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DETERMINATION OF CARBOSULFAN COMPOUNDS, ITS IMPURITIES AND BIO EVALUATION ON COTTON LEAF WORM

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ABSTRACT

The aim of this study to investigate the degradation of carbosulfan 25% WP wettable powder (New 2019, old 2017, i.e. validity), carbosulfan 20% EC emulsion concentrate (New 2019, old 2017,i.e validity) and Technical carbosulfan 86.5% (TC)from the same source before and after storage 21 days at 54± 2°C. Beside the effect of storage on the formation of toxic impurity (carbofuran) was studied. The data showed that carbosulfan 20% EC (new) was more stable than carbosulfan 20%EC validity, whereas the percentage loss 4.21% for 20% EC(new) and the percentage loss 9.6% for 20% EC (old) after 21 days of storage at 54± 2°C. While carbosulfan 25%WP (New, old) and technical carbosulfan (86.5%TC) were less stable for storage, which have percentage loss were (29.86, 24.2%) and 26.22%, respectively after 21 days of storage at 54± 2°C. Moreover, the carbofuran impurity in carbosulfan 20%EC, carbosulfan 25%WP and carbosulfan 86.5% were more than the maximum permissible concentrate of impurity recommended by FAO specification before and after storage. On the other hand, GC-Ms was used to compare the fragmentation of the test carbosulfan and result showed that breakdown of carbofuran (main product) in marshal 25% WP (New, Old), marshal 20% EC (New, Old) and carbosulfan Technical 86.5%. Other fragmentation carbofuran phenol and dibutyl amine (minor product) due to hydrolysis and oxidation of carbosulfan, while N-nitrose dibutyl amine was found in marshal 25% (WP) due to nitrosating agents in manufacturing process or in active substance. Toxicity bioassay on *Spodoptera littoralis)* Boisd **(**.fourth instar larvae revealed noticeable differences in LC_{50} of 2019 than 2017 of carbosulfan WP or EC formulated product and between unstored and the stored formulations under storage conditions. Where toxicity values was 158, 684, 120, 413 and 506 ppm for Technical grade (86.5% TC 25, 2019) WP 2019, EC 25%, 2019 20% WP 2017, and EC 2017 20% products unstored **.**In addition, the formation of carbofuran impurities during storages intervals were increased toxicity at all formulation variety. The technical material was the most toxic because of higher concentration.

***Keywords* :** Carbosulfan–carbofuran–impurities- GC/MS and Fragmentation.

Introduction

Carbosulfan (2,3-dihydro-2,2 dimethyl-7- benzofuranyl (di-n-butyle amino sulfenyl) methyl one of the carbamate systemic insecticide, nematicide and miticide that are used to absorb insects in some countries in a wide range of crops Sucheta and Khokar, 1996.

Carbofuran (2,3- dihydro-2,2 dimethyl (-benzofuranyl-7yl- methyl carbamate) is main degradation of carbosulfan in plant and itself pesticide Guillet *et al.* (2001) when, it reach water supplies and becomes a problem in the environment Tariq *et al.* (2010). It is considered a neurotoxic pesticide acting as cholinesterase inhibitor in nervous system Giri *et al.* (2002) and Dobsikova R. (2003). Carbofuran is also power full endocrine disruptors that can cause transient alterations in the concentration of many hormones in animals and humans even at extremely low dose. The variation may consequently lead to serious reproductive toxicities following repeated exposure Lau *et al.* (2007).

Carbofuran is found to be more persistent and toxic than carbosulfan itself. Trevisan *et al.* (2004). In addition Carbofuran is highly toxic if inhaled or ingested and moderately through dermal absorption Lamb Thomas *et al.*, 2016, Rauzi and Krieger, 1986.

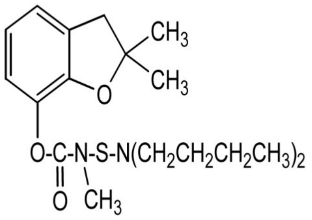
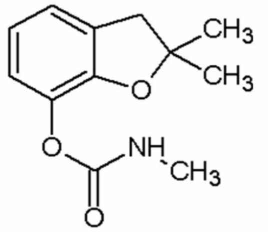
Carbofuran and Carbosulfan could contaminate the ground water with their residue in water and crops, there is probable chance of the allowable, this pose an acute risk to human from consumption of crops. For then Carbosulfan has hazardous potentially genotoxic metabolites. One of its carcinogenic impurities (e.g. N. nitrose dibutyl amines) was found in the active substance sold in market PANAP, 2017. In pesticide, nitroseamine are formed in several ways by nitrosating agents on secondary amine in manufacturing process example nitrogen oxide or their precursors nitrite or as nitrate which use as container corrosion inhibition or as impurities in amine reagents used in synthesis of pesticide. Although the nitrose amine occur at low levels (ppm) many have been found to cause cancer in laboratory animals and may present a hazard to pesticide uses probst G.W. (1981).

