Effect of Storage Temperature on Degradation of Two Herbicides and Their Sub-Acute Toxicity on Albino Rats

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Abstract: Degradation of active ingredient for herbicides sencor 60% SC (metribuzin) and turonex 50% SC (isoproturon) was determined by HPLC-DAD to study their resistance against decomposition when stored at zero temperature and under accelerated storage temperature when stored for 14 days at 54±2°C. According to FAO specification the physical properties for sencor and turonex herbicides such as suspensibility, pH values, pourability, persistent foam spontaneity of dispersion and wet sieve test were studied before and after the storage. Results illustrated that thermal storage at 54±2°C affected on metribuzin active ingredient more than isoproturon active ingredient which become conformity with FAO specification, but metribuzin become nonconformity with FAO specification after 10 days of storage. All physical properties for metribuzin and isoproturon formulations under thermal storage passed successfully except wet sieve test of metribuzin. On the other hand, some components of biochemical which carried out on albino rats were evaluated on the initial time at 23±2°C and at 54±2°C after 14 days of storage for both herbicides using 1/20 of their LD₅₀. The results at the initial time for both herbicides under the study showed highly decreasing in the content of total soluble protein with highly increasing in glucose, urea, creatinine, bilirubin, cholesterol and triglycerides levels. Aspartate aminotransferase, Alkaline Phosphatase and Alanine Aminotransferase activities showed high results compared to control. Where, storage at $54\pm2^{\circ}$ C leaded to slightly decreasing on content of total soluble protein, but bilirubin, serum glucose, cholesterol, urea, triglycerides and creatinine were low increase compared to health normal control. Activities of AST, ALT and ALP were the stimulated under the effect of the both herbicidescompared with the normal. Storage at 54±2°C until 14 days for both herbicides sencor 60% SC and turonex was lower harmful than their effect at initial time at room temperature 23±2C°, the influences of metribuzin after thermal storage at $54\pm^{\circ}C$ and at initial time $23\pm^{\circ}C$ on the biochemical study was more than that of isoproturon.

Keywords: Degradation, Isoproturon, Metribuzin, Sub acute toxicity.

INTRODUCTION

Triazine herbicides are one of the world's largest groups of pesticides (Salman et al., 2012; Hamza et al., 2015). For crops such as potatoes, soybeans and other vegetable plants, triazinones such as metribuzin (4amino-6-tert-butyl-4,5-dihydro-3methyltio-1,2,4-triazin-5-one) are used worldwide to combat widespread weeds (El Saved and Prasher, 2013). Metribuzin has paid more attention because of its extensive use, finding it to be a potential environmental contaminant (Mehdizadeh, 2014). Medidoub has been observed that Metribuzin having high dangerous effect on humans (Medjdoub et al., 2011), hatmful effect on domestic animals (EPA, 2003) and fish (Štèpánová et al., 2012; Maksymiv et al., 2015). However, Medjdoub has been reported that metribuzin leaded to immunotoxicity on the vitro (Medjdoub et al., 2011). Isoproturon is a phenyl urea compound that is used as herbicides, generally have high effect and used against unwanted herbs. Human health may be affected by the frequency of certain diseases is increased from pesticides environmental exposure. Their toxic effects are clearly mediated by reactive oxygen species which can react with biological molecules and initiate oxidative damage including protein oxidation, reduced lipid peroxidation (LPO) and glutathione (GSH) depletion (Otuechere et al., 2012). Over the past few decades, the use of pesticides in agriculture to preserve crops for humans and animals has resulted in their undesirable accumulation in the environment (Dbira *et al.*, 2014). The Pesticides and herbicides are poisonous, xenobiotic, and sometimes non-biodegradable and can lead to a many serious health and environmental problems. (Hamza *et al.*, 2015). In a study, it was shown that the commercial formulation containing isoproturon and metribuzin induced changes during male wistar rats ' metabolic parameters. Metribuzin was confirmed to be toxic to rats and the target organs were the liver, muscles, adipose tissue and intestines (Chiali *et al.*, 2013). This investigation was carried out to study stability of these two pesticides.

