%, diazole 50%, diazot 60% and octadat 60% show it's relatively moderate in its stability compared with other tests which have a percentage loss ranged from 1.33 to 27.2% after 14 days of storage at the same conditions.

Moreover, the monothiono-tepp impurity (O, S- TEPP) in diazinon 95%, diazinonox 5% and diazot 60% was more than the maximum permissible concentration of impurity recommended by FAO specifications before and after storage. But diazinon 15%, diazole 50% and octadat 60% was This level are allowed to limit according to FAO before and after storage. While sulfotep impurity in diazinon 95% was more than the maximum permissible concentration of impurity recommended by FAO specifications before and after storage. On the other hand, diazinon 15%, diazinonox 5%, diazole

*Corresponding author: E-mail: nahed_slem@yahoo.com;

50%, diazit 60% and octadat 60% were This levels are allowed to limit according to FAO before and after storage.

Finally, GC – MS analysis gives the same separation before storage whereas it gives a new separation peak after storage and results showed that of the breakdown of the 2-Isopropyl-6-methyl-pyrimidin-4-ol main product in diazinon and diazinon. Also, IR analysis showed that the % match of all tested was more than 90 before and after storage.

Keywords: *Diazinon; diazinon; sulfotep (s, s-tepp) and monothiono-tepp (o, s-tepp).*

1. INTRODUCTION

Diazinon (DZ) and its active metabolite, diazoxon (DZO), are members of a ubiquitously-used class of insecticides known as organophosphorus insecticides (OPs) [1].

Diazinon is widely used in agriculture and animal husbandry to control pests [2]. Diazinon is high – effective pesticides that are extensively applied in agriculture [3]. Diazinon is an organophosphate insecticide that can inhibit acetyl-cholinesterase competitively and increase the production of Reactive Oxygen Species (ROS) [4].

Diazinon enters the food cycle by releasing into the environment and, by entering water resources, affects the life of aquatic organisms. It can be stored in their body tissues. Diazinon through the dosage of residues in crops and forage can enter the human body and animals and cause poisoning Nima Nezami [5]. Diazinon (DZN) is one of the most dangerous hepatotoxic organophosphorus insecticides used in veterinary practices which induces oxidative stress [6].

The widely-used OP diazinon (DZN) and its oxygen analog, diazoxon (DZO), are considered neurotoxic, but the mechanism of toxicity is not well understood. While diazinon was banned for residential use in the U.S. in 2004, it is still commonly used in agriculture in the U.S and abroad [1]. Diazinon (DZ) is an organophosphate pesticide that induces oxidative damage in different organs[7].

DZN is an organophosphate pesticide that is extensively used to control households' insects and vegetable crops. Indiscriminate use of pesticides including DZN has been one of the problems of current society, especially in the major centres of agriculture Fatemeh et al. [8].

Li et al. [9] reported that the degrading compounds from diazinon had identified as 2-isopropyl-6-methyl-4-hydroxypyrimidine (IMHP); IMHP is a

major degradation product when a low concentration of diazinon was studied in soil and water. Although (IMHP) is found to be potentially leachable, it is less toxic than diazinon. Moreover, diazinon-O-analog (or diazoxon), 1,3-dimethyl-2-nitrobenzene, O,O,O,O-tetraethyl dithiopyrophosphate can also be produced as hydrolytic degradation.

During synthesis and/or storage of diazinon, there may be the formation of a highly toxic impurity, sulfotepp (O,O,O,O-tetraethyl dithiopyrophosphate)[10,11].

The food and agricultural organization (FAO) Specification for plant protection products lists the main impurities of diazinon is Sulfotep, monothiono-TEPP and water [12].

Sulfotep (S,S-TEPP) and Monothiono- TEPP (O,S-TEPP) are potent cholinesterase enzyme inhibitors and highly toxic. The cholinesterase inhibition activity of monothiono- TEPP (O,S-TEPP) was found to be about 14,000 times higher than that of diazinon [13,14]. Sulfotep is also resistant to hydrolysis and therefore is a concern in the disposal of waste or spilt diazinon [15].

In the present study aimed to evaluate the effect of storage on the degradation of diazinon 95% TC, diazinon 15% EC, diazinon 5% WG, diazole 50% EC, diazit 60%, octadat 60% and their content of impurities of sulfotep (S,S-TEPP) and monothiono-TEPP (O,S-TEPP). Also, diazinon and diazinon formulation have been determined by IR and GC/MS.

2. MATERIALS AND METHODS

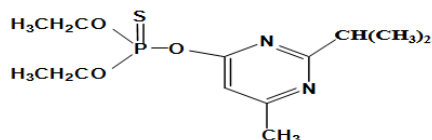
1. The structure of diazinon and its impurities are obtained from [11] and [16]:

1.1. Diazinon

IUPAC: O,O-Diethyl-O-[2-Isopropyl-4-Methyl-6-Pyrimidinyl] Thiophosphate

Empirical Formula: C₁₂H₂₁N₂O₃PS

Chemical Structure:



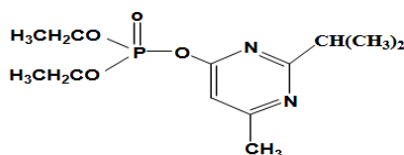
Formulation name: Diazinon, Diazole, Diazit, or octadat

1.2. Diazinon

IUPAC: O,O-Diethyl-O-[2-isopropyl-4-Methyl-6-Pyrimidinyl] phosphate

Empirical Formula: $C_{12}H_{21}N_2O_4P$

Chemical Structure:



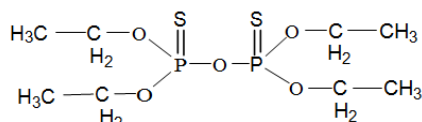
1.3. Impurities of diazinon and Diazinon

a. Sulfotep (S, S-EPP)

IUPAC NAME: O,O,O',O'-tetraethyl dithiopyrophosphate

Empirical Formula: $C_8H_{20}O_5P_2S_2$

Chemical Structure:



FAO maximum Permissible level:

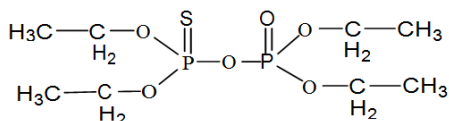
2.8XA mg/kg, where A is the diazinon content in g/l

b. Monothiono-TEPP (O, S- TEPP)

IUPAC Name: O,O,O',O'-tetraethyl thiopyrophosphate

Empirical Formula: $C_8H_{20}O_6P_2S$

Chemical Structure:



FAO maximum Permissible level:

0.22XA mg/kg, where A is the diazinon content in g/l

2. Storage stability test:

The samples of diazinon, diazinon, diazole, diazit, and octadat were stored at $54 \pm 2^\circ\text{C}$ for 21 days according to FAO specifications [12,17]. The active ingredient and the toxic impurities content of the samples were determined at 0, 7 and 14 days of the storage.