The common cutterpillar crop pest *S. littoralis* (Boisduval) (Lepidoptera: Noctuidae), represent the most pest infestation assortment especially in cotton plant in our country Egypt Abo Elghar *et al.* (2005). Information about high damages to plant foliage required a great variation in insecticides spray of the organophosphate, pyrethroid and carbamate insecticides Abul-Nasr and Naguib (1968). Although the common existence of resistance to insecticides is danger to corporate insecticide with other control and management tools of *S. littoralis.* The unfaithful Pyrethrins uses and search about replacement with other insecticide itinerary, push idea that survey about ignored and stored insecticide packages to reuse that call Expired insecticides. Moreover, the toxicological evaluation of the new, expired and the technical grade of carbosulfan insecticide wettable powder and emulsifiable concentrate at different duration of storage under 54±2 °C heat oven temperature; against cotton leafworm larvae were completed.

Material and methods

1. PESTICIDE USED:

Table 1 : Structure of carbosulfan and its impurity carbofuran are tested in trade name:

Trade name	Structure of carbosulfan	Structure of carbofuran
1. Marshal 20% EC 2. Marshal 25% WP 3. Carbosulfan 86.5% TC		

EC: Emulsion Concentrate. **WP: wettable powder.** **TC : Technical.**

2. Storage stability test

The samples of Marshal 25% wp (old 2017 and new 2019), Marshal 20% EC (old 2017 and new 2019) and technical carbosulfan 86.5% (TC) are the same source were stored at 54 ± 2 °C for 21 days according to FAO (1995). The active ingredient (a.i) and toxic impurity of the samples were determined at 0, 3, 7, 14 and 21 days of storage.

3. Preparation of sample:-

3.1. Standard preparation:

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

3.2. Sample preparation for tested pesticides

Accurately weighed sufficient sample formulation equivalent to 10 mg of carbosulfan standard in a different 25 ml of volumetric flask for each sample, and slowly mixed with methanol and the volume was completed with methanol.

1.3. Sample preparation for impurities:-

1 g of tested formulation samples (carbosulfan 25% wp, carbosulfan 20% EC and technical carbosulfan 86.5% TC) were weighed which contain 0.25 gm, 0.2 gm and 0.865 gm, respectively in different 25 ml volumetric flask dissolved and completed to the final volume with methanol.

2. Determination of carbosulfan and its impurity by HPLC instrument.

Equipment HPLC (Agilent technologies 1260 Infinity II) was used UV-detector. The wave length detector at 210 nm, respectively. A C18 column was used and the flow rate was 1 ml/min. The mobile phase for carbosulfan and carbofuran was acetonitril: methanol (70: 30). At this condition the retention time (RT) of carbosulfan and carbofuran were 3.265 and 1.735 min. This method was carried out according to a modified method of CIPAC (1991).

3. Gas-chromatography-Mass spectrometry analysis of carbosulfan TC, Marshal 25% WP (old and new) and Marshal 20% EC (old and new).

Apparatus Agilent 7890 B, 5977 A MSD gas chromatography equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column (30 m ×0.025 mm HP-5-0.25 microm -60 to 325/325 ºC) was used. Samples were injected under the following conditions: Helium was used as carrier gas at approximately 1 ml/min, pulsed splitl mode, splitl ratio (10:1), split flow 10 mL/min. The solvent delay was 4 min and the injection size was 1 µL. oven temperature program, 50°C for 0.5 min, then 10°C/min ramp to 190°C followed by a 10°C/min ramp to 210°C for 1 min followed by a 10°C/min ramp to 300°C and held for 2 min (total run time: 29.5 min) the injector temperature was set at 280°C. Wiley mass spectral data base was used in the identification of the separated peaks.

4. Kinetic study:

The rate of degradation of the tested active ingredient and half lives periods ($T_{0.5}$) for the tested pesticides were calculated according to equation (Moye *et al.,*1987).

$$T_{0.5} = \ln 2/ K= 0.6932K \text{ and } K = \frac{1}{tx}\ln\frac{a}{(bx)}$$

Were K = rate of decomposition
a = initial residue
tx = Time in days of hours
bx = residue at x time

5. Biological assessments

7.1. Insects and bioassay:

A susceptible strain of cotton leafworm *S. littoralis* (Boisd.) that used on this study were obtained from insect mass reared from egg to moth emergence about many years in rearing lab chambers under lab conditions without insecticide exposure according to (Shaaban *et al.*, 1985). Larvae feeding on leaves of castor bean *Ricinus communis* and moths were fed on a solution containing sucrose. This patch directly subjected for concentration-response bioassay.

7.2. Toxicity Bioassay:

Bioassay for toxicity tests were accomplished by a cotton plant leaf dipping method according to Paramasivam and Selvi (2017). Leave pieces individually and efficiently dipped in subsequently diluted solutions of the insecticide concentrations, 5 to 6 dilution were used, and leaves left to dry, alongside controls were dipped in water only. Afterward, treated leaves were placed in individually in 9 diameter petri dish filled with the newly molted 10 4th instars larvae and three replicate were done for each concentration. The dishes preserved in laboratory chamber controlled conditions at 25 ± 2ºC and 16:8 h light: dark. Mortality was recorded 24 h after insecticides treatment. Mortality were corrected using Abbott's (1925) formula and data were analyzed Polo Pc Program analysis (Rusell *et al.*, 1977) that statistically analyzed data using Finney (1971). The slope and LC_{50} values in ppm and their 95% confidence limits were estimated.