MATERIALS AND METHODS

Chemicals

All organic solvent were HPLC grade and purchased from Fisher Scientific Ltd. Milli-Q water purification system was used to prepare deionized water. Anhydrous calcium chloride and magnesium chloride were of analytical grade and purchased from Merck Ltd. Solution of buffer pH 4 and pH 7 received from Panreac Applichem from Germany. Both isoproturon and metribuzin standard received from Sigma-Aldrich. The commercial product of metribuzin ($C_8H_{14}N_4OS$) herbicide formulation imported from Bayer Crop Science Germany. The commercial name for metribuzin was Sencor 60% SC in which metribuzin was the active substance in the sum of 60%. Isoproturon ($C_{12}H_{18}N_2O$) herbicide was imported from Agriphar S A Belgium. The commercial name for Isoproturon was Turonex 50% SC in which the isoproturon active substance in the sum of 60%. Biochemical serum sample analysis was carried out using consumer kits by Pelobiotech GmbH Am Klopferspitz, Germany, purchased from Sunred Company Ibn Salama st. hajar building, vectoria, Alex, Egypt. Alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), fructose, urea, triglycerides, creatinine, cholesterol, bilirubin, and total protein was assessed biochemical components.

Apparatus

UHPLC system UltiMate 3000 dionex with quadrennial pump systems GmbH, Germany combined with photodiode array detector as described by Gamon *et al.* (1998) with conditions, metribuzin and isoproturon mobile phase were water: methanol (10/90 v/v) and flow rate 1.0 mL/min with detection wavelength 210 nm and retention time was 3.31 and 3.76 min, respectively.

Determination of active ingredient

Isoproturon and metribuzin formulations evaluated according to (CIPAC E, p. 110) and (CIPAC D, 283/SC/(M)/3, p.140) before and after storage, respectively. Isoproturon and metribuzin total content shall be declared (g/kg), when evaluated the content obtained shall not differ from that declared by more than (±5% and ±2.5%) of the declared content, respectively.

Stability at 54±2°C

According to FAO specification the storage until 14 days at $54\pm2^{\circ}$ C performed by method (MT 46.1, CIPAC 1C, p. 951) and after storage at $54\pm2^{\circ}$ C the samples was determined and the average of active ingredient percentage must not be lower than 97% relative to the determined average content found before storage, the product shall continue to comply with physical properties pH values, Pourability, Wet sieve test, Spontaneity of dispersion and suspensibility.

Stability at zero °C

Storage for 7 days at 0°C determined according to FAO specification (MT 39.1, CIPAC 1C, p. 930).

Physical properties

Physical properties determined before one day of storage and after different storage periods as the following, change of pH values (MT 75, CIPAC 1A, p. 1589), Pourability (CIPAC 1C, MT 148, p. 2282), Spontaneity of dispersion (CIPAC 1C, MT 160, p. 2291), Suspensibility (CIPAC 1C, MT 161, p. 2294), Wet sieve test (CIPAC 1C, MT 59.3, x, p. 981) and Persistent foam (CIPAC 1C, MT 47.2, p. 2249).

Animals test

Albino male rats weighed 120±10g which obtained from breeding house of animal in National Research Centre (NRC, 1996), Dokki, Cairo, Egypt. Plastic cages were used in breeding animals and allowed for one week before starting the work to adjust with environment. The rats were fed on standard food and tap

water and housed at $23 \pm 2^{\circ}$ C in daily for one week dark/light cycle. Rats were used in this work after agreement of animal care and in accordance with the guidelines for care and use animals in laboratory (NRC, 1996).

Experimental Design

The animals were divided to three groups (10 rats in each). 1. Untreated group, the rats used drinking water only. 2. Metribuzin-treated group (Mez), the rats ingested daily oral 1/20 from LD50 mg/kg body weight. 3. Isoproturon (Isot) group the rats ingested daily oral 1/20 from LD50 mg/kg body weight.

Preparation of the dose

A soluble concentrate (SC) from metribuzin and isoproturon were used in oil (5 ml) direct before orally ingestion into rats by esophageal tube. Sub-lethal dose 1/20 from LD₅₀ were calculated for metribuzin and isoproturon which 510 and 1826 mg/kg b.w., respectively, during experimental period the sub-lethal dose were used for dosing daily to rats albino male.