3. Preparation of sample:

i. Standard preparation

10 mg of the analytical standard from tested insecticide were weighted inside a 25 ml volumetric flask then dissolved and completed to the final volume with methanol.

ii. Sample preparation for tested pesticides

Accurately weighed sufficient samples formulation to equivalent to 10 mg of diazinon, diazinon, diazole, diazit and octadat Standard in a different 25 ml volumetric flask for each sample and slowly mixed with methanol and the volume was completed with methanol.

iii. Sample preparation for impurities

One ml of tested formulation samples was weighed which contain 0.95 g, 0.15 g, 0.05 g, 0.6 g and 0.6 g of diazinon 95% (TC), diazinon 15% (EC), diazinon 5% (WG), diazole 50% (EC) and diazit 60% and octadat 60%, respectively in six different 25 ml volumetric flasks, dissolved with methanol and completed to the final volume with methanol.

4. Determination of Diazinon, Diazinon, Diazole, Diazit, and octadat and its impurities by GLC instrument:

Diazinon and its toxic impurities sulfotep (s, s-tepp) and monothiono-tepp (o,s-tepp) were determined according to the method of Allender and britten [18] with some modification by using gas-liquid chromatography. A Hewlett-Packard G. C. Model 6890 instrument equipped with a flame ionization detector (FID), capillary column $15\text{ m} \times 0.53\text{ mm}$. Nitrogen was used as carrier gas at a flow rate of 7 ml/min. The detector and injector temperature was set at 300 and 250°C , respectively. The oven temperature was 200°C

and 160°C for diazinon and impurities. The retention of diazinon, sulfotep (s,s-tepp) and monothiono-tepp (o,s-tepp) under the described conditions were 2.85, 3.46 and 3.62 minutes, respectively. The quantitative determination by comparison with the standard of known purity under the identical GLC condition.

5. Determination of water impurity in Diazinon 15% and Diazit 60%:

Water content as relevant impurities for diazinon 15% and diazit 60% was determined by Dan streak method as mentioned in CIPACF [19].

6. The absorbance of Diazinon, Diazinox, Diazole, Diazit and octadat in infrared (IR spectra):

The Fourier transform infrared (Avtra 330 Thermo Nicolet) was used to study the effect of storage on the absorbance of function groups and the fingerprint of Organophosphorus insecticide formulations according to the method of Barbara [20] with some modification. samples were prepared by homogenized 0.01 gm of the sample with 0.1 gm of dry potassium bromide (KBr) by agate mortar and pestle, and then 0.03 gm from the above mixture was transferred with forceps to a clean stainless steel slide and placed in the piston to make a clear and thin film of a sample.

7. Gas–Chromatography-mass spectrometry analysis of Diazinon, Diazinox, Diazole, Diazit, and octadat:

The GC– MS analysis was performed with Agilent of 6890 gas chromatography equipped with an Agilent mass spectrometric detector, with the direct capillary interface and fused silica capillary column HP- 5MS (30 mx 320 mm × 0.25 mm film thickness). Helium was used as carrier gas at approximately 1.0 ml/min pulsed splitless modes. The solvent delay was 3 min, and the injection volume was 1 µL. The mass spectrometric detector was operated in electron impact ionization mode with the ionizing energy of was 70 e.v scanning from m/z 50 to 500. The ion source temperature was 230°C and quadruples temperature 50°C. The electron multiplier voltage (EM voltage) was maintained 1050 v above autotune. The instrument was manually tune using perfluorotributylamine (PFTBA). The GLC temperature program was held at 80°C for 3 min, then elevated to 260°C

at a rate of 80°C/ min, the detector and injector temperature were set at 280 and 250°C, respectively according to the method of saad et al. [21].

3. RESULTS AND DISCUSSION

1. Effect of storage at 54 ± 2°C on the stability of Diazinon 95% TC and its contents:

The data illustrated in Tables (1,2,4,5,6) show the effect of storage at 54±2°C on the stability of diazinon active ingredient (a.i) and their content of sulfotep (S,S-TEPP) and monothiono-TEPP (O,S- TEPP).

The data indicate that diazinon (a.i) in its formulation was relatively influencing during storage at 54± 2°C, where the percentage loss of diazinon after 14 days of storage was 1.14%. these results are in line with Olfat [22] who reported that the percentage of diazinon active ingredient was 59.31% after storage 14 days in an oven at 54± 2°C.

Also, the results in Table 1 showed their content of sulfotep (S,S-TEPP) and monothiono-tepp (O,S-TEPP) before storage were 3.95 and 0.37 g/l of diazinon contents, respectively. This level is higher matching the maximum level defined by FAO [12], the data also showed decreasing the amount of both impurities during storage periods, which become 3.05 and 0.26 g/l of diazinon contents after 14 days of storage at 54± 2°C for sulfotep and monothiono-TEPP, respectively. Nevertheless, such amount is still higher matching the maximum level. The obtained results are in line with Richard and Barry [23] found that the highest level of sulfotep was 0.53 g/l a.i of diazinon in technical formulated diazinon obtained in Canada between 1984 and 1986.

2. Effect of storage at 54 ± 2°C on the stability of Diazinon 15% EC and its contents:

Data presented in Table 2 show that the stability of Diazinon 15% EC active ingredient in its formulation was relatively influencing during storage at 54± 2°C, where the percentage loss of diazinon after 14 days of storage was 2.14%. Also, the results in Table 2 showed that the amount of sulfotep (S,S-TEPP) before storage was 99.0 and was UND for monothiono-TEPP (O,S-TEPP). This level is allowed to limit according to FAO [12]. Where the maximum level

for sulfotep and monothiono-tepp in diazinon 15% were 418.6 mg/l and 32.89 mg/l before storage.

The obtained results are in line with Meier et al. [10] found that sulfotep was identified as an impurity in all of the diazinon formulation understudied also they found that the oldest samples of diazinon formulation had sulfotep concentration lower than some of the other formulations analyzed. This showed that the concentration of sulfotep was not a function of the age of the formulation. Also, Margot & Gysin [24] reported that sulfotep is formed during the manufacturing process, and it is not a hydrolysis product.

3. Effect of storage at 54±2°C on the stability of Diazinon 5% WG and its contents:

The data illustrated in Table 3 show the effect of storage at 54± 2°C on the stability of Diazinon 5% WG and their content of sulfotep (S,S-TEPP) and monothiono-TEPP (O,S- TEPP).

The data indicate that diazinon (a.i) in its formulation was relatively influencing during storage at 54± 2°C, where the percentage loss of diazinon after 14 days of storage was 38.5%.

