Egypt. J. Chem. **Vol. 67**, No. 10 pp. 21 - 33 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Assessment of Toxicological Effect of Ethyl Formate on Male Albino Rats after Fumigating Dates in a Prototype

Mahmoud M. Abo-El-Saad[a], Reda K. Abdel-Razik[b], Yasser H. Hegazi[a], Samar M. S. Shawir[c]*, Nadia H. Hamed[b]

[a] *Department of Pesticide Chemistry and Technology, Faculty of Agriculture, El-Shatby, Alexandria University, Alexandria, Egypt*

[b] *Department of Pesticides Toxicity to Mammals and Aquatic Organisms, Central Agricultural Pesticide Laboratory, Agricultural Research Center, Giza, Egypt*

[c] *Home Economics in food and food science Department, Faculty of Specific Education, Alexandria University, Alexandria, Egypt*

Abstract

Ethyl formate (EF) was selected as a promising alternative to methyl bromide, a harmful substance for humans and the environment, against the common insect pests that attack Egyptian dates in the storehouse. EF, carbon dioxide (CO_2), and their EF/CO_2 mixture (16.7%: 83.3%) at a concentration of 420 g/m^3 were tested on El-Wady semi-dry and Ghazaly dry dates in the designed prototype (fumigation pilot chamber) to evaluate their insecticidal activity. After 24 hours of exposure, the treated dates were used to study the toxicological effect of ethyl formate on male albino rats. The rats were fed on treated El-Wady and Ghazaly dates for 28 days. Rats fed on El Wady showed a substantial gain in body weight of 52.6% and 40.8% in the control, and EF/CO_2 mixture compared with 34.8%, and 34% for Ghazaly. Results showed that the EF, CO_2, and EF/CO_2 mixture significantly didn't affect the hematological parameters: red and white blood cell counts, hemoglobin, and hematocrit values. Hepatic and renal toxicity were monitored by measuring blood biochemical parameters. The activity levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) were 33.10, 172.50, and 346.40 U/L, respectively in El Wady EF/CO_2 treatment group. While in Ghazaly EF/CO_2 treatment group, the corresponding activity levels were determined to be 31.90, 163.00, and 358.10 U/L, respectively. No significant change was notable between treatments in the levels of liver enzymes, bilirubin, total protein, urea, and creatinine. Histological investigation validated the preceding data, showing that the liver and kidney had normal and pathology-free histoarchitecture. The findings concluded that no risk is associated with consuming El-Wady and Ghazaly dates 24 hours after fumigating with an EF/CO_2 mixture.

Keywords: Date fruits; Ethyl formate; Liver; Kidney; Hematology

1. Introduction

Ethyl formate (CH_2H_5OCHO) is a transparent ester that is a somewhat oily liquid with a nice aroma reminiscent of rum and the taste of raspberries. It may be produced by reacting ethanol with formic acid. EF is utilized as a fumigant for pest management in many agricultural goods (Lee et al., 2018) and as a flavoring additive, with no indication of it being harmful to the public (Ryan and De Lima, 2014). By combining the advantages of hermetic storage and ethyl formate, especially, the benefits of utilizing ethyl formate (EF) instead of methyl bromide fumigant include brief fumigation holding durations of up to 6 hours (Damcevski et al., 2010).

The benefits of utilizing ethyl formate (EF) instead of methyl bromide fumigant include brief fumigation holding durations of up to 6 hours (Damcevski et al., 2010; Kwon et al., 2023), and a threshold limit value (TLV) of 100 ppm. In comparison, the TLV for methyl bromide is 3 ppm and for phosphine is 0.3 ppm, respectively. Methyl bromide and phosphine are 30 and

*Corresponding author e-mail: samar.mohamed.shawir@alexu.edu.eg.

Receive Date: 09 March 2024, Revise Date: 17 April 2024, Accept Date: 23 April 2024

DOI: 10.21608/ejchem.2024.275597.9426

330 times more poisonous than EF (Coetzee et al., 2019), indicating low toxicity to animals.

EF quickly decomposes into ethanol and formic acid, which are natural chemicals, with little to no impact on the environment (Haritos et al., 2003; Ren et al., 2014). This process does not impair product quality and ensures that residual levels remain below the maximum residue limit (MRL) (Ambrus et al., 2023). The MRL of EF for use on dried fruits is 1.0 mg/kg (Reuss et al., 2001). Storage conditions and package design significantly impact dates sustainability and quality. Hermetic storage is a key strategy for controlling postharvest pests (El-Kholy and Kamel, 2021; Elsawy et al., 2023).

The downside of EF is its low explosive limit (LEL) of 96 g/m^3, which can be mitigated by mixing it with an inert gas like carbon dioxide. The non-flammable mixing range of EF in CO_2 was chosen at 1:6 by volume to reduce the risk of flammability and prevent explosions (Ma, 2015). CO_2 also has a synergistic impact within the ideal range of 5-20%. This led to the creation of a commercial mixture of EF dissolved in liquid CO_2, which was then compressed to 40 bar pressure in an industrial gas cylinder, primarily to address EF flammability issues (Ryan and De Lima, 2014).

EF is a volatile liquid with a boiling point of 54°C, necessitating vaporization to decrease adsorption and provide even dispersion during fumigation. The EF/CO_2 combination, either pre- or post-(onsite), is transformed into a gaseous mixture using a specialized high-pressure vaporizer. This mixture is precisely delivered into the fumigation chambers by weight (De Lima et al., 1994).

The work by Haritos et al. (2003) and Ivantsova et al. (2023) extensively documents the effect of EF on insect respiration, demonstrating its inhibition of mitochondrial cytochrome C oxidase. Kim et al. (2019) discovered that EF has the ability to suppress cholinesterase and carboxylesterase activities.

The toxicological processes of EF and Phosphine (PH_3) are suggested to be the same (Haritos et al., 2003). They conducted experiments on several animal species to assess the acute toxicity of EF by oral and inhalation exposure. The oral administration of EF results in minimal acute toxicity, with an LD_{50} of around 2 g/kg body weight. Additionally, EF has lesser acute toxicity in comparison to dichlorvos or phosphine, as demonstrated by Haritos et al. (2006).

The World Health Organization's expert panel established the recommended acceptable daily intake (ADI) of ethyl formate at 3 mg/kg body weight/day during its routine evaluations of food additives in 1997(World Health Organization (WHO), 1997). Maille (2019), found that 1.88, 15.06, and 16.04 mg/liter of ethyl formate were required to cause 50% mortality in adults, larvae, and pupae.

It was suggested that Ethanedinitrile posed a greater threat than EF to all developmental stages of *L.serricorne*. Although EF is effective in controlling a wide range of stored goods insects, there is less understanding of its toxicological impact on mammals, which is crucial for evaluating its safety in different uses. Because there is a necessary need to find an alternative to methyl bromide at the global level to combat warehouse pests, and since ethyl formate is one of the most promising compounds as an alternative in this regard. Accordingly, this study aims to design a prototype for the use of this compound for date fumigation, then study its toxicological effects on laboratory animals, and study the potential effects of EF on blood parameters, hepatic and renal function, and histology in male rats.