Sampling and Blood Collection

At the beginning of the experiment, four mice from each group were randomly used at 23 \pm 2 °C to collect serum by capillary tube after anesthesia of ether for biochemical tests. Also at the end of the experiment after 14 days at 54 \pm 2 °C, blood samples were collected for the same previous chemical tests using the same anesthetic and then slaughtered mice to obtain blood samples. Blood samples were collected in anticoagulant tubes and left for 20 minutes at room temperature and centrifuged at 3000 rpm at 4 °C for 10 minutes using BOECO model C-28, Germany, to separate the serum from blood samples.

RESULTS

I. Stability at elevated 54±2°C for 14 days

A. Degradation of Active ingredients under storage at 54±2°C

Degradation of isoproturon and metribuzin active ingredients for two tested herbicides under thermal storage mentioned in Table (1). Isoproturon percent value at initial period before one day of storage was 49.87% with decomposition ratio 0.26 % and it was 59.91% showed loss 0.15% for metribuzin active ingredient at initial period. Increasing time of thermal storage effected on active ingredient through 14 days of storage and decomposed it to reach 47.72% with decomposition ratio 4.56% of isoproturon whilst, metribuzin decomposition reached 58.44% with loss 2.60% through 12 days of storage. Tolerances of isoproturon and metribuzin were $(\pm 5\%)$ and $(\pm 2.5\%)$ respectively as reported in FAO specifications (1990) and (1991). The used turonex 50% SC formulation for isoproturon was compatible with FAO specifications under 14 days of storage but in contrast metribuzin formulation was not compatible with FAO specifications after 10 days of storage. Shereen (2008), El-badry and kamal El-din (2007), Ismail (2010) and Mohamed (2013) founded similar results on both isoproturon and metribuzin.

| Storage period | | ctive ingredient % SC | Metribuzin active ingredient 60% SC | | |
|----------------|-------|--------------------------|--|--------|--|
| (days) | % | Loss % | % | Loss % | |
| Initial | 49.87 | 0.26 | 59.91 | 0.15 | |
| 2 | 49.69 | 0.62 | 59.70 | 0.50 | |
| 4 | 49.33 | 1.34 | 59.47 | 0.88 | |
| 6 | 49.06 | 1.88 | 59.19 | 1.35 | |
| 8 | 48.67 | 2.66 | 58.99 | 1.68 | |
| 10 | 48.19 | 3.62 | 58.71 | 2.15 | |
| 12 | 47.96 | 4.08 | 58.44 | 2.60 | |
| 14 | 47.72 | 4.56 | 58.32 | 2.80 | |

| Table (1): Degradation of isoproturon and metribuzin under stored at $54\pm2^{\circ}$ C for 14 days | Table | (1): Degradation | of isoproturon and | l metribuzin under | stored at 54±2°C fo | or 14 days |
|--|-------|------------------|--------------------|--------------------|---------------------|------------|
|--|-------|------------------|--------------------|--------------------|---------------------|------------|

B. Effect of storage at 54±2°C on change of pH values of isoproturon and metribuzin

pH value under thermal storage reported in table (2) which showed that isoproturon pH value was 7.23 and 6.64 for metribuzin 1 day before thermal storage. Both tested formulation slightly effected with heat of storage after 14 days and became 6.73 for isoproturon and 6.31 for metribuzin. Two herbicides became compatible with FAO specifications (1990) and (1991) which mentioned that isoproturon pH from 6 to 8.5 and metribuzin from 6 to 7. Sencor 60 % SC and turonex 50% SC formulations can be stored under thermal storage for 14 days. Results agree with El-badry and Mohsin (2007), Kamal El-Din and Ola (2007), Ola and Shereen (2007), Mohamed (2009), Mohamed (2013) and Mohamed *et al.* (2016).