Also, the results in Table 3 showed their content of sulfotep (S,S-TEPP) was UND and monothiono-TEPP (O,S-TEPP) before storage was 86.31 g/l of Diazinon contents, this level is higher matching the maximum level defined by

FAO [12], the data also showed decreasing the amount of impurities during storage periods, which become 86.31 g/l of diazinon contents after 14 days of storage at 54± 2°C for monothiono-tepp. Nevertheless, such amount is still higher matching the maximum level. Where the maximum level for sulfotep and monothiono-tepp in diazinon 5% were 130.2 mg/l and 10.25 mg/l before storage.

4. Effect of storage at 54±2°C on the stability of Diazole 50% EC and its contents:

Data presented in Table 4 show that the stability of diazole 50% EC active ingredient in its formulation was relatively influencing during storage at 54± 2°C, where the percentage loss of diazole after 14 days of storage was 2.1%. Also, the results in Table 4 showed their content of sulfotep and monothiono-TEPP before storage were 0.046 and 0.59 mg/l of diazole contents, respectively. This level is allowed to limit according to FAO [12]. Where the maximum level for sulfotep and monothiono-tepp in diazole 50% were 1398.04 mg/l and 109.8 mg/l before storage.

5. Effect of storage at 54±2°C on the stability of Diazit 60% and its contents

The data indicate that diazit (a.i) in its formulation was relatively influencing during storage at 54± 2°C, where the percentage loss of diazit after 14 days of storage was 27.2%.

Table 1. Influence of storage at 54 ± 2°C on the stability of Diazinon 95% TC and its contents of sulfotep and monothiono-TEPP

Storage periods (days)	Diazinon 95 g/l	Loss %	Sulfotep g/l	*FAO max	Monothiono-TEPP g/l	**FAO max
0	945.4	-	3.95	2.6	0.37	0.2
7	941.7	0.39	3.54	2.6	0.36	0.2
14	934.6	1.14	3.05	2.6	0.26	0.2

Zero: One hour before storage; * FAO Maximum: 2.8XA mg/kg, where A is the diazinon content in g/l

** FAO Maximum: 0.22XA mg/kg, where A is the diazinon content in g/l

Table 2. Effect of storage at 54 ± 2°C on the stability of Diazinon 15% EC and its contents

Storage periods (days)	Diazinon 15% g/kg	Loss %	Sulfotep mg/l	*FAO max	Monothiono-TEPP mg/l	**FAO max
0	149.5	-	99.0	418.6	UND	32.89
7	148.8	0.47	101.4	416.73	UND	32.74
14	146.3	2.14	100.2	409.64	UND	32.19

Zero: One hour before storage

* FAO Maximum: 2.8XA mg/kg, where A is the diazinon content in g/l

** FAO Maximum: 0.22XA mg/kg, where A is the diazinon content in g/l

Table 3. Effect of storage at 54±2°C on the stability of Diazinox 5% WG and its contents:

Storage periods (days)	Diazinox 5% g/kg	Loss %	Sulfotep mg/kg	*FAO max	Monothiono-TEPP mg/kg	**FAO max
0	46.5	-	UND	130.2	89.29	10.25
7	43.7	6.02	UND	122.36	95.24	9.61
14	28.6	38.5	UND	80.08	86.31	6.292

Zero: One hour before storage

* FAO Maximum: 2.8XA mg/kg, where A is the diazinon content in g/l

** FAO Maximum: 0.22XA mg/kg, where A is the diazinon content in g/l

Table 4. Effect of storage at 54±2°C on the stability of Diazole 50% EC and its contents

Storage periods (days)	Diazole 50% g/l	Loss %	Sulfotep mg/l	*FAO max	Monothiono-TEPP mg/l	**FAO max
0	499.3	-	0.046	1398.04	0.59	109.8
7	497.6	0.34	0.061	1393.28	0.25	109.5
14	488.9	2.1	0.055	1368.9	0.39	107.56

Zero: One hour before storage

* FAO Maximum: 2.8XA mg/kg, where A is the diazinon content in g/l

** FAO Maximum: 0.22XA mg/kg, where A is the diazinon content in g/l

Also, the results in Table 5 showed their content of sulfotep (S, S-TEPP) and monothiono-TEPP (O, S- TEPP) before storage were 420.19 and 4873.2 g/l of diazit contents, respectively. This level for monothiono-tepp is higher matching the maximum level defined by FAO [12], the data also showed decreasing the amount of both impurities during storage periods, which become 4809.52 mg/l of diazit contents after 14 days of storage at 54± 2°C for monothiono-TEPP, Nevertheless, such amount is still higher matching the maximum level. But for sulfotep, this level is allowed to limit according to FAO [12]. Where the maximum level for sulfotep and monothiono-tepp in diazinon 600 g/l were 1659 mg/l and 130.35 mg/l before storage, respectively.

6. Effect of storage at 54±2°C on the stability of octadat 60% and its contents

The data indicate that octadat (a.i) in its formulation was relatively influencing during storage at 54± 2°C, where the percentage loss of diazinon after 14 days of storage was 1.33%.

Also, the results in Table 6 showed their content of sulfotep (S, S-TEPP) and monothiono-TEPP (O, S- TEPP) before storage was 79.62 and 125.6 mg/l of diazinon contents, respectively.

This level is allowed to limit according to FAO [12]. Where the maximum level for sulfotep and monothiono-tepp in diazinon 600 g/l were 1663.8 mg/l and 130.72 mg/l before storage.

7. Estimation of water impurities for diazinon 95%, diazinon 15%, diazinon 5%, diazole 50% and diazit 60% and octadat 60%

The water content for impurities diazinon 95%, diazinon 5%, diazole 50% and octadat 60% were undetectable, while water content in both diazinon 15% and diazit 60% was 0.25 g / kgs 3.33g / kg, respectively according to FAO [12] the maximum limit of water content in diazinon and diazit is 2g / kg, the data showed increasing the amount of impurity of water in diazit 60% from the maximum level defined by FAO [12]. The results were similar to those obtained by Sovocol et al. [25] and might exist either as the impurity in the manufacture of diazinon or as a breakdown product in the presence of trace water in the pesticide formulation. According to Fishel and Ferrell [26], the rate of hydrolysis will increase, also the time the pesticide to degrade is in contact with water.