2. Materials and Methods

2.1. Fumigant

2.1.1. Ethyl formate (≥98%) was obtained from *Merck KGaA*, Darmstadt, *Germany.*

2.1.2. EF/CO_2mixture

Filling and mixing of EF/CO_2 was done by Auf industrial gases Company, at New Borg Al-Arab- 3rd region, Alexandria, Egypt. Ethyl formate was mixed with CO_2 under pressure (50 bar). At a rate of EF: CO_2 (16.7%: 83.3 %).

2.2. Dates varieties

Two date varieties among the most popular date cultivars in Egypt, El-Wady (semi-dry cultivar) and Ghazaly (dry cultivar) were obtained from date stores for fumigation trials.

2.3. Animals

Forty-five male albino rats weighing 100±5g were acquired from the Faculty of Science at Alexandria University. Rats were given 14 days to adjust to laboratory settings before starting the experiment, which included a 12-hour light and dark cycle, temperatures ranging from 22 to 26 °C, and humidity between 60-70%. Rats were kept in separate plastic hanging wire-mesh cages to make it easier to assess their food consumption. Rats were nourished with a basal diet formulated following the guidelines of Reeves et al. (1993). The composition includes 20% casein (protein), 10% sucrose, 5% corn oil, 0.25% choline chloride, 1% vitamin mixture, 3.5% salt mixture, and 5% cellulose. Corn starch is used to balance the ingredients to make up 100%. After a two-week acclimatization period, the rats were separated into three main experimental groups. Food and water are supplied freely and checked regularly. All Rats procedures were conducted in compliance with the standards outlined in the Organisation for Economic Co-operation and Development (OECD) (2008) guidance. The experimental protocol (AU 08 23 01 24 4 125) was approved by the Institutional Animal Care and Use Committee (IACUC) at Alexandria University.

2.4 . Date fumigation in prototype chamber (the optimal operating condition)

Fumigation pilot chamber (prototype) designed by Techno lab Science (capacity 1.5x1.2x1.9m= 3.42 m^3). Constructed with heavy duty and proper metal with a sealed cover. The prototype contains a distribution fan, and thermostat to adjust the temperature, a light bulb for vision and follow-up, a glass window for observation from the outside, internal pipes for the distribution of gas, an opening to extract air and gas from inside the prototype (blower), an opening to enter the gas connected to the gas source (cylinder) fitted with a barometer to control pressure, heater and a precision balance used to determinate the quantity of injected EF/CO2 mixture. Fifty crates (boxes 40x30x15 cm^3 dimensions) containing 250 kg of two date cultivars, Ghazaly dry or El-wady semi-dry) were used for the fumigation trial (**photo 1)**. Artificial infestation of dates by the three insects; almond moth, *Ephestia cautella*, the sawtoothed grain beetle *Oryzaephilus surinamensis* and the red flour beetle, *Tribolium castaneum*)was performed. The promising concentration of EF/CO_2 mixture corresponding to complete insect mortality was 420 g/m^3, equivalent to 70.14 g/m^3 EF, and 349.86 g/m^3 CO_2, released into the enclosure at estimated optimum time and temperature, 24 h and 25ºC.After 24 hours of exposure, the treated cultivars of dates were used to study the toxicological effect of ethyl formate on dates using male albino rats.

Photo 1. Closed prototype (left) and opened prototype showing dates in boxes (right)

2.5. Experimental setting

Experimental diets were prepared from chopped treated and untreated El-Wady semi-dry or Ghazaly dry date fruits for 1 min. using Moulinex Blender, 800 Watt, 2 Liters, XL Chopper - LM42X2EG, France made and mixed at a rate of 30% with the basal diet.

Forty-five male albino rats (100±5g) were used to estimate the toxicological effect of consuming treated dates of El-Wady and Ghazaly that fumigated with EF, CO_2, and/or EF/CO_2 mixture. Rats were divided into three main groups, as follows: first group (5 rats) is negative control of rats fed on a basal diet, the second, El-Wady group (20 rats) contains four sub groups; rats fed on unfumigated date plus basal diet (served as El-Wady positive control), rats fed on fumigated El-Wady date by CO_2 plus basal diet, rats fed on fumigated El-Wady date by EF plus basal diet and rats fed on fumigated El-Wady date by EF/CO_2 plus basal diet. The third, Ghazaly group (20 rats) contains four sub groups; rats fed on unfumigated date plus basal diet (served as Ghazaly positive control), rats fed on fumigated Ghazaly date by CO_2 plus basal diet, rats fed on fumigated Ghazaly date by EF plus basal diet and rats fed on fumigated Ghazaly date by EF/CO_2 plus basal diet. Rats fed on treated dates at 30% (300 g of date/kg of basal diet) for 28 consecutive days

2.6. Biological evaluation

During the experimental period, the consumed diet and body weight were recorded every week. At the end of experiments the body weight gain (BWG%) and the relative weight of organs (%) were calculated.

2.7. Blood sample collection

At the end of the treatment period, animals abstained from food overnight with free access to water. Then, rats were euthanized using light anesthesia of sodium pentobarbital. Blood samples were obtained using a heart puncture. Two tubes were used to collect blood: one with EDTA for hematological analysis and the other without anticoagulant for serum production. Unclotted blood samples were examined for several peripheral blood cell parameters including white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin content (Hb), hematocrit percentage (Hct), and total platelet (PLT) count using the methods outlined by Dacie and Lewis (1991). Erythrocyte indicators such as mean corpuscular volume (MCV) calculated as Hct (%) x 10/RBC count (million/µL), mean corpuscular hemoglobin (MCH) calculated as Hb (g/dL) × 10/RBC

count (million/μL) and mean corpuscular hemoglobin concentration (MCHC) calculated by dividing the hemoglobin by the hematocrit.

2.8. Biochemical measurement in serum

The collected blood was left to coagulate for 30 minutes at room temperature, then centrifuged at 4,000 g for 15 minutes using Sigma 3K30 bench centrifuges to extract the serum. The serum was maintained at -20°C until it was utilized to determine biochemical parameters with BioMed diagnostic kits from Germany.

2.8.1. Liver functions:

2.8.1.1. *Aspartate aminotransferase* and alanine aminotransferase activities

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated by Reitman and Frankel (1957) method. The formation of hydrazone resulting from the reaction of 2,4-dinitrophenylhydrazine in both transaminase processes was measured at 546 nm.

2.8.2. Alkaline phosphatase activity

Alkaline phosphatase (ALP) levels were determined using the Belfield and Goldberg (1971)technique. Phenol is produced by enzymatic hydrolysis of disodium phenyl phosphate under certain circumstances of time, temperature, and pH. Phenol undergoes a reaction with 4-aminoantipyrine in the presence of an alkaline oxidizing agent to form a red product that is measured at 520 nm compared to a blank reagent.

2.8.3. Bilirubin level

Walter and Gerade (1970) method served to estimate serum total bilirubin. Serum bilirubin undergoes a reaction with diazotized sulfanilic acid to form azobilirubin, resulting in a pink color. DMSO catalyzes the conversion of free bilirubin into azobilirubin. The pink color's intensity increases in direct proportion to the bilirubin content when measured at 546 nm.