C. Effect of storage at 54±2°C during 14 days on pourability limits of isoproturon and metribuzin

Results in Table (2) showed that the residue percentage of isoproturon and metribuzin before one day of storage were 0.2% and 0.1%, respectively. By increasing long time of storage the percentage increased to reach 0.60% and 0.40% after 14 days of storage for isoproturon and metribuzin, respectively. This test pass successfully according to FAO specification (1990) for isoproturon which reported maximum rinsed residue 0.8% and according to FAO specification (1991) for metribuzin which reported maximum rinsed residue 0.5% the used isoproturon and metribuzin formulations become conformity with FAO specifications.

Table (2): Thermal stability effect on change of pH values, pourability and persistent foam of isoproturon and
metribuzin during storage at 54± 2°C for 14 days

| Storage period _ | | Isoproturo | on | | Metribuzi | n |
|------------------|------|-----------------------|-----------------------|------|-----------------------|-----------------------|
| (days) | рН | Pourability residue % | Persistent foam Ml | рН | Pourability residue % | Persistent foam ml |
| Initial | 7.23 | 0.20 | 3.5 | 6.64 | 0.10 | 7 |
| 2 | 7.20 | 0.20 | 3.5 | 6.58 | 0.10 | 7 |
| 4 | 7.16 | 0.30 | 3.0 | 6.51 | 0.15 | 6 |
| 6 | 7.11 | 0.35 | 2.0 | 6.48 | 0.20 | 5 |
| 8 | 7.02 | 0.45 | 2.0 | 6.45 | 0.30 | 4 |
| 10 | 6.92 | 0.50 | 1.5 | 6.39 | 0.35 | 3 |
| 12 | 6.80 | 0.50 | 1.0 | 6.34 | 0.40 | 3 |
| 14 | 6.73 | 0.60 | 1.0 | 6.31 | 0.40 | 2 |

D. Storage effect on persistent foam limits of isoproturon and metribuzin at 54±2°C during 14 days

Data in Table (2) showed that foam volume one day before storage after 1 min was 3.5 ml and 7 ml for isoproturon and metribuzin respectively. According to FAO specifications (1990) which reported maximum 5 ml of foam after 1 min for isoproturon and FAO specifications (1991) which reported after 1 min maximum 20 ml of foam for metribuzin. The used isoproturon and metribuzin formulations become conformity with FAO specification when stored for 14 days at $54\pm2^{\circ}$ C. The obtained results are agree those of Ismail (2010), Kamal El-Din and Ramadan (2011) and Mohamed *et al.* (2016) finding for isoproturon and metribuzin herbicides.

E. Storage effect for 14 days at 54±2°C on limits of wet sieve test of isoproturon and metribuzin

From data in Table (3) the residue percentage one day before storage of isoproturon was none but it was 0.20% for metribuzin and became 0.70% for isoproturon at the end of experiment and 1.00% for metribuzin after 12 days of storage. According to FAO specification (1990) and (1991) which reported a maximum of 1.0% for isoproturon and metribuzin retained on a 63 μ m and a 75 μ m test sieve the isoproturon formulation become conformity with FAO specification when stored for 14 days but metribuzin become non conformity with FAO specification when stored after 12 days. The results are in line with Smith (1976), Morpeth (1995), Mohamed (2009), Mohamed (2013) and Mohamed *et al.* (2016) data for the both herbicides for limits of wet sieve test.

Table (3): Thermal stability effect on wet sieve test, spontaneity of dispersion and suspensibility of isoproturon and
metribuzin when storage during 14 days at 54±2°C

| | | Isoproturon | Isoproturon | | Metribuzin | |
|-----------------------------|-----------------------|--|-----------------------|----------------------|--|-----------------------|
| Storage period (days) | Wet sieve test (%) | Spontaneity of dispersion (minute) | Suspensibility (%) | Wet sievetest (%) | Spontaneity of dispersion (minute) | Suspensibility (%) |
| Initial | None | 100 | 100 | 0.20 | 100 | 98 |
| 2 | None | 100 | 100 | 0.20 | 100 | 98 |
| 4 | None | 100 | 98 | 0.35 | 100 | 98 |
| 6 | None | 100 | 96 | 0.60 | 100 | 97 |
| 8 | 0.20 | 99 | 95 | 0.90 | 100 | 95 |
| 10 | 0.20 | 99 | 93 | 0.95 | 97 | 93 |
| 12 | 0.50 | 97 | 90 | 1.00 | 93 | 91 |
| 14 | 0.70 | 97 | 89 | 1.15 | 92 | 91 |