8. Effect of storage temperatures on the absorbance of diazinon, diazinon, diazole, diazit and octadat in infrared

The data in Tables 7 and 8 and Figs. (1,2,3,4 and 5) showed the IR spectra of diazinon, diazinon, diazole, diazit and octadat before and after storage at 54°C. All the IR spectra of the samples showed the same bands. But with different intensity due to the degree of the degradation of the active ingredient during the storage at 54 +2°C for 14 days of storage. The

samples showed the characteristic bands of as follows:

- The peaks of R-O-R bonds appeared at 1020-1080 cm^{-1} and the peaks of P-O-C bonds appeared at 970-1050 cm^{-1} .
- The peaks of P=S bonds appeared at 580-750 cm^{-1} .
- The C-H bonds aliphatic and aromatic appeared their peaks at $2930 \pm 10 \text{ cm}^{-1}$ and $2955 \pm 10 \text{ cm}^{-1}$, respectively.

The infrared analysis of different diazinon and diazinon 5% were presented in Tables 7 and 8. Characterized the structure of diazinon 95%, diazinon 15%, diazinon 5%, diazole 50%, diazit 60% and octadat 60% were appeared bands of nitrogen (N) atom at 3415.86, 3412.75, 3410.69, 3408.57, 3430.48 and 3436.27 cm^{-1} and shifted about -4.51, -11.64, -11.93, -0.22,

21.91 and 17.53 after storage at 54°C, respectively.

9. Identification of Diazinon and Diazinon by chemical ionization GC/MS spectroscopy:

The data present in Table 9 and Figs. (6,7,8 and 9) showed the GC/MS analysis for Diazinon and Diazinon before and after storage. The results summarized in Table 9 and Figs. 7 and 8 showed the degradation process for diazinon is chemical oxidation. This process known as oxidative desulfuration, involves the addition of OH radicals to P=S bond, resulting in a P=O bond and eventually diazoxon, which is more toxic than diazinon [27- 30].

The general pathways of diazinon and diazinon are given in Fig. 7.

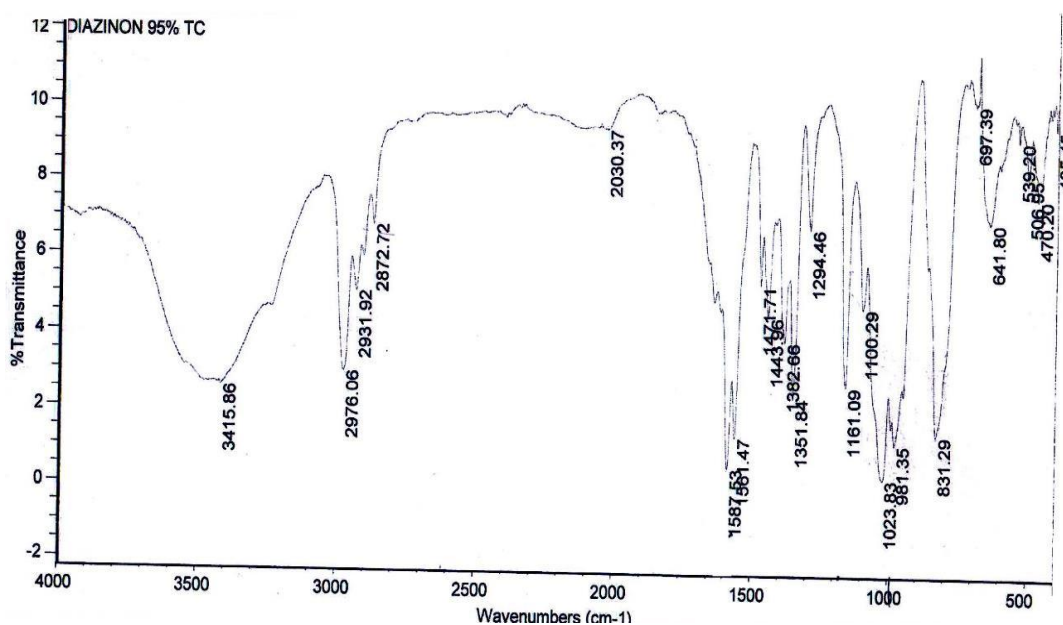


Fig. 1. IR chromatogram of diazinon 95% before storage

Table 5. Effect of storage at 54±2°C on the stability of Diazit 60% and its contents:

Storage periods (days)	Diazit 60% g/l	Loss %	Sulfotep mg/l	*FAO max	Monothiono-TEPP mg/l	**FAO max
0	592.5	-	420.19	1659	4873.2	130.35
7	561.7	5.2	417.77	1572.8	4880.95	123.57
14	431.4	27.2	422.60	1207.9	4809.52	94.91