Results and discussion

Influence of storage at 54 ±2°C on stability of wettable powder carbosulfan (New 25% WP), validity (old) and its content of carbofuran.

The data presented in Table (2) showed that carbosulfan 25%WP (New formulation and old validity formulation) in this experiment was determinate the (a.i) for carbosulfan, its content of impurities (carbofuran), calculated percentage of FAO max. for impurities and calculated $T_{0.5}$ for carbosulfan formulation. The active ingredient of carbosulfan 25% WP (new and old) were 23.68% and 16.96% at the beginning of experimental and were degrade to 16.61% and 12.86% after 21 days of storage, respectively. Data also in Table (2) showed that the levels of carbofuran as % of carbosulfan content before storage were 1.204%, 21.4% and increased to 10.69, 57.9% during storage periods for carbosulfan 25% WP (new), carbosulfan 25% WP (validity), respectively . These levels of carbofuran were not defined by FAO (1995) for carbosulfan 25% WP.

Influence of storage at 54 ±2°C on stability of Emulsion Concentrate carbosulfan (New 20% EC), validity (20% EC old) and its content of carbofuran.

The data presented in Table (3) revealed that carbosulfan 20% EC (New formulation and old validity formulation) in this experiment was determinate the (a.i) for carbosulfan, its content of impurities (carbofuran), calculated percentage of FAO max. for impurities and calculated $T_{0.5}$ for carbosulfan formulation. The percentage of active ingredient loss of the carbosulfan 20% EC (New formulation) and 20% EC old validity were 4.21% and 9.6% after 21 days of storage at 54°C ± 2.

Also, it is found that in Table (3), the levels of carbofuran as % of carbosulfan content before storage were 8.12,15.6 % and increased to 12.7,19.32% after 21 days of storage for carbosulfan 20% EC(new), carbosulfan 20% EC(old), respectively. Nevertheless, these levels are still higher the matching the FAO (1991) maximum level, (2%).

It is founded that carbosulfan show higher degradation due to transformation to carbofuran, when exposed to 54°C with in the period of the experiment the former one is more toxic than carbosulfan, so it is represents a great risk to the human Soler *et al.* (2006).

Influence of storage at 54 ±2°C on stability of Technical carbosulfan 86.5% TC and its content of impurity carbofuran.

The data presented in Table (4) revealed that carbosulfan 86.5% TC. The percentage of active ingredient loss of the carbosulfan 86.5% TC was degraded to 26.22% after 21 days of storage at 54 ±2°C.

Also, the results in Table (4) showed that the amount of carbofuran before storage 19.96 g/kg and increased to 23.7 g/kg of carbosulfan Technical 86.5%. This level is higher matching the maximum levels (20g/kg) which define by FAO (1991). Decomposition of these materials can be calculating follows first order reaction. However, the half lives $T_{0.5}$ of these materials of carbosulfan 25%WP (new, old), of carbosulfan 20% EC (new, old) and carbosulfan (TC) were (164.08, 108.52), (444.6, 72.53) and 41.853 days. Respectively as shown in Tables (2,3 and 4).

According to Chang *et al.* (2016). It is found carbosulfan are hard to detect in environmental and hydrolysis with time to carbofuran during the analytical process due to thermal dacompostion.

Identification of carbosulfan by chemical ionization GC/MS.

Figure (1) and Figure (2) described two possible reaction pathways leading to degradation of carbosulfan M.F $C_2OH_{32}N_2O_3S$ m/z 380.6 g/mol. Firstly cleavage N-S bond by oxidation. Results in the formation C_{16} H_{23} N_2O_3S m/z 323 g/mol by loss butyl group C_4H_9 followed by C_4H_9 N to give peaks at m/z 252 g/mol. The protonated molecule ion of m/z 252 g/mol loss atom (S) to give peaks at m/z 221 g/mol M.F C_{12} H_{15} NO_3 (carbofuran) and dibutyl amine thio $[S N (CH_2)_3 (CH_3)_2]^+$ at m/z 160 g/mol as main fragmentation of carbosulfan. Also, at m/z 118 g/mol corresponds to $S N CH_3 (CH_2)_3 CH_3^+$ by loss propen C_3H_6 as showen in fig (1) and (2). Other pathway, cleavage of the C-O bond of carbosulfan by hydrolysis to form intermidate 2,2- dihydro 2,2- dimethyl benzofuran -7-ol (carbosulfan phenol) at m/z 164 g/mol by loss CH_3 CON. The major ions is mass spectra are as follows m/z 149 by loss CH_3N followed m/z 132 by loss 2 (CH_3). Other signification ions are m/z 91 g/mol corresponding to the benzyl ion C_7 H_7^+ and m/z 77gm/mol corresponding to C_6 H_5^+ as shown in figure (1) and figure (2). According to Abass *et al.* 2010. The primary metabolic pathway were the initial oxidation of sulfur to carbosulfan sulfanamide and the cleavage the nitrogen sulfur bond (N-S) to give carbofuran and dibutyl amine as shown in fig (3).