F. Storage effect during 14 days at 54±2°C on suspensibility percentage of isoproturon and metribuzin

Data in Table (3) showed that the suspensibility percentage before one day of storage for isoproturon was 100% and it was 98 % for metribuzin and started decease by increasing the time of storage to reach 89 % and 91% after 14 days from storage at $54\pm2^{\circ}$ C for isoproturon and metribuzin respectively. According to FAO specification (1990) and (1991) which reported a minimum of 70% and 90% of the isoproturon and metribuzin shall be in suspension after 30 min respectively. The two herbicides formulations become conformity with FAO specification when stored for 14 days at $54\pm2^{\circ}$ C. Results are agree those of Duraipandian and Regupathy (1989), Morpth (1995), Kamal El-Din (2007), El-badry and Mohsin (2007), Kamal El-Din and Ramadan (2009), Mohamed (2009) and Mohamed (2013) they observed similar results.

G. Storage effect during 14 days at 54±2°C on spontaneity of dispersion percentage of isoproturon and metribuzin

Data in Table (3) showed that the spontaneity of dispersion percentage one day before storage after 5 min for isoproturon and metribuzin were 100 % and it started decease by increasing long time of storage to reach 97% and 92% at end of the experiment after 14 days of storage at $54\pm2^{\circ}$ C for isoproturon and metribuzin, respectively. According to FAO (1990) and (1991) which reported a minimum of 95% and 60% of isoproturon and metribuzin shall be in suspension after 5 min respectively. The used herbicides formulations become compatible with FAO specification when stored for 14 days at $54\pm2^{\circ}$ C.

II. Stability during 7 days of storage at zero °C according to FAO specification.

A. Storage effect at zero °C on limits of wet sieve test of isoproturon and metribuzin during 7 days of storage

Data in Table (4) showed that wet sieve test limits for isoproturon and metribuzin passed successfully. Where, the retained percentage of isoproturon was none and 0.20% for metribuzin one day before storage and until the end of experiment after 7 days of storage at zero °C. According to FAO specification (1990) and (1991) which reported a maximum of 1.0%, retained on a 63 μ m and a 75 μ m test sieve for isoproturon and metribuzin. The used herbicides formulations become conformity with FAO specification. These results are in agreement with Smith (1976), Morpeth (1995), Mohamed (2009) and Mohamed (2013).

B. Effect of storage at zero °C for 7 days on suspensibility percentage of isoproturon and metribuzin.

Data in Table (4) showed that the suspensibility percentage of isoproturon was 100% and was 98% for

metribuzin one day before storage until the end of experiment after 7 days of storage at 0°C. According to FAO specification (1990) and (1991) which reported a minimum of 70% and 90% of the isoproturon and metribuzin shall be in suspension after 30 min respectively. The two herbicides formulations become conformity with FAO specification when stored for 7 days at 0°C. Results agreement with Duraipandian and Regupathy (1989) and Morpth (1995), El-badry and Mohsin (2007) and Mohamed *et al.* (2016).

C. Effect of storage at zero °C for 7 days on spontaneity of dispersion percentage of isoproturon and metribuzin

Data in Table (4) showed that the spontaneity of dispersion percentage after 5 min for isoproturon and metribuzin were 100% one day before storage until the end of experiment after 7 days of storage at 0 °C. According to FAO (1990) and (1991) which reported that a minimum of 95% and 60% of isoproturon and metribuzin shall be in suspension after 5 min respectively. The used herbicides formulations become conformity with FAO specification when stored for 14 days at $54\pm2^{\circ}$ C.