2.8.4. Total protein level

Lowry et al. (1951) method was used to determine total protein concentrations**.**

2.8.5. Determination of Kidney function:

2.8.5.1. Urea concentrations

Urea and creatinine are possible indicators for renal disease. Urea levels were determined using the Fawcett and Scott (1960) technique. Urease hydrolyzes serum urea to produce ammonium and carbonate. Under basic conditions, the ammonium ions combine with salicylate and HCl to produce a green-colored compound known as indophenol (2,2-dicarboxylindophenol). The color intensity is exactly proportional to the concentration of urea/BUN that measured at 578 nm.

2.8.5.2. Creatinine concentrations

Serum creatinine was determined using the Shlipak et al. (2013) method. When serum creatinine is in an alkaline solution, it interacts with picrate to form an orange-colored compound that absorbs light at a wavelength of 492 nm. Absorbance rate increases in direct proportion to the concentration of creatinine in the samples.

2.9. Histology examination:

Carleton's histology method was employed for the histological analysis (Carleton et al., 1980). The liver and kidney tissues from both control and testing rats were fixed in 10% buffered formalin for 10 hours and then thoroughly rinsed in 70% alcohol. After fixation, specimens were dehydrated in ethanol of varying concentrations and then embedded in paraffin using conventional methods. Light microscopy was utilized to analyze the 5 μm thick haematoxylin and eosin (H & E)-stained sections.

2.10. Statistical analysis:

The experiment design was Complete Randomize Design (CRD). Data were analyzed using IBM SPSS software version 25.0 by IBM Corp (2017). The data were presented as mean ± standard error (SE). The data was evaluated using one-way analysis of variance (ANOVA) followed by the student-Newman-Keuls test to establish significance across various groups. The criterion for statistical significance was established at $p < 0.05$.

3.Results

3.1. Effect of fumigated date treatments on body weight gain and organ weight relative to body weight of rats

The obtained results showed no signs of toxicity or mortality in any of the treated rats. Table (1) displays the impact on body weights after feeding rats two different date cultivars treated with carbon dioxide (CO_2), ethyl formate (EF), and a combination of both (EF/CO_2 mixture) for 28 days. According to the data, rats fed the El Wady cultivar saw a substantial rise in body weight of 52.6%, 37.2%, 45.1%, and 40.8% for the El-Wady control, CO_2, EF, and EF/CO_2 mixture, respectively. Rats that were fed Ghazaly dates saw weight gains of 34.8%, 31.7%, 34%, and 34% for the Ghazaly control, CO_2, EF, and EF/CO_2 mixture, respectively. However, rats fed El-Wady dates acquired more weight than those given Ghazaly dates.

The results in Table 2 show no significant changes in the absolute and relative weights of the liver and kidney in all treatment groups of El-Wady and/or Ghazaly dates compared to the negative control groups.

Table 1. Effect of El-Wady and Ghazaly dates fumigated with carbon dioxide, ethyl formate and EF/CO_2 mixture on body weight and body weight gain % of male albino rats

Treatment groups		Body weight		
		Initial (g)	Final (g)	Gain %
Negative control		$94.66^{b}\pm1.76$	$139.0^{b}\pm5.2$	$46.7^{ab}\pm2.8$
El Wady	Control	$105.3^{a}\pm2.72$	$160.6^{a}\pm2.3$	$52.6^{a}\pm2.1$
	CO_2	$102.0^{ab}\pm1.96$	$140.0^{b}\pm5.8$	$37.2^{bc}\pm3.9$
	EF	$98.66^{ab}\pm1.76$	$143.3^{b}\pm5.8$	$45.1^{abc}\pm3.5$
	CO_2 / EF	$98.00^{ab}\pm1.15$	$138.6^{b}\pm6.0$	$40.8^{abc}\pm5.3$
Ghazaly	Control	$99.66^{ab}\pm2.18$	$134.3^{b}\pm1.2$	$34.8^{bc}\pm2.1$
	CO_2	$100.6^{ab}\pm1.45$	$132.6^{b}\pm2.6$	$31.7^{c}\pm0.7$
	EF	$100.0^{ab}\pm1.0$	$134.0^{b}\pm1.15$	$34.0^{bc}\pm1.7$
	CO_2 / EF	$98.00^{ab}\pm3.0$	$131.3^{b}\pm3.3$	$34.0^{bc}\pm0.9$

Values are expressed as means (5 rats) standard error (SE).
Values in columns with different letters are significantly different at ($p \leq 0.05$).

$$\text{Gain}(\%) = \frac{\text{Final weight} - \text{initial weight}}{\text{intial weight}} \times 100$$

Table 2. Effect of El-Wady and Ghazaly dates fumigated with carbon dioxide, ethyl formate and EF/CO_2 mixture on organs weight relative to body weight of male albino rats

Treatment groups		Final body weight (g)	Organs weight (g)		Relative organ weight (%)	
			Liver	Kidneys	Liver	Kidneys
Negative control		$139.0^{b}\pm5.2$	$5.57^{a}\pm0.28$	$1.21^{a}\pm0.029$	$4.01^{a}\pm0.20$	$0.87^{a}\pm0.052$
El Wady	Control	$140.0^{b}\pm5.8$	$5.64^{a}\pm0.10$	$1.25^{a}\pm0.030$	$4.07^{a}\pm0.19$	$0.90^{a}\pm0.028$
	EF	$143.3^{b}\pm5.8$	$5.84^{a}\pm0.52$	$1.28^{a}\pm0.034$	$4.06^{a}\pm0.22$	$0.90^{a}\pm0.017$
	CO_2 / EF	$138.6^{b}\pm6.0$	$5.93^{a}\pm0.75$	$1.24^{a}\pm0.029$	$4.24^{a}\pm0.34$	$0.90^{a}\pm0.06$
Ghazaly	Control	$134.3^{b}\pm1.2$	$6.36^{a}\pm0.18$	$1.20^{a}\pm0.036$	$4.73^{a}\pm0.14$	$0.89^{a}\pm0.035$
	CO_2	$132.6^{b}\pm2.6$	$6.15^{a}\pm0.01$	$1.21^{a}\pm0.010$	$4.64^{a}\pm0.10$	$0.91^{a}\pm0.020$
	EF	$134.0^{b}\pm1.15$	$6.04^{a}\pm0.16$	$1.23^{a}\pm0.003$	$4.50^{a}\pm0.13$	$0.92^{a}\pm0.010$
	CO_2 / EF	$131.3^{b}\pm3.3$	$6.14^{a}\pm0.49$	$1.24^{a}\pm0.006$	$4.67^{a}\pm0.31$	$0.94^{a}\pm0.020$

Values are expressed as means (5 rats) standard error (SE).
Values in columns with different letters are significantly different at ($p \leq 0.05$).

$$\text{Relative weight of organs } (\%) = \frac{\text{Organs weight}}{\text{Body final weight}} \times 100$$

3.2. Effect of fumigated date treatments on hematological parameter and hematological erythrocytes indices of male albino rats

A complete blood count (CBC) is utilized for the diagnosis and monitoring of many disorders. The hematological parameters (WBC and RBC counts, HGB, Hct, and PLT values) in Table 3 did not exhibit any statistically significant changes ($p < 0.05$) in rats that were given El-Wady and Ghazaly dates treated with CO_2, EF, and EF/CO_2 mixture. No significant changes were seen in the hematological erythrocyte indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), or mean corpuscular hemoglobin concentration (MCHC) when compared to the negative control, as shown in Table 4. The study showed that exposure to ethyl formate at a concentration of 420 mg/m^3 did not have a negative impact on the red blood cell count and hemoglobin levels in the treated rats.