It is similar that carbamate group CH_3CONH is cleaved from carbofuran molecule to form intermidate 2,3- dihydro-2,2-dimethyl benzofuran-7-ol and carbamic acid by hydrolysis. Unstable carbamic acid rapidly degradation to methyl amino and carbon dioxide gases at room temperature. The formation of the gaseous product is quickly for this degradation according to John and Howard 1999. In The Table (5) it is found that retention time (Rt) of carbofuran were ranged from 19.304 to 19.341 minutes and dibutylamin were ranged from 6.729 to 6.779 for carbosulfan 25% WP (New and old), carbosulfan 20% EC (New and old) and carbosulfan Technical (86.5% TC) before and after 21 days of storage at 54 ±2°C due to oxidation and cleavage of N-S bond carbosulfan as shown in figure (3).

Also in Table (5) it is noticed that (Rt) of carbofuran phenol were ranged from 13.365 to 13.483 minutes for the above sample by hydrolysis of carbofuran according to Abass *et al.* (2010).

Retantion time (Rt) of carbosulfan in the above samples were ranged from 26.045 to 26.061 minutes before and after 21days of storage at 54 ±2°C as shown in Table (5).

Table 2 : Influence of storage at 54 ±2°C on stability of wettable powder carbosulfan (New 25% WP), validity (old) and its content of carbofuran.

	Carbosulfan 25% WP (New)					**Carbosulfan 25% WP (Old validity)**				
Storage period (days)	**(a.i) Carbosulfan 25% WP**	**% loss**	**% Carbofuran**	**% increase**	**% FAO MAX**	**(a.i) Carbosulfan 25% WP**	**% Loss**	**% Carbofuran**	**% increase**	**% FAO MAX**
0	23.68	0.00	0.285	0.00	1.204	16.96	0.00	3.63	0.00	21.4
3	23.56	0.51	0.953	70.09	4.04	16.84	0.71	4.023	9.77	23.9
7	22.9	3.29	1.066	73.3	4.65	15.23	10.2	5.036	27.92	33.07
14	20.2	14.69	1.426	80.01	7.06	13.57	19.99	7.013	48.24	51.68
21	16.61	29.86	1.78	83.99	10.69	12.86	24.2	7.45	51.3	57.9
$T_{0.5}$	164.08					108.52				

0 = initial concentration before storage. (a.i) = active ingredient. $T_{0.5}$ = half life.

Table 3 : Influence of storage at 54 ±2°C on stability of Emulsion Concentrate carbosulfan (New 20% EC), validity (old 20% EC) and its content of carbofuran.

	Carbosulfan 20% EC (New)					**Carbosulfan 20% EC (Old validity)**				
Storage period (days)	**(a.i) Carbosulfan 20% EC**	**% loss**	**% Carbofuran**	**% increase**	**% FAO MAX**	**(a.i) Carbosulfan 20% EC**	**% loss**	**% Carbofuran**	**% increase**	**% FAO MAX**
0	19.95	0.00	1.62	0.00	8.12	19.81	0.00	3.09	0.00	15.6
3	19.89	0.30	1.65	1.82	8.3	19.20	3.07	3.19	3.13	16.61
7	19.75	1.00	1.093	16.06	9.8	18.78	5.2	3.33	7.21	17.73
14	19.24	3.56	2.21	26.7	11.5	18.34	7.42	3.43	9.91	18.7
21	19.11	4.21	2.43	33.3	12.7	17.91	9.6	3.46	16.7	19.32
$T_{0.5}$	444.6					72.53				

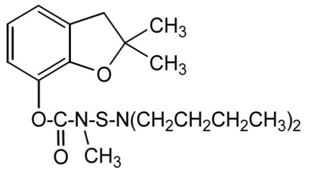
0 = initial concentration before storage. (a.i) = active ingredient. $T_{0.5}$ = half life.

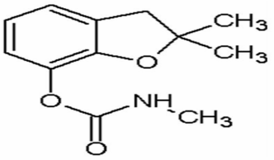
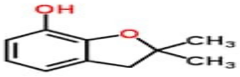
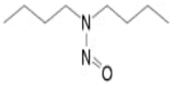
Table 4 : Influence of storage at 54 ±2°C on stability of Technical carbosulfan 86.5% TC and its content of impurity carbofuran.

Storage period (days)	(a.i) Carbosulfan 86.5 % TC	% Loss	Carbofuran		% increase
			%	FAO MAX g/kg	
0	85.05	0.00	1.996	19.96	0.00
3	78.03	8.25	2.009	20.09	0.65
7	74.77	12.09.	2.174	21.74	8.19
14	71.89	15.47	2.324	23.24	14.1
21	62.75	26.22	2.37	23.7	15.78
$T_{0.5}$	41.853				

0 = initial concentration before storage. (a.i) = active ingredient. $T_{0.5}$ = half life.

Table 5 : Identification of carbosulfan by chemical ionization GC/MS spectroscopy.