Table (4): Cold stability of isoproturon and metribuzin formulations during 7 days of storage at zero °C

| | Isoproturon | | | Metribuzin | | | | |
|-----------------------------|--------------------------|--|-----------------------|----------------|--------------------------|--|-----------------------|----------------|
| Storage period (days) | Wet sieve test (%) | Spontaneity of dispersion (minute) | Suspensibility (%) | Cold stability | Wet sieve test (%) | Spontaneity of dispersion (minute) | Suspensibility (%) | Cold stability |
| Initial | None | 100 | 100 | None | 0.20 | 100 | 98 | None |
| 1 | None | 100 | 100 | None | 0.20 | 100 | 98 | None |
| 2 | None | 100 | 100 | None | 0.20 | 100 | 98 | None |
| 3 | None | 100 | 100 | None | 0.20 | 100 | 98 | None |
| 4 | None | 100 | 100 | None | 0.20 | 100 | 98 | None |
| 5 | None | 100 | 100 | None | 0.20 | 100 | 98 | None |
| 6 | None | 100 | 100 | None | 0.20 | 100 | 98 | None |
| 7 | None | 100 | 100 | None | 0.20 | 100 | 98 | None |

III. Effect of metribuzin and isoproturon on serum biochemical components of albino male rats in initial time at and after 14 days from storage.

A. Effects of sub-acute dose $(1/20 \text{ LD}_{50})$ of tested herbicides at initial time $23\pm2^{\circ}C$.

The data of Table (5) observed that the ingestion of the both herbicides resulted high reduction in content of total soluble protein with high increase in serum glucose, urea, creatinine, bilirubin, cholesterol and triglycerides relative to those of health normal control. Also, the activities of AST, ALT and ALP stimulate were high under the effect of the both tested herbicides compared with the normal control.

B. The sub-acute effect of storage tested herbicides which storage at 54±2°C for 14 days.

The results of Table (6) showed that effects of metribuzin and isoproturon slight decreasing in content of total soluble protein but the levels of serum glucose, urea, creatinine, bilirubin, cholesterol and triglycerides were increased relativity to health normal control. Also, the activities of AST, ALT and ALP (liver function enzymes) stimulated under the effect of the both herbicidescompared with the normal control.

| Table (5): Effect sub-acute toxicity of ingestion metribuzin and isoproturon on components of albino male rats at initial | |
|---|--|
| time at $23\pm 2^{\circ}C$ | |

| Components | Experimental Groups | | | | |
|------------------------|--------------------------|---------------------------|--------------------------|--|--|
| Components | Control | Metribuzin | Isoproturon | | |
| Glucose (mg/dl) | 108.21±6.09 ^e | 114.5±2.20 ^a | 111.3±1.30 ^b | | |
| Urea (mg/dl) | 38.3 ±6.70 ^e | 56.62±7.59 ^b | 75.22±1.30 ^a | | |
| Creatinine (mg/dl | $0.48{\pm}0.08^{c}$ | $0.85{\pm}0.10^{a}$ | 0.72±2.30 ^b | | |
| Cholesterol (g/l) | 0.94±0.13 ^c | 1.24±0.18 ^a | 1.18±1.20 ^b | | |
| Triglycerides (g/l) | 0.93±0.08 ^b | 1.12±0.09 ^a | 1.10±1.10 ^a | | |
| Total protein (g/l) | 70.45±4.12 ^a | 65.27±5.41 ^b | $52.23 \pm 2.30^{\circ}$ | | |
| Total bilirubin (mg/l) | 5.50±0.60° | 7.24±0.91 ^a | 6.2±0.9 ^b | | |
| AST (U/L) | 78.52±5.83° | 90.87±4.85 ^a | 86.65±1.07 ^b | | |
| ALT (U/L) | 31.61±4.93° | 44.12±7.64 ^a | 38.75±2.07 ^b | | |
| ALP (U/L) | 144.17±9.33° | 224.62±49.14 ^a | 186.16±1.05 ^b | | |