Table 3. Effect of El-Wady and Ghazaly dates fumigated with carbon dioxide, ethyl formate and EF/CO_2 mixture on hematological parameters in the blood of male albino rats

Treatment groups		WBCs ($10^3/\mu l$)	RBCs ($10^6/\mu l$)	HGB (g/dl)	Hct (%)	PLT ($10^3/\mu l$)
Negative control		10.90 ^{a} ± 1.64	6.536^{a} ± 0.28	11.36^{a} ± 0.45	29.67 ^{a} ± 0.15	741.33 ^{b} ± 47.93
El Wady	Control	10.40^{a} ± 1.21	6.33 ^{a} ± 0.13	11.40 ^{a} ± 0.75	32.13 ^{a} ± 1.97	693.33 ^{a} ± 64.01
	CO_2	11.37^{a} ±0.92	6.13^{a} ± 0.29	10.73 ^{a} ± 0.58	30.33 ^{a} ± 1.79	788.0 ^{ab} ± 89.91
	EF	11.9^{a} ± 3.67	6.34^{a}± 0.16	11.60 ^{a} ± 0.42	32.4 ^{a} ± 0.84	918.67 ^{ab} ± 52.90
	CO_2 / EF	10.67^{a} ± 0.73	6.37^{a} ± 0.43	10.0 ^{a} ± 0.90	30.66 ^{a} ± 0.55	881.66 ^{ab} ± 64.18
Ghazaly	Control	7.83^{a} ± 1.20	5.97 ^{a} ± 0.37	11.07 ^{a} ± 0.82	30.63 ^{a} ± 1.72	932.0 ^{ab} ± 106.93
	CO_2	6.77 ^{a} ± 0.67	5.87^{a} ± 0.21	10.46 ^{a} ± 0.52	29.77 ^{a} ±1.03	909.67 ^{a} ±113.33
	EF	6.2^{a} ± 0.78	6.26^{a} ± 0.28	11.67 ^{a} ±0.26	31.8 ^{a} ±0.91	838.33 ^{ab} ± 87.04
	CO_2 / EF	5.97^{a} ± 0.50	5.87^{a} ± 0.13	10.13 ^{a} ± 0.15	30.07 ^{a} ± 0.42	977.33 ^{ab} ± 151.05

Values are expressed as means (5 rats) standard error (SE).
Values in columns with different letters are significantly different at ($p \leq 0.05$).

Table 4. Effect of El-Wady and Ghazaly dates fumigated with carbon dioxide, ethyl formate and EF/CO_2 mixture levels on hematological erythrocytes indices of male albino rats

Treatment groups		M.C.V. (*fL*)	M.C.H. (*Pg*)	M.C.H.C. (*g/dL*)
Negative control		53.57 ^{a} ± 1.51	18.60 ^{a} ± 0.40	34.90 ^{a} ± 0.78
El Wady	Control	50.80 ^{a} ± 2.08	17.96 ^{a} ± 0.84	35.40 ^{a} ± 0.20
	CO_2	49.53 ^{a} ± 1.42	17.47 ^{a} ± 0.44	35.36 ^{a} ± 0.15
	EF	51.13 ^{a} ± 0.20	18.23 ^{a} ± 0.22	35.73 ^{a} ± 0.43
	CO_2 / EF	58.13 ^{a} ± 5.94	18.53 ^{a} ± 0.23	32.70 ^{a} ± 3.42
Ghazaly	Control	51.47 ^{a} ± 1.29	18.46 ^{a} ± 0.34	36.00 ^{a} ± 0.78
	CO_2	50.83 ^{a} ± 0.20	17.76 ^{a} ± 0.29	35.07 ^{a} ±0.73
	EF	50.93 ^{a} ±1.13	18.63 ^{a} ±0.59	36.66 ^{a} ± 0.38
	CO_2 / EF	52.37 ^{a} ± 0.62	18.83 ^{a} ± 0.33	36.03 ^{a} ± 0.19

Values are expressed as means (5 rats) standard error (SE).
Values in columns with different letters are significantly different at ($p \leq 0.05$).
M.C.V: Mean corpuscular volume
M.C.H: Mean corpuscular hemoglobin
M.C.H.C: Mean corpuscular hemoglobin concentration

3.3. Effect of fumigated date treatments on biochemical serum parameter of male albino rats

Biochemical blood tests can identify inflammation, damage, and the general performance of these essential organs. The data in Table (5) indicated that the activity of ALT, AST, and ALP in the El Wady control group were 58.60, 176.00, and 374.20 U/L, respectively. In the Ghazaly control group, the corresponding values were 58.06, 168.20, and 374.40 U/L. The levels of ALT, AST, and ALP enzymes in the El Wady EF/CO_2 treatment were measured as 33.10, 172.50, and 346.40 U/L, respectively. In the Ghazaly EF/CO_2 treatment, the corresponding values were 31.9, 163.00, and 358.10 U/L. No significant changes ($p < 0.05$) in the serum ALT, AST, and ALP activities, as well as total protein and bilirubin levels. Kidney biomarkers (creatinine and urea levels) did not show any significant difference compared to the negative control rats ($p < 0.05$). The EF/CO_2 combination at 420 g/m^3 had no impact on liver and renal functioning.

Table 5. Effect of El-Wady and Ghazaly dates fumigated with carbon dioxide, ethyl formate and EF/CO_2 mixture on biochemical serum parameter of male albino rats

Treatment groups		Creatinine (mg/dl)	Urea (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Protein (g/dl)	Bilirubin (g/dl)
Negative control		0.44^{ab}±0.02	30.3^{ab}±2	58.6^{ab}±2.8	144.7^{ab}±4.5	340.7^{abc}±28.5	6.0^{ab}±0.19	0.09^{abc}±0.02
El Wady	Control	0.42^{ab}±0.06	32.4^{b}±0.87	61.1^{b}±2.0	176.0^{d}±7.9	374.2^{d}±3.6	6.1^{ab}±0.17	0.13^{c}±0.01
	CO_2	0.46^{b}±0.01	29.9^{a}±0.98	59.6^{ab}±0.98	173.4^{cd}±4.7	392.2^{ab}±6.6	5.7^{ab}±0.1	0.11^{c}±0.02
	EF	0.46^{b}±0.01	29.3^{b}±0.64	62.4^{b}±5.5	173.7^{a}±7.6	324.5^{abc}±29.4	6.2^{b}±0.14	0.06^{b}±0.01
	CO_2 / EF	0.42^{ab}±0.02	32.1^{a}±1.16	58.6^{a}±0.81	168.2^{abc}±10.4	374.4^{bc}±15.6	6.3^{b}±0.06	0.04^{ab}±0.01
Ghazaly	Control	0.42^{ab}±0.01	33.1^{b}±0.75	67.0^{b}±1.16	172.5^{cd}±1.03	346.4^{d}±22.7	6.0^{ab}±0.08	0.12^{c}±0.01
	CO_2	0.40^{b}±0.01	29.5^{a}±1.45	66.4^{b}±2.6	165.8^{bcd}±1.1	308.6^{cd}±38.9	5.6^{a}±0.19	0.13^{c}±0.2
	EF	0.46^{b}±0.02	32.7^{b}±1.00	59.3^{ab}±2.4	169.6^{abcd}±4.8	348.6^{abc}±25.1	6.3^{b}±0.08	0.05^{b}±0.01
	CO_2 / EF	0.43^{b}±0. 05	31.9^{b}±2.00	58.7^{a}±2.0	163.0^{bcd}±4.7	358.1^{a}±12.2	6.0^{ab}±0.09	0.04^{ab}±0.01