Pesticides used **Degradation compounds**	**Wettable powder carbosulfan**				**Emulsion concentrate carbosulfan**				**Technical carbosulfan**	
	New		**OLD**		**New**		**OLD**		**Rt (minutes)**	
	Rt (minutes)		**Rt (minutes)**		**Rt (minutes)**		**Rt (minutes)**			
	Before storage	**After storage**	**Before storage**	**After storage**	**Before storage**	**After storage**	**Before storage**	**After storage**	**Before storage**	**After storage**
Carbosulfan M.F $C_2H_{32}N_2O_3S$ M/Z 380.6 g/MOL 	**26.056**	**26.059**	**26.045**	**26.053**	**26.058**	**26.060**	**26.050**	**26.059**	**26.061**	**26.059**

Carbofuran M.F $C_{12}H_{15}NO_2$ M/Z 221g/MOL 	**19.309**	**19.318**	**19.308**	**19.315**	**19.305**	**19.306**	**19.304**	**19.305**	**19.337**	**19.341**
Dibutyl amine M.F $C_8H_{19}N$ M/Z 129.24 g/M 	**6.739**	**6.729**	**6.779**	**6.743**	**6.751**	**6.737**	**6.779**	**6.751**	**6.740**	**6.747**
Carbofuran phenol M.F $C_{10}H_{12}O_2$ M/Z 164.2 g/MOL 	**13.390**	**13.436**	**13.389**	**13.437**	**13.365**	**13.392**	**13.36**	**13.384**	**13.376**	**13.485**
N.nitrose dibutyl amine M.F $C_8H_{18}N_2O$ M/Z 150.24 g/MOL 	**12.557**	**12.552**	**12.552**	**12.557**	**UND**	**UND**	**UND**	**UND**	**UND**	**UND**

UND- non detectable

As shown in Table (5) Rt of N-nitrose dibutyl amine was 12.55 minut before and after 21 days in the wettable powder carbosulfan, while Emulsion concentrate, Technical carbosulfan were undetectable.

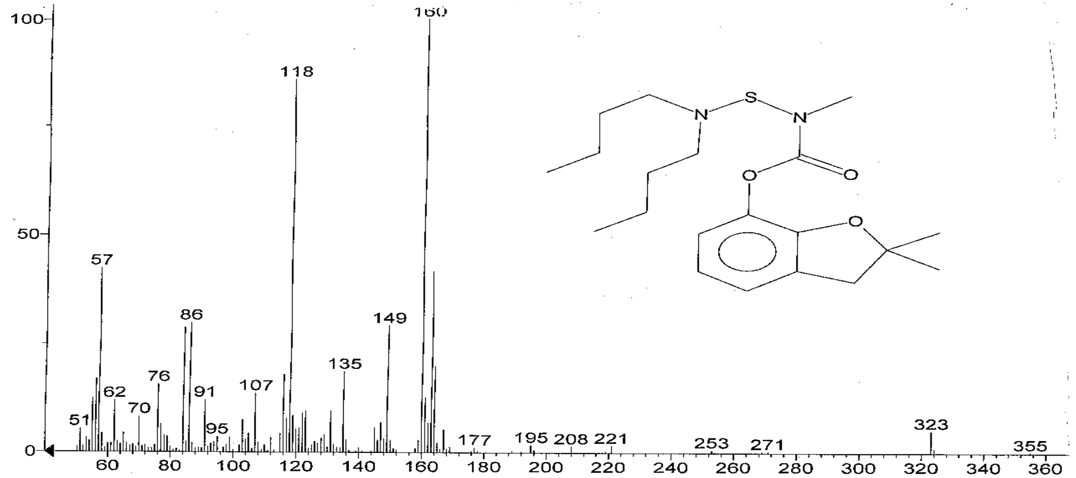


Fig. 1 : Mass spectrometry (MS) of carbosulfan.

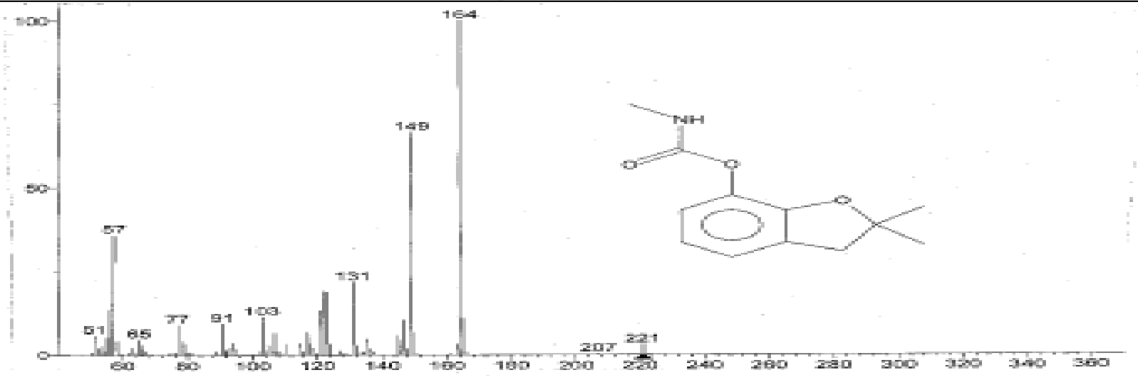


Fig. 2 : Mass spectrometry (MS) of carbofuran.