Values are mean ± SD for groups of 8 animals each; Means that do not share a letter are significantly different

| biochemical components on albino male rats | | | | | |
|--|--------------------------|--------------------------|--------------------------|--|--|
| Components | Experimental Groups | | | | |
| Components | Control | Metribuzin | Isoproturon | | |
| Glucose (mg/dl) | 108.21±6.09 ^c | 111.5±1.20 ^a | 110.3±1.20 ^b | | |
| Urea (mg/dl) | 38.3±6.70 ^c | 44.23±3.59 ^b | 55.12±2.30 ^a | | |
| Creatinine (mg/dl) | 0.48±0.08 ^c | 0.65±0.20 ^a | 0.52±0.10 ^b | | |
| Cholesterol (g/l) | 0.94±0.13 ^c | 1.11±0.19 ^a | 0.99±0.30 ^b | | |
| Triglycerides (g/l) | 0.93±0.08 ^b | 0.99±0.11 ^a | $0.97{\pm}0.05^{a}$ | | |
| Total protein (g/l) | 70.45±4.12 ^a | 67.27±3.21 ^b | 62.22±3.40° | | |
| Total bilirubin (mg/l) | 5.50±0.60° | 6.65±0.81 ^a | 5.98±0.7 ^b | | |
| AST (U/L) | 78.52±5.83° | 86.17±2.21 ^a | 83.25±2.17 ^b | | |
| ALT (U/L) | 31.61±4.93° | 39.11±4.14 ^a | 36.15±3.11 ^b | | |
| ALP (U/L) | 144.17±9.33° | 192.12±4.22 ^a | 176.12±4.12 ^b | | |

 Table (6): Effect sub-acute toxicity of ingestion metribuzin and isoproturon after 14 days from storage at 54±2°C on biochemical components on albino male rats

Values are mean ± SD for groups of 8 animals each; Means that do not share a letter are significantly different

From the present results the increasing in blood glucose level by ingestion of herbicides may be due to disturb in the metabolism of carbohydrates by the elevation in liver glycogen break down under the effect of increasing of adrenocorticotropic and glucagon hormones with decreased insulin activity or content (Mehra *et al.*, 2014). The induction of metribuzin resulted hyperglycemia in rats (Chiali *et al.*, 2013). The

elevation of serum urea and creatinine content on present studies under the ingestion of metribuzin considered as renal dysfunction (Chiali *et al.*, 2013) that may be due to the influences of pesticides on kidney function and also liver function in which urea is the end product of protein catabolism (Mehra *et al.*, 2014).The increasing in serum cholesterol and triglycerides content may be relate to elevate in cholesterol biosynthesis in liver tissues or related to liver damage which increased the cell permeability but for triglyceride may be related to lipolysis inhibition in liver triglycerides or blood lipoprotein (El-Demerdash and Nasr, 2014). The similar results were observed by other pesticides intoxication (Yassa et al., 2011; Mehra et al., 2014). The ingestion of metribuzin and isoproturon into albino rats resulted hepatic dysfunction that ALT, AST and ALP activities were increased significantly these hepatocytes dysfunction caused alteration in the permeability of liver membrane (Khalil et al., 2014). Our results are in agreement of those of (Chiali et al., 2013) who found that the administration of metribuzin significantly increased the activities of serum transaminases associated with oxidative damage in liver tissues. Also, this liver damage elevated ALP enzymes activity related liver necrosis in which serum function enzymes (ALT, AST and ALP) activity were significantly increased may be due to the leakage of these enzymes into blood (Rahman et al., 2000). In addition, serum bilirubin content was increased significantly under the both herbicides induction into albino rats. These results from metribuzin and isoproturon ingestion reduced liver uptake, conjugation and increased bilirubin synthesis from hemolysis (El-Demerdash and Nasr, 2014). Also, the decreasing of total soluble protein of serum may be due to the reduction in protein biosynthesis were its catabolism (El-Demerdash and Nasr, 2014).

CONCLUSION

The decomposition rate for active ingredient under thermal storage at 54 ± 2 °C was slightly affected on isoproturon formulation but for metribuzin it was more than allowed maximum lime after 10 days of thermal storage. All physical properties for metribuzin and isoproturon under both thermal storage and 0 °C passed successfully except wet sieve test of metribuzin. Storage at 54 ± 2 °C until 14 days for both herbicides was lower harmful than their effect at initial time at room temperature 23 ± 2 °C. Influences of metribuzin after thermal storage at 54 ± 2 °C for 14 days and at initial time 23 ± 2 °C on the biochemical study was more than that of isoproturon.