Values are expressed as means (5 rats) standard error (SE).
Values in columns with different letters are significantly different at ($p \leq 0.05$).
ALT: Alanine Aminotransferase (ALT/GPT)
AST: Aspartate Aminotransferase (AST/GOT)
ALP: Alkaline phosphatase

3.4. Effect of fumigated date treatments on histological changes in liver and kidney tissues of male albino rats

Figure (1) shows that the liver section of control rats (A) given El-Wady dates had a histological structure with well-defined hepatocytes (H) and sinusoidal spaces (S) arranged in a radial fashion around the central vein (CV). Rats fed fumigated El-Wady dates with an EF/CO_2 combination (B) showed normal and regular Kupffer cells in the sinusoid walls. (Hematoxylin and eosin stain at 100x magnification). Figure (2) displays the tissue of the control group (A) and rats that were given fumigated Ghazaly dates with an EF/CO_2 combination (B). The control rats had a typical histological structure characterized by well-defined hepatocytes and a regular arrangement of sinusoids in a radial pattern around the central vein. Rats fed Ghazaly dates fumigated with an EF/CO_2 combination (B) exhibited typical characteristics, including clear hepatocytes with distinct basophilic nuclei, nucleoli, and eosinophilic cytoplasm (arrows). Additionally, a typical amount of Kupffer cells may be seen in the sinusoid walls. (Hematoxylin and eosin stain at 100 times magnification). Figure (3) displays the rat renal cortex from the control group (A) and the group treated with fumigated El-Wady dates with the EF/CO_2 mixture (B). This demonstrates the typical histological structure of the renal parenchyma. The Bowman's capsule consists of visceral epithelial cells encircling the glomerulus (G) and parietal epithelial cells enclosing the urine space (U). The proximal convoluted tubule is indicated by a thick arrow, whereas the distal convoluted tubule is indicated by a thin arrow in the observation under an H&E stain at 100x magnification. Figure (4) comparing the rat renal cortex of the control group (A) with the fumigated group exposed to EF/CO_2 combination and Ghazaly dates (B). The images display the typical appearance of capsules in both groups, including their globuli (G), lumen space (S), proximal (PX), distal (DS), and collecting ducts (CT) tubes. Rats that consumed El-Wady and Ghazaly dates treated with an EF/CO_2 combination for 28 days showed healthy liver and kidney tissues without any pathological abnormalities.

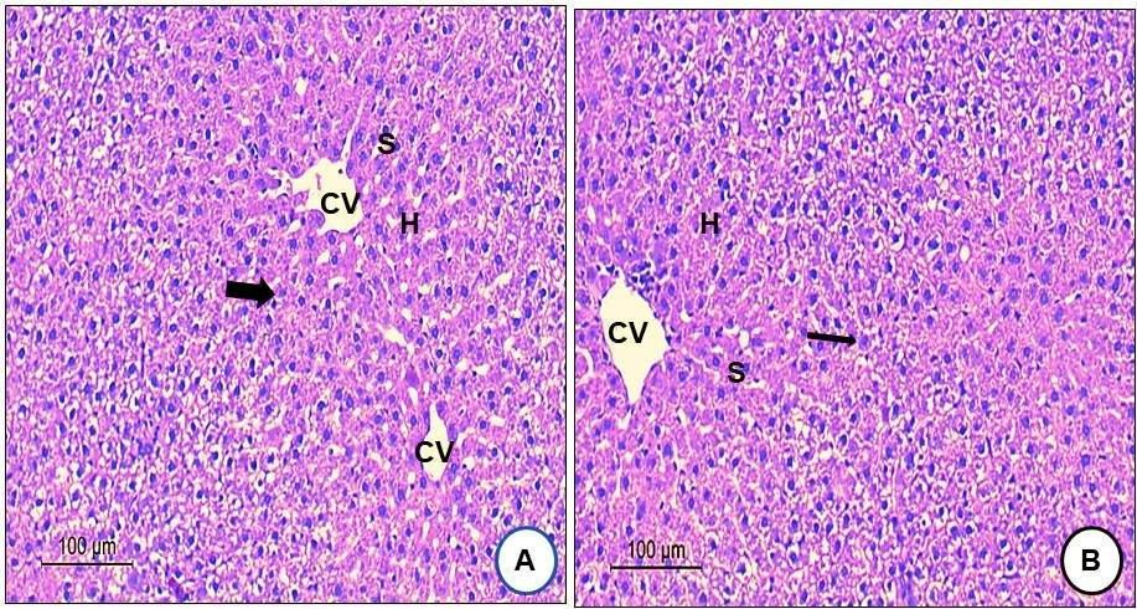

Figure 1. Photomicrograph of the liver tissues of male albino rats fed on El-Wady date at 30% of the basal diet for 28 consecutive days. (A): rats fed on El-Wady date (serve as control group). (B): rats that were fed fumigated El-Wady dates with an EF/CO_2 mixture (CV): central vein, (H): hepatocyte, (S): sinusoidal gaps. (H&E X 100).

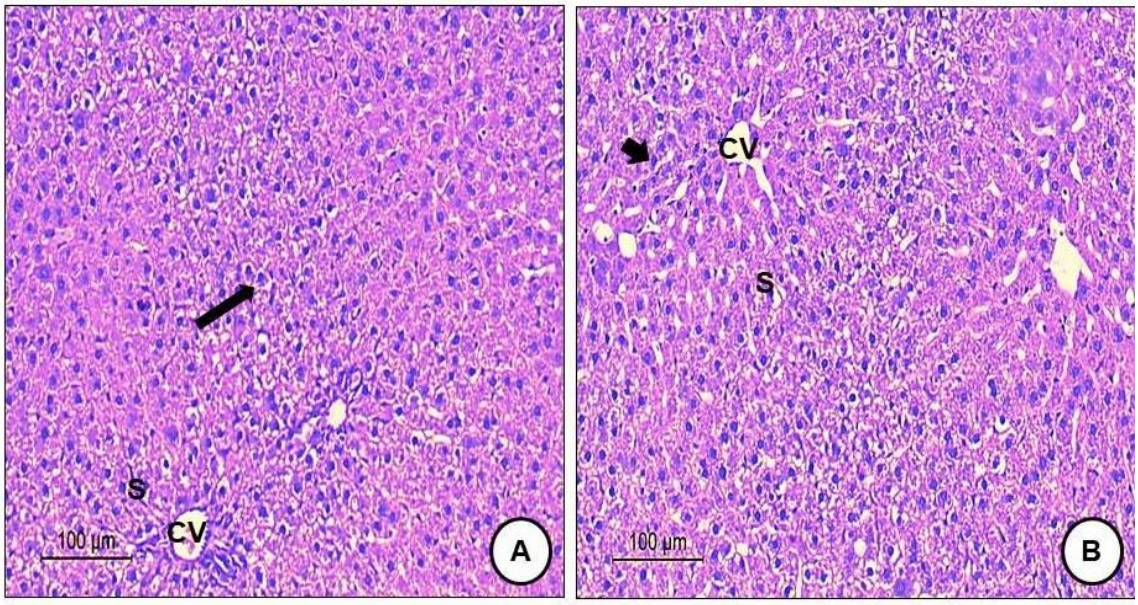

Figure 2. Photomicrograph of the liver tissues of male albino rats fed on Ghazaly date at 30% of the basal diet for 28 consecutive days. (A): rats fed on Ghazaly date (serve as control group). (B): rats that were fed fumigated Ghazaly dates with an EF/CO_2 mixture (CV): central vein, (H): hepatocyte, (S): sinusoidal gaps. (H&E X 100).