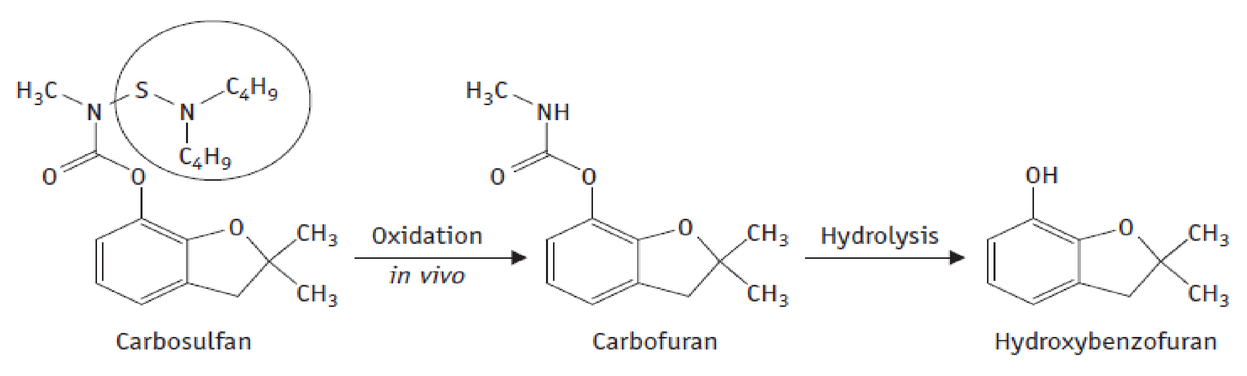


Fig. 3 : Degradation of carbosulfan to carbofuran to carbofuran phenol.

Concentration mortality bioassay

Around the insecticide formulation of the expired and unexpired insecticides, WP or EC at two manufactured dates 2019 and 2017 and the technical grades, results of toxicity evaluation of the biological survey against the fourth instar larvae of the cotton leafworm *S. littoralis* larvae data was found in table (6).

Each category of the tested formulation were exposed to heat 54 ±2°C at different intervals, 0,3,7,14 and 21 days. results showed visible shift in LC_{50} values before exposure at 0 time and after exposure at 3,7,14 and 21 gradually, because of heat changes the molecule properties that affect toxicity, and showed biological differences in toxicity toward the *S. littoralis* larvae, was clearly detected whereas toxicity were declined through the higher exposure duration at 21 days.

Table 6: Toxicity data of the different carbosulfan formulations stored under heat conditions 54 ±2°C at time intervals against fourth instar larvae of *S. littoralis.*

Duration of heat exposure effect (Slope, LC_{50}(FL))					**Row**	**Expire Date**
21 day	**14 day**	**7day**	**3 day**	**0 day**		
0.882±0.229	0.86±0.269	1.38±0.193	1.34±0.165	1.07±0.20*	Slope	13-2-2019 86.5% TC
2945(1046-8291)	3249(965-3290)	1437(602-3432)	362.6(180-796)	158(64-392)	LC_{50}	
2.8±0.087	1.99±0.105	1.65±0.117	1.6±0.12	1.66±0.12	Slope	15-5-2019 20% EC
2234(1507-3312)	1680(1046-2699)	530(312-901)	686(396-1186)	684(395-1182)	LC_{50}	
1.25±0.17	0.93±0.22	1.9±0.11	1.7±0.126	0.199±0.11	Slope	13-6-2019 25% WP
1458(670-3172)	1174(433-3179)	671(397-1134)	597(338-1055)	120(73-198)	LC_{50}	
1.6±0.12	2.2±0.1	2.28±0.1	2.5±0.09	2.65±0.087	Slope	24-5-2017 25% WP
658(370-1170)	555(350-881)	542(345-851)	457(303-690)	413(278-612)	LC_{50}	
0.88±0.29	1.2±0.19	1.1±0.19	1.5±0.13	1.6±0.13	Slope	02-7-2017 20% EC
13045 (3448-49349)	3999(1650-9691)	1363(574-3234)	1075(579-1995)	506(277-924)	LC_{50}	

FL= Fiducial limits of the lethal values from the statistical analysis
*=Standard error of the slope values

The results of LC_{50}'s of the technical grade was the most powerful formulation due to the carbosulfan active ingredient concentration in the formulation. Similarly, the WP agent were more effective than EC formulated insecticide and clearly at 2019 than 2017. However, data of the expired formulation at different dates exhibit more toxic to the larvae compared to the newly manufactured, although after expiry date, this because the impurities in the formulation content.

The toxicity values resulted from biological assay of the technical material of carbosulfan (86.5% TC) and 25% WP (New and old), carbosulfan 20% EC (New and old) at 0 time of storages and after 21 days of storage at 54 ±2°C were also illustrated in Figure 4 and showed noticeable decreases in EC than WP type. Such as carbosulfan 25%WP 2017 was 413, 457, 542, 555 and 658 at 0,3,7,14 and 21 day of storage respectively. While the LC_{50} of toxicity values differences between carbosulfan produced in 2017 and 2019 were found in Figure 5. where the new product were more toxic than old except some dates of storages that exhibit the beginning of the metabolite formation in the formulation and after that the compound return to stability e.g. for technical grade 86.5% TC the LC_{50} were declined from 3249 to 2945 ppm . This mean that the carbosulfan insecticide product remain toxic after time but also the new product was the most effective formulation than old production date.