Zero: One hour before storage

* FAO Maximum: 2.8XA mg/kg, where A is the diazinon content in g/l

** FAO Maximum: 0.22XA mg/kg, where A is the diazinon content in g/l

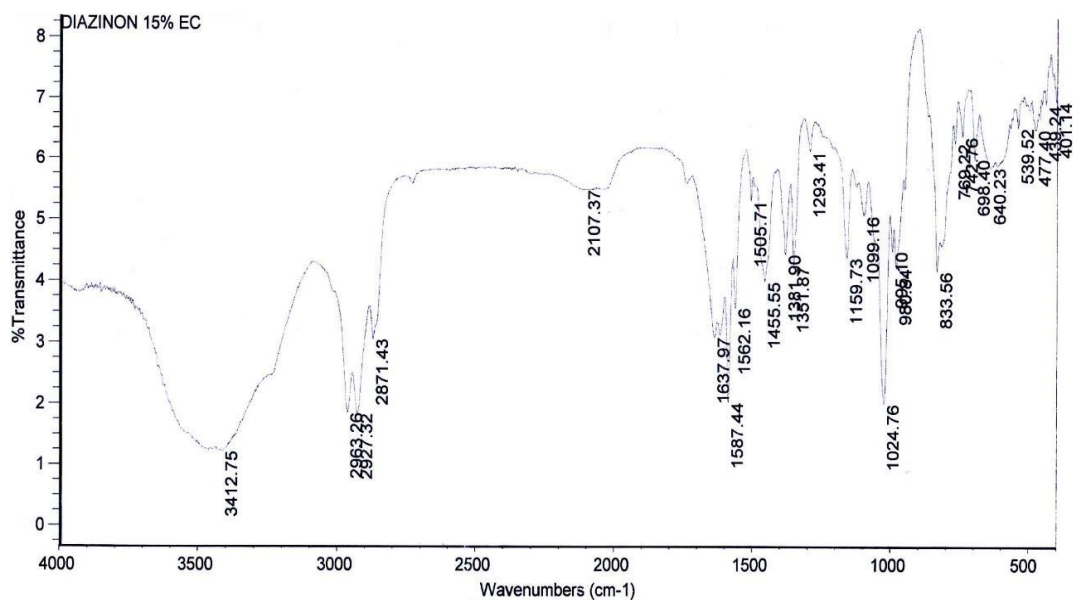


Fig. 2. IR Chromatogram of Diazinon 15% before storage

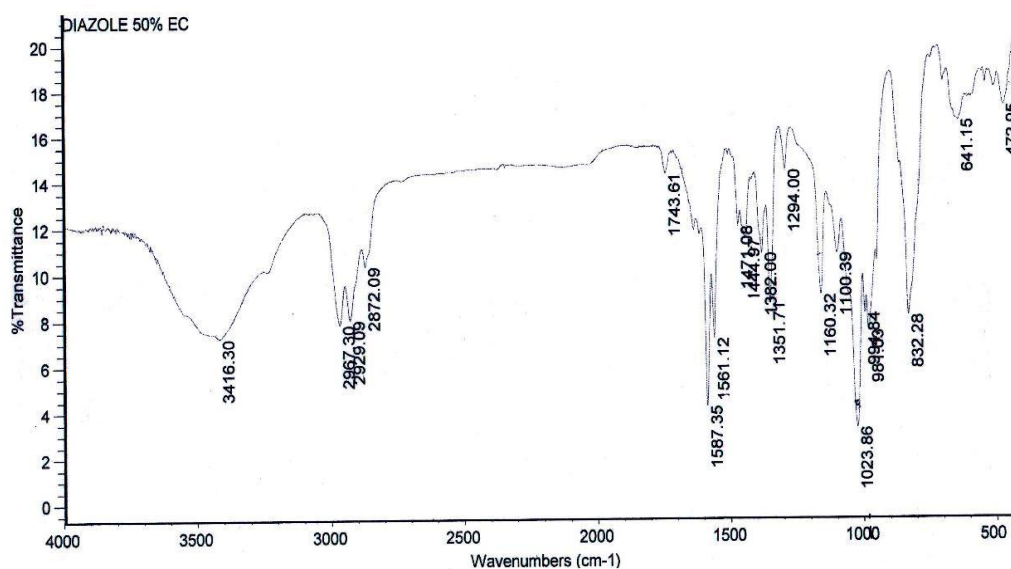


Fig. 3. IR Chromatogram of Diazole 50% before storage

Table 6. Effect of storage at 54±2°C on the stability of octadat 60% and its contents

Storage periods (days)	octadat 60% g/l	Loss %	Sulfotep mg/l	*FAO max	Monothiono-TEPP mg/l	**FAO max
0	594.2	-	79.62	1663.8	125.6	130.72
7	591.2	0.5	89.32	1655.4	112.3	130.064
14	586.3	1.33	79.4	1641.64	105.7	128.99

Zero: One hour before storage

* FAO Maximum: 2.8XA mg/kg, where A is the diazinon content in g/l

** FAO Maximum: 0.22XA mg/kg, where A is the diazinon content in g/l

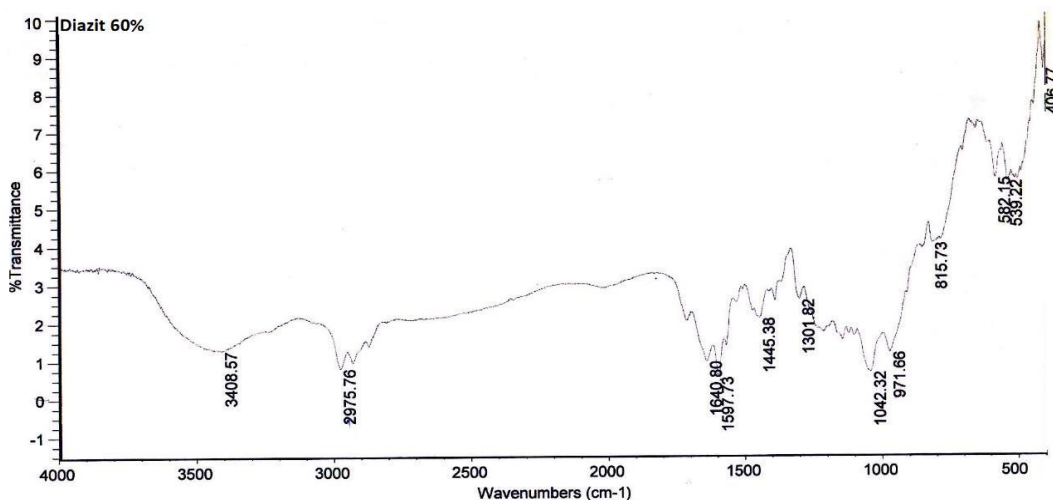


Fig. 4. IR Chromatogram of Diazit 60% after storage

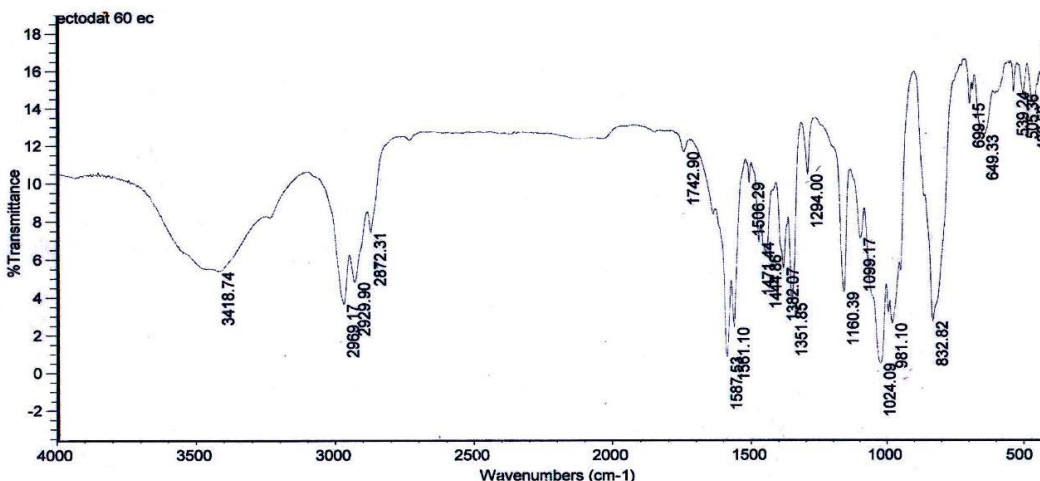


Fig. 5. IR Chromatogram of Octadat 60% after storage

Table 7. Effect of storage temperatures on a fingerprint of different diazinon concentrate by using IR spectrum