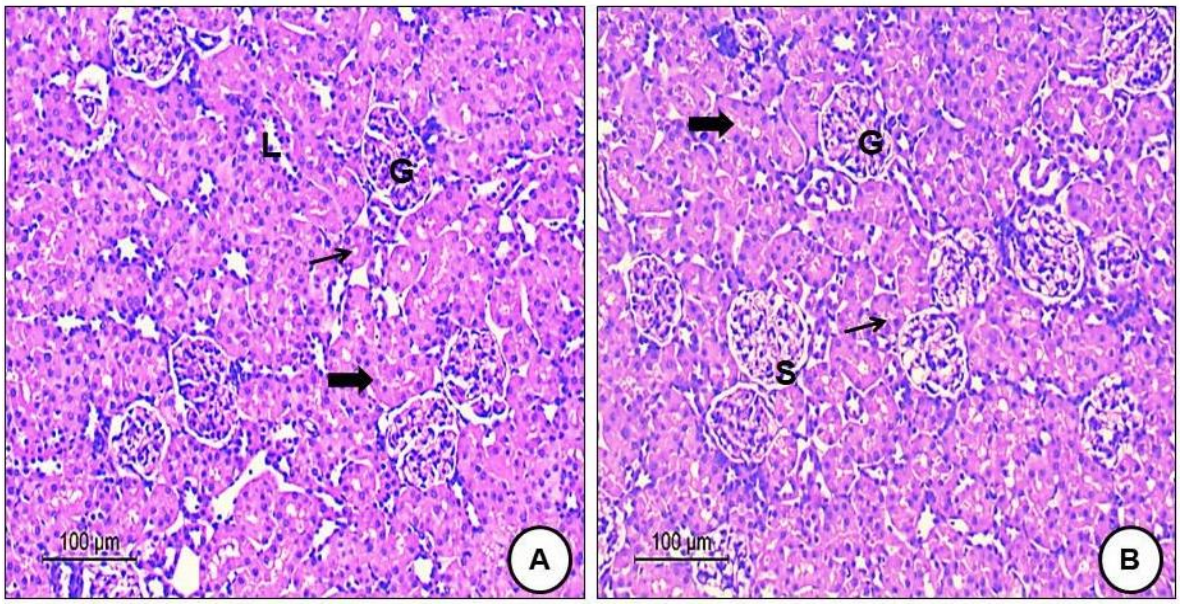

Figure 3. Photomicrograph of the kidney cortex of male albino rats fed on El-Wady date at 30% of the basal diet for 28 consecutive days. (A): rats fed on El-Wady date (serve as control group). (B): rats that were fed fumigated El-Wady dates with an EF/CO_2 mixture. (H&E X 100).

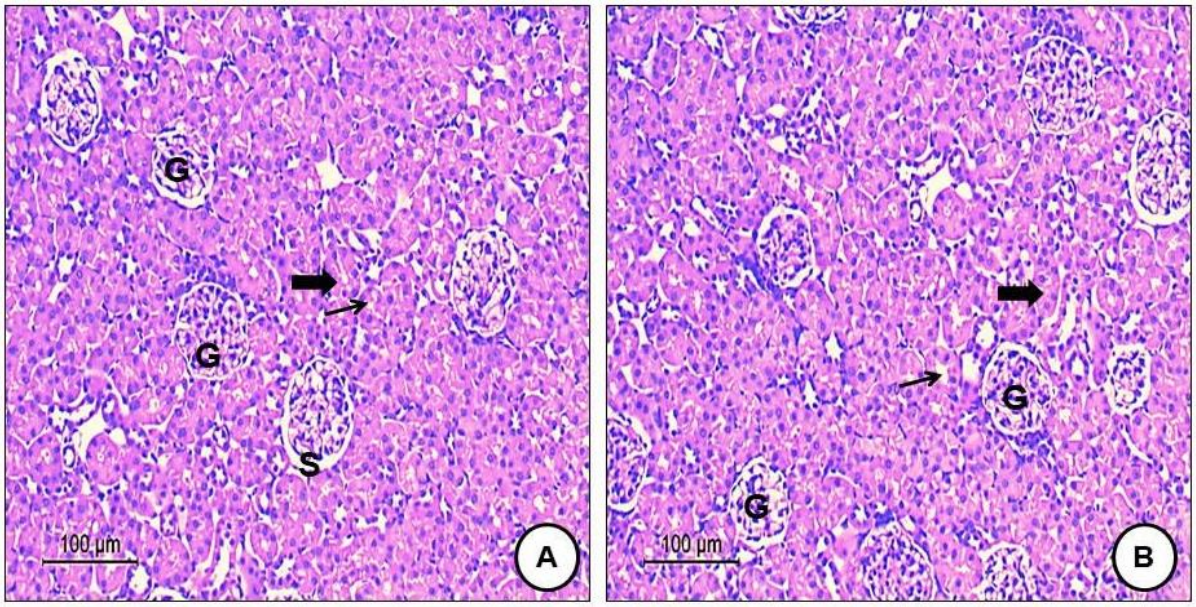

Figure 4. Photomicrograph of the kidney cortex of male albino rats fed on Ghazaly date at 30% of the basal diet for 28 consecutive days. (A): rats fed on Ghazaly date (serve as control group). (B): rats that were fed fumigated Ghazaly dates with an EF/CO_2 mixture. (H&E X 100).

4. Discussion

Ethyl formate is generally recognized as safe by US FDA (FDA, 2023). The liquid has a boiling point of 54.1 ºC. and exhibits moderate solubility in water, approximately 88.3 g/L at 25ºC (Haritos et al., 2003). It occurs naturally in ocean, soil, vegetation, and a range of food products, such as fruits, vegetables, grains, milk, cheese, beer, wine, and spirits (Ren et 2014). Ethyl formate levels in grains vary based on grain type, storage time, temperature, and moisture content. Newly harvested wheat, barley, oats, and canola have 0.1-0.2 mg/kg, 0.3-0.4 mg/kg, respectively (Bharath and Jaya 2024). The acceptable daily intake of ethyl formate is 3 mg/kg of body weight/d (WHO, 1997). Ethyl formate, a flavoring agent in food, has FDA approval levels of 0.05% in baked goods (FDA, 2023). Ethyl formate breaks down into formic acid and ethanol. Many or-ganisms undergo further metabolism of formic acid, converting it into carbon dioxide and water. These end products

can then be excreted, partially metabolized within tissues, or integrated into proteins, lipids, and nucleic acids, and subsequently distributed throughout the body (Haritos et al., 2003).