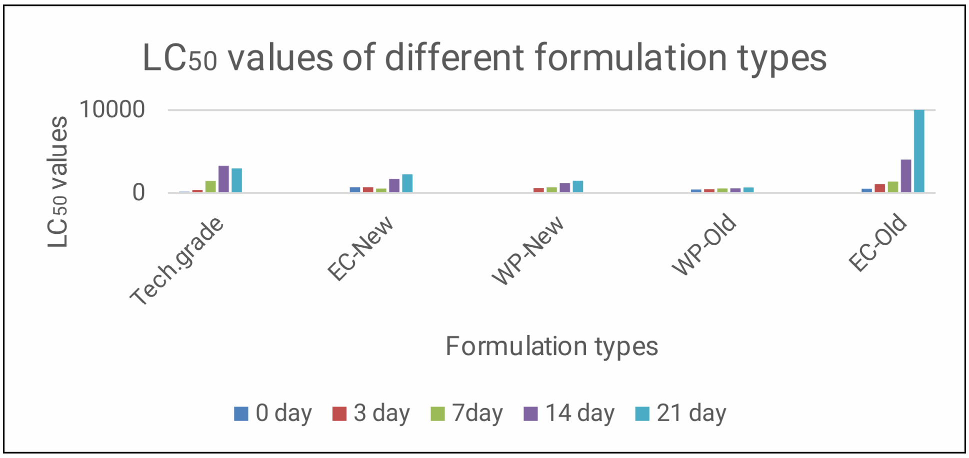


Fig. 4: LC_{50} toxicity values in ppm of the different carbosulfan EC and WP formulation type unstored and stored at 54 ±2°C in several days intervals.

Our biological toxicity data represented as LC_{50} resulted from statistical analysis of the biological assay, were agree with some authors like; Satyavani *et al.*, 2011, that said some pesticide formulations as carbosulfan were more toxic after shelf life expiry and remained toxic but variation exists when compared with fresh respective formulation.

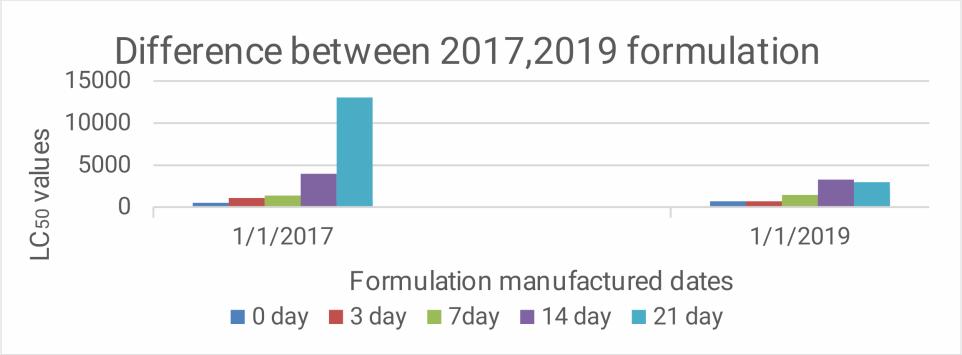


Fig. 5: LC_{50} values of carbosulfan 2017 and 2019 product stored under heat conditioned 54 ±2°C at several intervals in days.

Based on the observed LC_{50} values in the study, EC formulation may cause more toxicity in expired condition rather than other type of formulation like WP, SC because of impurities. The same author in 2012, investigate expired pesticide formulations belonged to various class and functional groups, results exhibited higher toxicity to tested algal species compared to unexpired pesticides. These data push pesticide product commercials to dispose expired pesticides. This auther explain the rise in toxicity grade story that the toxicity depends on the pesticides nature and their environmental affects as temperature, humidity, and pH and oxygen concentration. The unsuitable storage conditions may lead to the pesticides degradation to product much more toxic than the original active ingredient named insecticide metabolite.

The technical material Carbosulfan is the ISO common name for 2,3-dihydro-2,2-dimethy7-lbenzofuranyl (dibutylaminothio) methyl carbamate (IUPAC), contains three impurities is carbofuran, 5- chlorocarbofuran and N-nitrosodibutylamine that was described in many searches as very toxic.

Metabolism of carbosulfan studied in sugar beet, soybean, maize was initiated by the cleavage of the S-N bond into carbofuran its first metabolite, and dibutylamine. Carbofuran was further metabolised by subsequent hydroxylation on the furane ring to 3-OH-carbofuran were found in foood. Other metabolites were generated from carbofuran by successive hydroxylation or hydrolysis and oxidation steps, amongst them 3-keto-carbofuran and phenol derivates of carbofuran such as carbofuran-7-phenol6, 3-hydroxy-7-phenol7 and 3-keto-7-phenol8, which were further conjugated. Dibutylamine was slowly degraded to minor levels however; its derivates N-formyldibutyl amine and acetyl-dibutylamine were identified in plant material. dibutylamine was found as a major aerobic soil metabolite Dharmarathne *et al*., 2015. Hydrolysis enzymes is the main path of carbosulfan transformation to carbofuran degradation in vivo, that no longer persistence in a low pH environment according to EFSA Scientific Report (2006).