Functions group	The position of bands cm^{-1}					
	diazinon 95%		diazinon 15%		diazinox 5%	
	Before storage	After storage	Before storage	After storage	Before storage	After storage
N-Atom	3415.86	3411.35	3412.75	3401.11	3410.69	3398.76
CH- Aromatic	2931.92	2932.05	2927.32	2927.54	2929.50	2929.76
CH- Aliphatic	2872.72	2872.11	2871.43	2871.43	2872.29	2872.31
C=C Aromatic	1561.47	1560.92	1562.16	1561.65	1561.49	1561.07
C-O	1294.46	1294.13	1293.41	1293.22	1294.61	1294.42
C=N	1587.35	1587.44	1587.44	1586.46	1587.55	1587.53
-C-N	1351.84	1351.84	1351.87	1351.52	1351.94	1351.80
Methylene group CH ₃	1443.96	1443.98	1455.55	1455.58	1444.30	1444.33
R-O-R	1023.83	1023.99	1024.76	1024.88	1024.42	1024.71
P-O-C	981.35	981.35	980.84	980.66	981.19	981.12
P=S	641.80	641.80	640.23	640.11	592.92	592.46

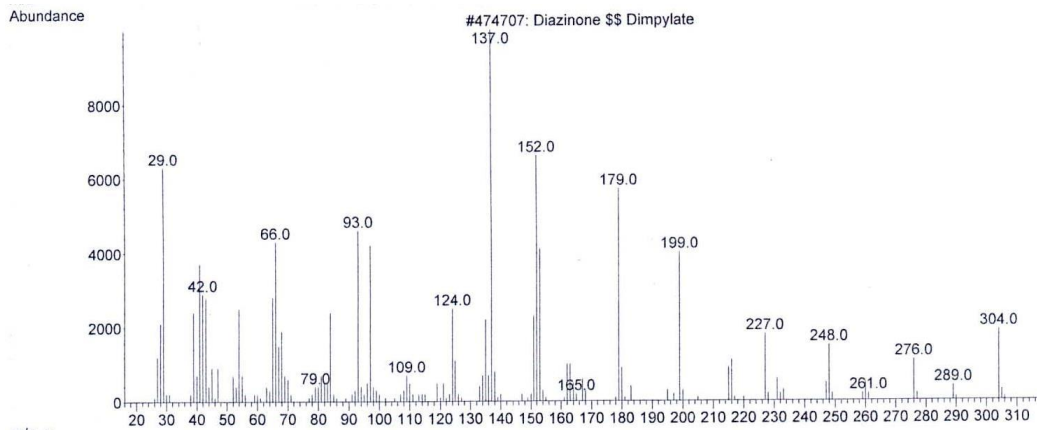


Fig. 6. GC/MS Chromatogram of Diazinon before storage

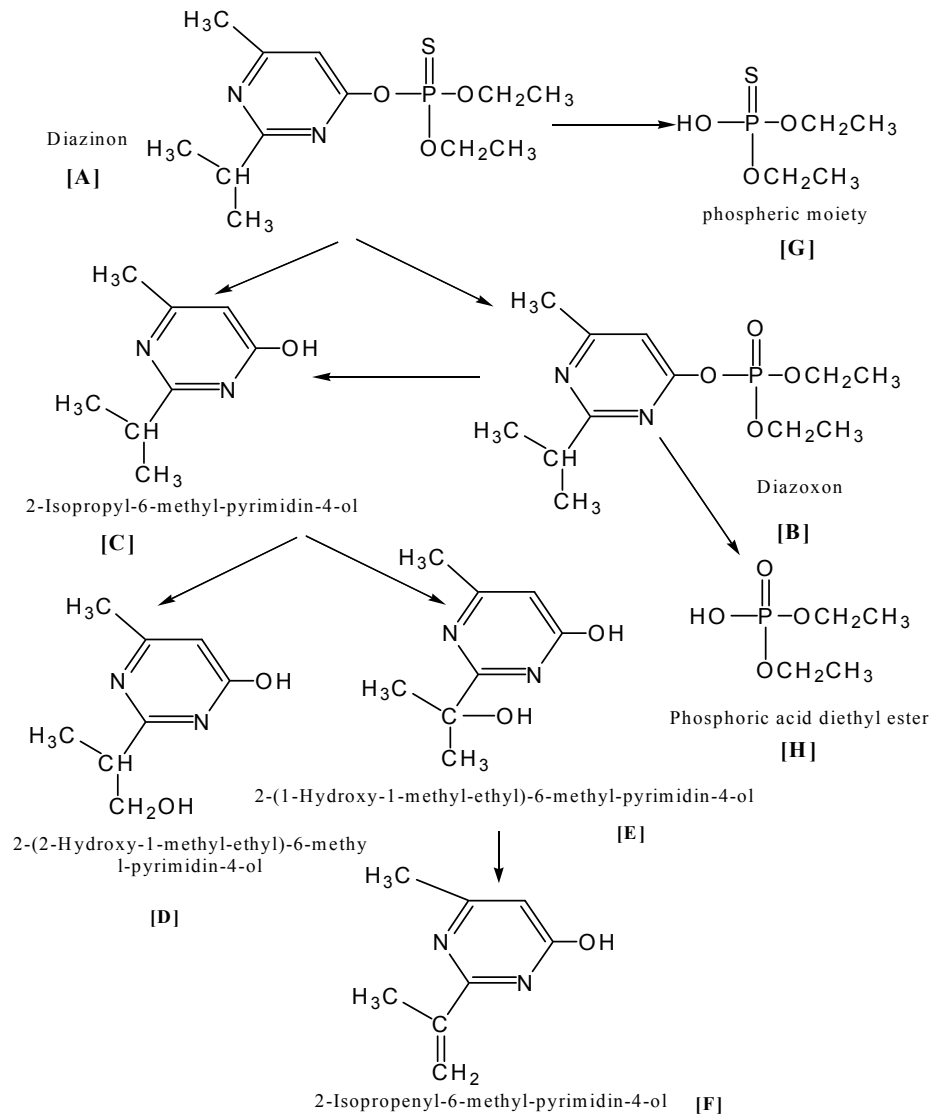


Fig. 7. The degradation pathway for Diazinon and Diazinonox

The main pathways of degradation of diazinon are:

- Transformation of P-S moiety in diazinon [A] to P-O derivative, leading to the active metabolite, diazoxon [B].
- Cleavage of the ester bond of diazinon or diazoxon leading to the hydroxypyrimidine derivatives (2-Isopropyl-6-methyl-pyrimidin-4-ol) [C].
- Oxidation of isopropyl substituent leading to the corresponding tertiary and primary alcohol derivatives [D], [E].
- Oxidation of the methyl substituent leading to the corresponding alcohol [F].

The hydrolytic and oxidative cleavage of the phosphorus ester bond, leading directly or via diazoxon to the pyrimidinyl derivative.

In Table 9 it was shown that retention time (Rt) of diazinon m/z (304.35) was 18.72 min before storage and shift to 18.13 min after 14 days. Also, the data showed that retention time (Rt) of diazoxon m/z (288.2) was 7.32 min before storage and shift to 7.29 min after 14 days at $54 \pm 2^\circ\text{C}$.

The presence of only trace amounts of water leads to the formation of the toxic impurities S, S-TEPP and, O, S-TEPP. The initial reaction diazinon hydrolysis gives diethyl-thiophosphoric acid and 2-isopropyl-4-methyl-6-hydroxypyrimidine Fig. 8. Two molecules of diethyl-thiophosphoric acid can combine in an acid-catalyzed or radical-initiated dimerization to form the S, S-TEPP, the finding is similar to that by Shemer et al. and Rahiminejad et al. [31,32].

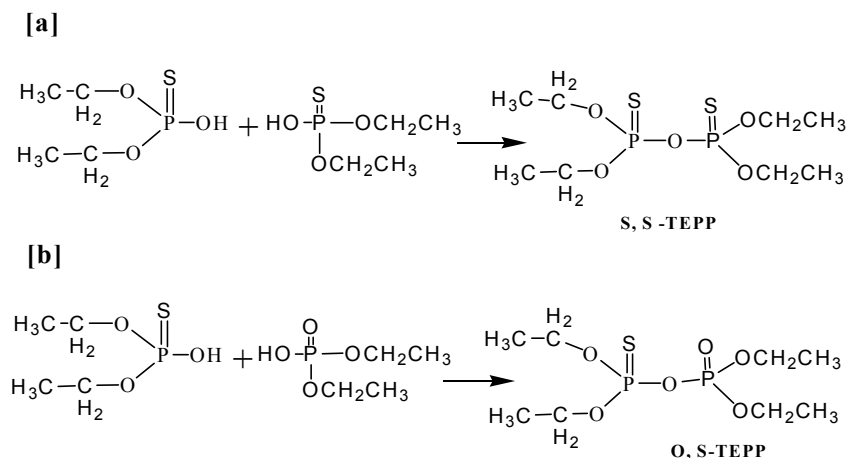


Fig. 8. The degradation products of Diazinon (a) S, S –TEPP and (b) O, S- STEPP

Table 8. Effect of storage temperatures on a fingerprint of different diazinon by using IR spectrum

Functions group	The position of bands cm^{-1}					
	diazole 50%		diazit 60%		octadat 60%	
	Before storage	After storage	Before storage	After storage	Before storage	After storage
N-Atom	3416.30	3408.35	3430.48	3408.57	3436.27	3418.74
CH-Aromatic	2929.09	2929.23	2929.61	2929.82	2931.56	2929.90
CH-Aliphatic	2872.09	2871.15	2975.28	2973.92	2873.39	2872.31
C=C Aromatic	1561.12	1560.32	1560.22	1560.01	1562.39	1561.10
C-O	1294.56	1294.00	1301.82	1301.70	1294.61	1294.00
C=N	1587.35	1587.21	1597.73	1589.46	1587.58	1587.53
-C-N	1351.71	1351.01	1346.73	1345.82	1351.83	1352.20
Methylene group CH3	1444.92	1444.97	1444.74	1445.38	1444.83	1444.86
R-O-R	1023.86	1023.91	1042.32	1042.81	1023.61	1024.09
P-O	981.03	981.03	971.69	971.66	981.85	981.82
P=S	641.15	641.01	582.15	581.85	651.30	649.53

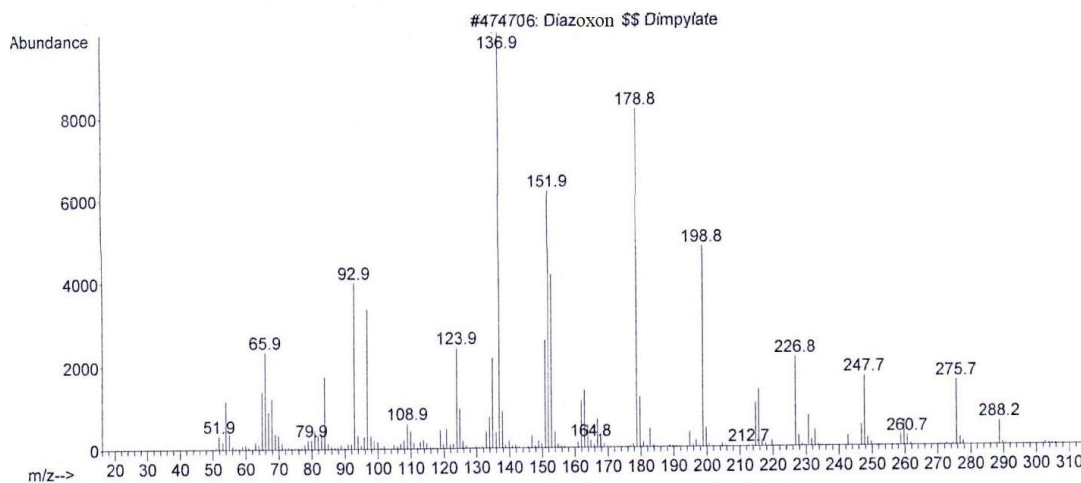


Fig. 9. GC/MS Chromatogram of Diazoxon before storage

Table 9. Separation and degradation of Diazinon by GC/MS:

Storage periods	Diazinon degradation	Retention Time (Min)	Formula	M.Wt
Initial	diazinon	18.72	C ₁₂ H ₂₁ N ₂ O ₃ PS	304.35
	diazoxon	7.32	C ₁₂ H ₂₁ N ₂ O ₄ P	288.2
After 14 days of storage at 54°C	diazinon	18.13	C ₁₂ H ₂₁ N ₂ O ₃ PS	304.35
	diazoxon	7.21	C ₁₂ H ₂₁ N ₂ O ₄ P	288.2
	2-isopropyl-6-methyl-pyrimidin-4-ol (IMP)	7.29	C ₈ H ₁₂ N ₂ O	152.19
	2-(2-hydroxy-1-methyl-ethyl)-6-methyl- pyrimidin-4-ol	11.05	C ₈ H ₁₂ N ₂ O ₂	168.19
	2-(1-hydroxy-1-methyl-ethyl)-6-methyl- pyrimidin-4-ol	11.87	C ₈ H ₁₂ N ₂ O ₂	168.19
	2-isopropenyl-6-methyl-pyrimidin-4-ol	13.96	C ₈ H ₁₀ N ₂ O	150.18
	S, S-TEPP	16.43	C ₈ H ₂₀ O ₅ P ₂ S ₂	322.32
	O, S-TEPP	9.04	C ₈ H ₂₀ O ₆ P ₂ S	306.25

Initial: one hour before storage

4. CONCLUSIONS

The foregoing study revealed that the tested commercial diazinon and diazinon formulations samples had some changes in chemical properties. The obtained results showed that diazinon 5%, was less stable than different concentrations of diazinon while, diazinon 95% was more stable for storage at the same conditions. The present study demonstrated the formation of a less toxic metabolite, 2-Isopropyl-6-methyl-pyrimidin-4-ol (IMP) as a result of the degradation of diazinon and

diazinon. Finally, diazinon and diazinon were used veterinary rather than as an insecticide.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. EPA US. Pesticides Industry Sales and Usage: 2006-2007 Market Estimates. Washington, D.C.;2011.