Ethyl formate shows significantly less acute toxicity compared to Dichlorvos (about 100 times more toxic) or phosphine (about 1000 times more toxic more toxic) (Haritos et al., 2003). Sorption of EF by grain mainly depends on the type, volume, and the properties of grain as well as the environmental conditions. Furthermore, Contrarily, Bessi et al. (2015) and, (Bharath and Jaya 2024) re-ported that the moisture content of the dates did not affect the sorption capacity of the samples dry (16% moisture content) and semi dry (20% moisture content) dates)

fumigated with different concentrations of ethyl formate (28.6, 57.3, 85.9 and 114.6 g/m^3) for 2 h. Shan et al, (2024), suggested that, although the toxicological mechanisms of EF and PH_3 share similarities, particularly in terms of inhibiting respiration by damaging the mitochondria.

The current study conducted to examine the negative impacts of feeding male rats for 28 days with two types of dates treated with an EF/CO_2 combination. The change in body and organ weights was used to evaluate the biological and physiological states of the treated and control animals. The results revealed that rats fed El-Wady dates gained more weight than those fed Ghazaly dates. According to research conducted by Ahmad and Abdoh (2015), rats showed a substantial increase in body weight (g/day) after consuming the Khalal cultivar compared to the Tamr cultivar. Based on previous research by Abo-El-Saad et al. (2023), the chemical composition of dry and semi-dry dates showed no significant variations in the average levels of crude protein, fat, fiber, ash, and carbohydrates, except for moisture content. This could make El-Wady dates more desirable for consumption because of their elevated moisture content, making them easier to consume.

Complete blood count (CBC) test is crucial for evaluating an animal's overall physiological health and well-being in different environmental circumstances. It is essential for distinguishing if hemolysis is caused by medications or chemicals (Atamanalp and Yanik, 2003). Obtained data revealed normal blood count. It suggests that there was no blood related disease or immunological reaction triggered by these fumigated substances (EF/CO_2). Many clinical researches demonstrated that alterations in liver enzyme activity can result from liver malfunction, leading to reduced enzyme synthesis and modifications in membrane permeability that allow enzymes to escape into the bloodstream (Cichoż-Lach and Michalak, 2014). A deficit in AST may make the liver more vulnerable to other infections or harmful substances (Nayak et al., 2023). ALP levels can aid clinicians in distinguishing between hepatobiliary illnesses such as primary or secondary liver cancer and cholestasis not originating in the liver (Han et al., 2013). Jubayer et al. (2020) suggested that alterations in ALT and AST values might be indicative of liver injury. The obtained results showed normal levels of AST, ALT, ALP, total protein, and bilirubin. Therefore, the fumigant (EF/CO_2) did not have any negative effects on liver and renal function. Consequently, there were no adverse impacts from such fumigant (EF/CO_2) on the liver and kidney function. The current results align with Kim et al. (2023) in demonstrating that EF fumigation at the optimal dose might be an effective, residue-free, and environmentally friendly method for managing agricultural pests in greenhouses. In addition US Food Drug Administration (FDA) (1984) stated that, EF is a volatile chemical found naturally in some goods and is considered Generally Recognized as Safe (GRAS).

Serum urea and creatinine values are commonly used as clinical indicators of kidney damage (Shawir et al 2024). No changes were observed in the two renal biomarkers according to our results.

It is customary to utilize histological examinations to identify organ alterations caused by chemical exposure. The liver, being a main organ for metabolism, is usually the initial major organ to encounter ingested poisons through its portal blood supply (Popp and Cattley, 1991). The liver sections of rats fed with dates fumigated with EF/ CO_2 mixture exhibit both normal and diseased hepatic lobular architecture, as indicated by the results. According to Lee and Kim (2017), breathed ethyl formate did not produce any changes in male or female rats subjected to 330 and 66 ppm, respectively. Therefore, they propose that exposure to ethyl formate at concentrations as low as 66 parts per million for a duration of 13 weeks is considered to be relatively safe. Also, the kidney sections of rats fed with dates fumigated with EF/ CO_2 mixture were found to have an intact histological structure of glomeruli and renal tubules, with a normal presence of interstitial collagen serving as structural support. The current histological examination of liver and kidney tissue from male rats that consumed dates fumigated with an EF/CO_2 mixture for 24-hour did not show any signs of ethyl-formate negative impacts, aligning with the results from the CBC test and biochemical assessments.

5. Conclusions

Ethyl formate is a promising compound as an excellent alternative for controlling pests of stored commodities, including dates. It offers minimal risk to human health due to its fast break down into ethanol and formic acid, which further degrade into carbon dioxide and water. The compound's high volatility aligns with laboratory research, suggesting it ceases to exist after three days. Our toxicological investigation shows no impact on experimental animals, making it

safe for national-scale pest control in warehouses. Accordingly, the experimental finding demonstrated that there was no risk associated with the exposure of ethyl formate, carbon dioxide, and their mixture EF/CO_2 at 420 g/m^3 on dates, as the hematological, biochemical, and histological findings, showed no negative effects. This finding is consistent with our preliminary data in which no residues were detected after three days from exposure due to the high volatility and degradation of EF.

6. Conflicts of interest

"There are no conflicts to declare".

7. Formatting of funding sources

The authors declares that they have no competing interests.

8. Acknowledgments

The authors would like to thank the Science & Technology Development Fund (STDF) for supporting this work under project ID 37143

9. References

Abo-El-Saad MM, Badawy ME, Mohammed YM (2023) Analysis and identification of microbial species associated with the Egyptian date fruits during postharvest storage. J Appl Biol Sci 17(1): 138-154.

Ahmad MN, Abdoh MJ (2015) The effect of date palm fruit (Phoenix dactylifera L.) on serum lipid and lipoprotein concentrations in rats fed cholesterol-supplemented diet. Mediterr J Nut Metabol 8(1): 51-60.

Ambrus Á, Doan VV, Szenczi-Cseh J, Szemánné-Dobrik H, Vásárhelyi A (2023) Quality Control of Pesticide Residue Measurements and Evaluation of Their Results. Molecules 28(3):954. https://doi.org/10.3390/molecules28030954.

Atamanalp M, Yanik T (2003) Alterations in hematological parameters of rainbow trout (Oncorhynchus mykiss) exposed to mancozeb. Turkish J Veter Animal Sci 27(5): 1213-1217.

Belfield A, Goldberg D (1971) Colorimetric determination of alkaline phosphatase activity. Enzyme 12(5): 561-568.

Bharathi VSK, Jayas DS (2024) Ethyl formate: A comprehensive review on its Function as a fumigant for stored products. J Stored Products Res 106: 102280. . https://doi.org/https://doi.org/10.1016/j.jspr.2024.102280.

Carleton HM, Drury RA, Wallington EA (1980) Carleton's histological technique. Oxford University Press, Oxford.

Cichoż-Lach H, Michalak A (2014) Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol.20(25):8082-8091. https://doi.org/10.3748/wjg.v20.i25.8082.

Coetzee EM, Newman J, Coupland GT, et al. (2019) Commercial trials evaluating the novel use of ethyl formate for in-transit fumigation of shipping containers. J Environ Sci Health B 54(8):717-727. https://doi.org/10.1080/03601234.2019.1631101.