Two important information about expired pesticide and biological toxicity were obtained from (Rajput 2012). The impurities may contribute to the toxicity of the pesticide or may alter the physical properties of the product. The common pesticide properties well known as bioaccumulation and biomagnification that lead to long lasting on the environment and the potential risk of pesticides prediction qualification.

After displaying results we can concluded that, the recent insecticide product can eradicate pest population foraging more than the longstanding one but some insecticide remains toxic after passing the half-life because the metabolite formation that more toxic than the original. Detection of metabolites in insecticide containers can predict harmfull product that hurt human and shelf life. Regularly when pests exposed to pesticide that can affect reproductive ability, then expired pesticide lead to insect pest biological changes such as fecundity, fertility or formation deformities lead to big decrease in pest population and abundance. Aldridge 1979, found isomalathion was produced during storage of the formulated malathion as impurities have potentiating the toxicity of malathion. Also O, O, S-trimethyl phosphorodithioate, tetramethyl thiodiphosphate, mixed ester, malaoxon. In addition, endosulfan sulfate, a metabolite product of endosulfan and aldicarb sulfone and aldicarb sulfoxide. All are metabolite produced from other pesticide by GLC method.

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الملخص العربي

تقدير مركبات الكاربوسلفان و شوائبها و تقيمها الحيوى على دودة ورق القطن أثناء التخزين

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الهدف من الدراسة التعرف علي تحطيم كاربوسلفان 25% القابلة للبلل (جديدة 2019 ،قديم 2017صلاحية)، كاربوسلفان 20% المستحلب (جديدة 2019 ،قديمة 2017 صلاحية)، كاربوسلفان الخام 86.5% من نفس المصدر قبل وبعد التخزين لمدة 21 يوم عند درجة حرارة 54م°. بالجانب دراسة تأثير التخزين علي تكوين الشائبة كاربوفيوران اثناء فترة التخزين.

أظهرت النتايج ذلك ان كاربوسلفان 20% مستحلب (الجديدة 2019) أكثر ثبات من كاربوسلفان 20% المستحلب (صلاحية 2017) ونسبة الفقد.29 4 % للكاربوسلفان 20% المستحلب (الجديدة 2019) أكثر ثبات من الكاربوسلفان 20% المستحلب (صلاحية2017) ونسبة الفقد 9.6% للكاربوسلفان 20% مستحلب (صلاحية) بعد 21 يوم م التخزين عند 54م°. بينما كاربوسلفان 25% القابل للبلل(الجديد 2019 و القديمة 2017) و كاربوسلفان الخام 86.5% أقل ثبات للتخزين التي لديها نسبة فقد (29.8و 24.2) و 26.22 %على التوالى بعد 21 يوم من التخزين عند 54م°. علاوة على ذلك ان شائبة كاربوفيوران فى كاربوسلفان 20% المستحلب و 25% القابلية للبلل كاربوسلفان الخام 86.5 % كانت اكبر من المسموح به طبقا لمواصفات منظمة الاغذية و الزراعة. استخدام التحليل الغازى الكروماتوجرافى و مطياف الكتلة لمقارنة تكسيرات الكاربوسلفان المختبرة .

أظهرت النتائج أن الكاربوفيوران هو ناتج التكسير الأساسى فى مارشال 25% القابلة للبلل(جديدة و صلاحية) مارشال 20% المستحلب (جديدة وصلاحية) والكاربوسلفان الخام 86.5% و نميزتكسيرات أخرى كاربوفيوران فينول و داى بيوتيل امين (نواتج قليلة) نتيجة الاكسدة و التحلل المائى للكاربوسلفان. بينما وجد أن نيتروز داى بيوتيل أمين فى مارشال 25 % القابلية للبلل نتيجة عوامل النيتروز فى عملية التصنيع أو وجودها فى المادة الفعالة .

اظهرت نتائج التقييم الحيوي الذي اسفر عن الجرعات النصفيه لمبيد الكاربوسلفان بمستحضريه البودر القابل للبلل والمستحلب الزيتي وايضا المستحضر الخام منه حيث هذه العبوات من انتاج شركه واحده بتاريخ 2017 و2019 بعد 21 يوم من التخزين عند 54م°. واجراء تقصي لفاعليه هذه المستحضرات علي يرقات دوده ورق القطن وجد ان المستحضر الجديد انتاج 2019 عالي السميه بينما القديم انتاج 2017 اقل منها فيما عدا بعض الايام من التخزين التي ارتفع فيها تكون الميتابوليت المسمي كاربوفوران الاشد سميه من الكاربوسلفان وسبب في ارتفاع السميه لليرقات المعامله بعدها عاد الي الاستقرار وقلت السميه وكانت قيم الجرعات النصفيه المميته كالتالي 158 ,684 ,120 ,413 ,506لباقي المستحضرات علي التوالي ppmمره اخري للمستحضر الخام و . بالاضافه الي ان المستحضر القابل للبلل كان اعلي في السميه عن المستحضر المستحلب وذلك لارتفاع نسبه الماده الفعاله في تلك عن ذلك بمقدار 5% بالاضافه الي ان المستحضر الخام يمثل الاكثر سميه لزياده تركيز الماده الفعاله فيه.