2. Aggarwal V, Deng X, Tuli A, Goh KS. Diazinon—chemistry and environmental fate: A California perspective. *Rev Environ Contam Toxicol.* 2013;223:40–107.
3. Liu YN, Jin D, Lu XP and Han PF. Study on degradation of dimethoate solution in an ultrasonic airlift loop reactor. *Ultrason. Sonochem.* 2008;15:775–760.
4. Ulansari S. Efi, Anna Roosdiana, Dyah Ayu Oktavianie AP. The toxicity effect of organophosphate (Diazinon) towards duodenum histopathology and the activity of Superoxide Dismutase (SOD) Serum in Rats (*Rattus norvegicus*). *J. Pure App. Chem. Res.* 2018;7(2):109-115.
5. Nima Nezami. Diazinon toxicity, fate and transport and environmental impact. International Conference on Sustainable Development With a focus on Agriculture, Environment and Tourism. Tabriz, Iran; 2015.
6. Emad W, Ghazy¹, AbdAllah A, Mokh¹, Doaa H, Abdelhady¹. The role of thymoquinone in ameliorating the hepatotoxic effect of diazinon in male rats. *Slov Vet Res.* 2019;56(Suppl 22):735–45.
7. Abdollahzadeh Estakhri MR, Shokrzadeh M, Jaafari MR, Karami M, Mohammadi HR. Organ toxicity attenuation by nano micelles containing curcuminoids: Comparing the protective effects on tissues oxidative damage induced by diazinon. *Iran J Basic Med Sci.* 2019; 22:17-24.
8. Fatemeh RA, Mohammad RN, Mehdi J, Hamid RS, Zinat S, Leila M. Single dose effect of diazinon on biochemical parameters in testis tissue of adult rats and the protective effect of vitamin E. *Iran J Reprod Med.* 2014;12:731-736.
9. Li PCH, Swanson EJ, Gobas FAPC. Diazinon and its degradation products in agricultural watercourses in British Columbia, Canada. *Bull. Environ. Contam. Toxicol.* 2002;69:59-65.
10. Meier EP, Dennis WH, Rosencrance AB, Randall WF, Cooper WJ, Warner MC. Sulfotepp, a toxic impurity in formulations of diazinon. *Bull Environ Contam Toxicol.* 1979;23:158–164.
11. Ambrus A, Hamilton DJ, Kuiper HA, Racke KD. Significance of impurities in the safety evaluation of crop protection products. *Pure Appl Chem.* 2003;75:937–973.
12. FAO. Specification and evaluation for diazinon emulsifiable concentrate 15/EC/S; 1988.
13. Soliman SA, Sovocool GW, Curley A, Ahmed NS, El-Fiki S, El-Sebae AK. Two acute human poisoning cases resulting from exposure to diazinon transformation products in Egypt. *Arch. Environ. Health.* 1982;37:207-212.
14. Singmaster JA. Old and nasty pesticides. *Agrichem Age.* 1990;4.
15. Dennis WJ, Rosencrance A, Randall WF, Meier EP. Acid hydrolysis of military standard formulations of diazinon. *J. Environ Sci, Health B.* 1980;15(1):47-60.
16. Tomlin CDS. The pesticide Manual, 13 ed. Edition: British Crop Protection Council, London UK. 2004;262.
17. FAO. Specification and evaluation for dimethoate emulsifiable concentrate. 59/EC; 2005.
18. Allender WJ, Britt AG. Analyses of liquid diazinon formulations and breakdown products: An Australia-wide survey. *Bull. of Envir. Cont. and Toxic.* 1994; 53(6):902-906.
19. CIPAC F. physicochemical methods for technical and formulated pesticides, V. F Collaborative international pesticides analytical Council Limited: MT 15. 1995; 1:45-48.
20. Barbara S. Modern infrared spectroscopy published on behalf of ACOL (University of Greenwich) by John Wiley & Sons Chichester, New York. Brisbane. Toronto. Singapore; 1985.
21. Saad MMI, Ali HM, Habiba RA. GCECD and GC/MS Analysis of profenfos residues and its biochemical effects in Tomatoes and Tomato Products. *J. Agri. Food. Chem.* 1993;41:610-615
22. Olfat RA. Effect of storage condition on the stability of physicochemical properties of diazinon insecticide formulation and its residue in *Oreochromis* notices. *J. of Agric. Res. Center*, in press; 2010.
23. Richard T, Barry L. Sulfotep in diazinon and other organophosphorus pesticides. *Bull. Environ. Contam. Toxicol.* 1987;38: 793-797.
24. Margot A, Gysin H. Diazinon, its decomposition products under its properties. *Helv Chim Acta.* 1957;40:562-573.

25. Sovocol GW, Harless RL, Bradway DE, Wright LH, Lores EM, Feige LE. The recognition of diazinon, an organophosphorus pesticide, when found in samples in the form of decomposition products. *J. Anal Toxicol.* 1981;5(2):73-80.
26. Fishel FM, Ferrell JA. Water pH and the Effectiveness of Pesticides. UF: University of Florida IFAS Extension publication. 2010;P1-156.
27. Glotfelty DE, Majewski MS, Seiber JN. Distribution of several organophosphorus insecticides and their oxygen analogues in a foggy atmosphere. *Environ Sci Technol.* 1990a;24:353-357.
28. Raina R, Hall P, Sun L, Occurrence and relationship of organophosphorus insecticides and their degradation products in the atmosphere in Western Canada Agricultural Regions. *Environ Sci Technol.* 2010;44:8541-8546.
29. US EPA. Environmental risk assessment for diazinon Office of Prevention, Pesticides and Toxic Substances, Washington, DC; 1999.
30. Zhang X, Starner K. Analysis of diazinon agricultural use in regions of frequent surface water detections. California Department of Pesticide Regulation, Sacramento; 2011.
31. Shemer H, Linden KG. *Journal of Hazardous Materials.* 2006;B136:553-559.
32. Rahiminejad S, Shahtaheri J, Ganjali A, MR Rahimi, Forushani F. Golbabaei Iran. *J. Environ. Health. Sci. Eng.* 2009;6(2):97-106.

© 2020 Selim and Ibrahim; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

*The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/54856>*