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تأثير درجة حرارة التخزين علي تحطم إثنين من مبيدات الحشائش والسمية تحت الحادة علي ذكور التثير درجة حرارة التخزين علي تحطم إثنين من البيضاء

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تهدف هذه الدراسة إلى إلقاء الضوء عن تأثير حرارة التخزين المختلفة طبقا لمواصفات الفاو لسنة (١٩٩٠) و (١٩٩١) على ثبات الخواص الكيميائية والطبيعية لمركبات الايزوبرتيورون والمتريبيوزين في المستحضرات وهي مركز معلق لمبيد تيورنكس ٥٠% ومركز معلق لمبيد سنكور ٦٠% على درجة حرارة ٤٥°م لمدة ١٤ يوم و درجة الصفر المئوي لمدة ٧ أيام لدراسة ثبات نسبة المادة الفعالة من الايزوبرتيورون والمتريبيوزين التي تم تقديرها بواسطة جهاز التحليل الكروماتوجرافي السائل HPLC ودراسة الخواص الطبيعية مثل اختبار التعلق ومدى pH واختبار النعومة المبتلة واختبار ثبات الانتشار واختبار ثبات الرغاوى وتقييم التأثيرات المختلفة لمبيدي الايزوبرتيورون والمتريبيوزين باستخدام ٢٠/١ من الجرعة المميتة لــ ٥٠% من الأفراد علي بعض المقاييس البيوكيماوية علي ذكور الجرزان البيضاء البداية على درجة حرارة الغرفة ٢٣±٢ درجة مئوية وبعد ١٤ يوم من التخزين على درجة حرارة ٤٥±٢ درجة مئوية. وتبين من النتائج المتحصل عليها ما يلي:- ١) لم تتأثر نسبة المادة الفعالة لمستحضر مبيد الايزوبرتيورون بحرارة التخزين ولكنها تأثرت لمستحضر مبيد المتريبيوزين بعد ١٠ أيام من التخزين على درجة حرارة ٤٥°م. الخواص الطبيعية للمستحضرين بعد التخزين لمدة ٤٤ يوماً على درجة حرارة ٤٥°م جميعها لم تتأثر بحرارة التخزين ماعدا اختبار النعومة المبتلة لمستحضر المتريبيوزين الذي تأثر بحرارة التخزين بعد ١٢ يوماً. ٢) جميع اختبارات الخواص الطبيعية مرت بنجاح عند تخزينها على درجة الصفر المئوي لمدة ٧ أيام. ٣)- أظهرت النتائج بعد استخدام ٢٠/١ من الجرعة الفمية المميتة لـ ٥٠% من الأفراد لمبيدي الايزوبرتيورون والمتريبيوزين الأتي:-أ) أن تقدير المقاييس البيوكيماوية في البداية علي درجة حرارة الغرفة ٢٣±٢ درجة مئوية سببت أنخفاض عالِ في مستوي البروتين الكلّي وزيادة عالية في مستوي كل من الجلوكوز واليوريا والكرياتنين والبليريوبن والكلويسترول والتراي جلسريد بالإصافة إلى زيادة مرتفعة في نشاط كل من إنزيم AST و ALT و ALP مقارنة بالكنترول. ب) بعد التخزين علي درجة ٤٤ مئوتي بعد ١٤ يوم سببت أنخفاضاً قليلاً في محتوي البروتين الكلي وزيادة بسيطة في مستوي كل من الجلوكوز واليوريا والكرياتنين والبليريوبن والكلويسترول والتراي جلسريد بالإضافة إلى زيادة بسيطة في نشاط كل من إنزيم AST وALT و ALP مقارنة بالكنترول. وأخيراً أوضحت النتائج أن مبيد المتريبيوزين كان أكثر سمية من الايزوبرتيورون في البداية على درجة حرارة الغرفة ٢٣±٢ درجة مئوية وبعد ١٤ يوماً من التخزين على درجة حرارة ٤٥ درجة مئوية على المقاييس البيوكيماوية محل الدراسة.