Dacie JV, Lewis SM (1991) Practical haematology 7th ed. Churchill Livingstone, London.

Damcevski KA, Dojchinov G, Woodman JD, Haritos VS (2010) Efficacy of vaporised ethyl formate/carbon dioxide formulation against stored-grain insects: effect of fumigant concentration, exposure time and two grain temperatures. Pest Manag Sci 66(4): 432-438. https://doi.org/https://doi.org/10.1002/ps.1895.

De Lima C, Every R, Jackson P. (1994). Improved procedures for fumigation of oaten hay in shipping containers. Paper presented at the Stored Product Protection: Proceedings of the 6th International Working Conference on Stored-product protection.

El-Kholy MM, Kamel RM (2021) Performance analysis and quality evaluation of wheat storage in horizontal silo bags. Int J Food Sci 2021: 1-10.

Elsawy HIA, El-Kholy MM, Mohamed AM, Kamel RM (2023) Efficacy of different multi-layer hermetic bags on the seed quality of the faba bean (Vicia faba L.) in outdoor storage condition. Sci Rep 13(1): 20653. https://doi.org/10.1038/s41598-023-47598-4.

Fawcett JK, Scott JE (1960) A rapid and precise method for the determination of urea. J Clin Pathol,13(2):156-159. https://doi.org/10.1136/jcp.13.2.156.

Han GJ, Lee NK, Kim S, et al. (2013) Septic liver: clinical relevance of early inhomogeneous enhancement of the liver in patients with acute pyelonephritis. Acta Radiol 54(8): 975-980. https://doi.org/10.1177/0284185113484645.

Haritos V, Damcevski K, Dojchinov G. (2003). Toxicological and regulatory information supporting the registration of VAPORMATETM as a grain fumigant for farm storages. Paper presented at the Proceedings of the Australian Postharvest Technical Conference.

Haritos VS, Damcevski KA, Dojchinov G (2006) Improved efficacy of ethyl formate against stored grain insects by combination with carbon dioxide in a 'dynamic' application. Pest Manag Sci 62(4): 325-333. https://doi.org/10.1002/ps.1167.

Ivantsova E, Konig I, Lopez-Scarim V, et al. (2023) Molecular and behavioral toxicity assessment of tiafenacil, a novel PPO-inhibiting herbicide, in zebrafish embryos/larvae. Environ Toxicol Pharmacol 98: 104084. https://doi.org/https://doi.org/10.1016/j.etap.2023.104084.

Jubayer F, Kayshar S, Rahaman M (2020) Effects of Ajwa date seed powder on serum lipids in humans: A randomized, double-blind, placebo-controlled clinical trial. J Herb Med 24: 100409.

Kim K, Kim C, Kwon TH, et al. (2023) Optimizing ethyl formate fumigation in greenhouse cucurbit crops for efficient control of major agricultural pests, Myzus persicae and Thrips palmi. Chem Biol Technol Agric 10(1): 112.

Kim K, Lee YH, Kim G, et al. (2019) Ethyl formate and phosphine fumigations on the two-spotted spider mite, Tetranychus urticae and their biochemical responses. Appl Biol Chem 62(1): 1-10.

Kwon T-H, Cho J-H, Kim D-B, et al. (2023) Ethyl Formate Fumigation for Control of the Scale Insect Asiacornococcus kaki, a Quarantine Pest on Sweet Persimmon, Diospyros kaki: Efficacy, Phytotoxicity and Safety. Insects 14(4): 341. https://doi.org/10.3390/insects14040341.

Lee B-H, Park C-G, Yang J-O, Kim K, Lee S-E (2018) Concurrent application of ethyl formate and 1-methylcyclopropene to control Tetranychus urticae on exported sweet persimmons (Diospyros kaki Thunb. 'Fuyu'). Entomol Res 48(3): 198-203. https://doi.org/https://doi.org/10.1111/1748-5967.12269.

Lee MJ, Kim HY (2017) A 90-Day Inhalation Toxicity Study of Ethyl Formate in Rats. Toxicol Res 33(4): 333-342. https://doi.org/10.5487/tr.2017.33.4.333.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193(1): 265-275.

Ma T (2015) Ignitability and explosibility of gases and vapors. Springer, New York.

Maille JM. (2019). The efficacy of propylene oxide, ethyl formate, and ethanedinitrile as fumigants to control Lasioderma serricorne (Fabricius, 1792) (Coleoptera: Anobiidae), Tyrophagus putrescentiae (Schrank, 1781) (Sarcoptiformes: Acaridae), and Necrobia rufipes (De Geer, 1775) (Coleoptera: Cleridae) (Master Thesis) [Thesis]. Kansas State University.

Nayak P, Sharma SN, Nayak S, et al. (2023) Biochemical changes in the gill, liver, and muscle tissue of Anabas testudineus on exposure to varying concentration of Lead acetate. Environ Quality Manag 1: 1-12. https://doi.org/https://doi.org/10.1002/tqem.22050.

Organisation for Economic Co-operation and Development (OECD) (2008) Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris.

Popp J, Cattley R (1991) Hepatobiliary system. In: Haschek WM, Rousseaux CG (Eds.) Handbook of Toxicologic Pathology. Academic Press, New York p.p. 279-314.

Reeves PG, Nielsen FH, Fahey GC, Jr. (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr123(11):1939-1951. https://doi.org/10.1093/jn/123.11.1939.

Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28(1): 56-63. https://doi.org/10.1093/ajcp/28.1.56.

Ren Y, Lee B, Mahon D, et al. (2014) Fumigation of wheat using liquid ethyl formate plus methyl isothiocyanate in 50-tonne farm bins. J Econ Entomol.101(2):623-630. https://doi.org/10.1603/0022-0493(2008)101[623:fowule]2.0.co;2.

Reuss R, Annis P, Khatri Y. (2001). Fumigation of paddy rice and rice products with ethyl formate. Paper presented at the International Conference on Controlled Atmosphere and Fumigation in Stored Products.

Ryan R, De Lima C. (2014). Ethyl formate fumigation an overview update. Paper presented at the Proceedings of the 11th International Working Conference on Stored Product Protection, Chiang Mai, Thailand.

Shan C, You X, Li L, et al. (2024) Toxicity of Ethyl Formate to Tribolium castaneum (Herbst) Exhibiting Different Levels of Phosphine Resistance and Its Influence on Metabolite Profiles. Agriculture 14(2): 323. https://doi.org/10.3390/agriculture14020323.

Shawir, S., Eltayip, A. A., & Lotfy, T. M. (2024). protective effect of pan bread fortified with ginger on liver and renal function of hepatotoxic and nephrotoxic albino rats. Egyptian Journal of Chemistry 67:361-369.

Shlipak MG, Matsushita K, Ärnlöv J, et al. (2013) Cystatin C versus creatinine in determining risk based on kidney function. N Engl J Med

369(10): 932-943. https://doi.org/10.1056/NEJMoa1214234.

US Food Drug Administration (FDA) (1984) Subchapter B-food for human consumption. FDA, Atlanta.

Walter M, Gerade R (1970) Bilirubin direct/total. Microchem J 15: 231-233.

World Health Organization (WHO). (1997). Evaluation of certain food additives and contaminants : forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization.