

Toxicity of Carbon Dioxide-Phosphine Combination to *Tribolium Castaneum* Inside Gastight Bins and Its Histological Effect on Albino Rats

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Abstract

Toxicity of Carbon dioxide (30%), Phosphine (100 ppm) and their combination against the developmental stages of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) were studied under two degrees of temperatures 20 and 30±2 °C, inside steel bins. The residual impact of the combination on the treated wheat was evaluated with respect to histological changes in different organs of rat fed on the treated wheat. Lethal time for 50, 90 and 99% (LT₅₀, LT₉₀ and LT₉₉) after exposure periods of 1, 2, 3 and 5 days were calculated. Results indicated that the pupae were the least sensitive stage while the larvae were the most susceptible one to the combination. In addition, the mortalities of the different stages of *T. castaneum* in the combination treatment were higher than those obtained from each treatment alone. Histological examination for the rats feeding treated wheat with carbon dioxide-phosphine combination was done. Overall, testis, epididymis, stomach and spleen of the treated rats were not affected while liver; kidney and lung were affected through the appearance of mild histological changes. It may be concluded that this combination is good effective and less harmful in the case of control *T. castaneum* in the stored grains and recommended feeding of animals on these fumigated grains after 3 days from fumigation.

Key words: Stored grains, carbon dioxide-phosphine combination, fumigation, *Tribolium castaneum*, histological changes, rat.

Introduction

Insect pests of stored products are responsible for considerable economic losses to stored grains. *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is important pest of stored grains in Egypt. Presents secondary eating habits and is cosmopolitan, can attack different products, as flour, bran, feed, grain, biscuits, etc. **Trematerra & Sciarretta (2004); Daghli (2006)**. Control of this insect relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to insecticides and lethal effects on non-target organisms in addition to direct toxicity to users **Jembere et al., (1995); Okonkwo and Okoye (1996) and El-Gizawy(2012)**.

Phosphine (PH₃) is the most popular fumigant in situations where rapid fumigations are not a priority. PH₃ has many advantages, it is effective against the most insect species on a very wide range, easy to use and apply, relatively inexpensive and most markets accept it as a residue free treatment. **El-Lakwah (2001)** showed that the dosage used of two-phosphine formulations was effective against the adults and immature stages of the tested insect species. However, the bio-toxicity of the gas was temperature and exposure period dependent. It was found that at 16-20°C grain temperatures, an exposure period of 7 days was required for complete mortality and this period was prolonged to 10 days

when the temperature of the grain fell to from 12 to 16°C.

Many studies were carried out whether fumigation treatments for stored product insects with different combinations of phosphine, Carbon dioxide and temperatures would be advantageous over PH₃ alone. Such studies involved use of gaseous mixtures of CO₂, ranging from low concentrations to almost 100 % with PH₃ as the remaining gas added at different temperatures. Several authors have suggested that the exposure period required to control insect pests with PH₃ could be reduced by combining it with CO₂, **Athie et al., (1998); Desmarchelier (1984); Kashi and Bond (1975); Liang (1989); Mueller (1998); Rajendran (1989); Rajendran and Muthu (1989); Ren et al.,(1998); El-lakwah et al.,(2005) and Darwish (1998)**.

PH₃ are highly toxic to mammals **World Health Organization(1988)**, including human **Chugh et al., (1996); Anger et al.,(2000)**.PH₃ induces oxidative toxicity in many species **Hsu et al., (2000); Chugh et al.,(1996); Anger et al.,(2000)**. **Dua et al. (2010)** have revealed that the brain and liver are two of the most susceptible tissues to phosphide toxicity, and an earlier study in which phosphide powder residue contaminated cowpea was fed to rats, revealed significant alterations in serum levels of hepatic indices.

The aim of the present study was to evaluate the effect of temperature on the toxicity of carbon dioxide; phosphine and their combination against the developmental stages of *T. castaneum* and identify the impact of 30% CO₂ and 100 ppm

PH₃ combination residue in wheat on histological changes in different rat organs.

Material and Methods

The experiments were conducted inside four gastight steel bins of 0.5 m³ volume. Each bin was filled with about 450 kg wheat grains. The bins were designed and constructed by the Military Factory for Airplanes at Helwan, Egypt. These bins were situated on the roof of the building of the Plant Protection Department at Faculty of Agriculture, Benha University.

1. Insect culture

Tribolium castaneum was reared in glass container (250 ml) containing wheat flour covered with a fine mesh cloth for ventilation. The cultures were maintained in the dark in an incubator at 27 ± 2 °C and 60 ± 5% RH. Adults were obtained from laboratory stock cultures maintained at the Plant Protection Dept. Faculty of Agric., Moshtohor, Benha University, Egypt.

The bioassay tests were carried out inside the gastight bins to prove the efficacy of gases in the different treatments against the developmental stages of *Tribolium castaneum* under two degrees of temperatures 30±2 and 20±2 °C. Thirty adult of 7-14 days old insects, twenty eggs, thirty 4th instar larvae and thirty pupae were used for fumigant toxicity tests. They were introduced to 50 g wheat flour in each cloth bag (10×16 cm). Each bag was closed well by a rubber band. Each treatment was replicated three times. Percentage of insect mortality was calculated using Abbott formula **Abbott (1925)**. The fumigation of sub lethal-time of insect was done as described above, using the method with concentrations viz. LT₅₀, LT₉₀ and LT₉₉ after 1, 2, 3 and 5 days from treatment. Control bin was done in the same way without gases.

2. Fumigation bioassay:

2.1. Gases used:

Carbon dioxide was provided as pure gases of around 99.9% in pressure steel cylinders. Each cylinder was connected with a pressure regulator. Phosphine pellets, produced by Detia-DEGESCH, Germany were used during this study. One pellet weighs 0.6 g. and produced 0.2 g PH₃. CO₂ at concentration 30 ± 5%, PH₃ concentration at 100 ppm and combination of 30% CO₂ plus 100 ppm PH₃ were used at two degrees of temperatures 20 and 30±2 °C, inside steel bins against the various developmental stages of the tested insect.

2.2. Exposure procedure of the insects inside the bins:

Insect bags were inserted in the grains inside gastight steel bins just before each treatment. The cover of the bin was closed tightly. Grain

temperature and the relative humidity inside the bins were recorded. After the exposure time, the bin was opened and aerated. Insect samples were then taken and transferred to the laboratory for mortality assessment.

2.3. Purging of the gases inside the bin:

CO₂: for obtaining 30% CO₂ concentration the cylinder of CO₂ was connected with the upper valve of the bin through a polyethylene tube, the valve of the cylinder was opened for about one minute and half, while the bottom valve of the bin was opened and closed after one minute from the initial purging of the gas. to measure the CO₂ conc. by using CO₂ gas analyser model 200-600 Gow-Mac-Instruments Company USA.

PH₃: After introducing the insect samples inside the grains, half phosphine pellet was put in a petri dish, which placed on the top of the grains. Then the cover and two valves of the bin were closed tightly. Measurements of PH₃ concentration inside the treated bins indicated that half pellet produced 100 ppm PH₃. A bin without treatment was used in this experiment as control. PH₃ concentration was measured using Draeger-gas detector tubes (50/a).

Histological study:

Twenty seven male Albino Wistar rats, weighing 150±25 g. were obtained from Rodents laboratory at Faculty of Agriculture, Moshtohor, Benha University. Experimental design and animal handling were approved by the Research Ethical Committee of Faculty of Veterinary Medicine, Benha University, Egypt. All efforts were made to minimize animal suffering. After one week acclimation period, rats were randomly assigned to two groups; first group fed on fumigant grain with combination of 30% carbon dioxide with 100 ppm phosphine for 5 days while the second group fed on untreated grain as control. Three replicates were carried out for each group. Specimens from vital organs (liver, kidney, spleen, testis, epididymis, lung and stomach) of treated male rats were collected at 1st, 3rd and 5th days after treatment. Specimens from these organs were taken at the same interval times from control rats for comparison. All specimens except testis were fixed in 10% neutral buffered formalin but tests were fixed in Bouin's fluid. The fixed specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylene, blocked in paraffin. Paraffin blocks were cut in sections of 5 micron thickness. Sections were stained with hematoxylin and eosin for general structure, periodic acid Schiff method for glycogen detection, and Masson's trichrome for identification of collagen fibers. All histological changes were examined and photographed by Leica microscope.

Results and discussion

Toxicity of 30% CO₂, 100 ppm PH₃ and their combination of 30% carbon dioxide and 100 ppm phosphine were investigated against the developmental stages of *Tribolium castaneum* (Herbst.) under two degrees of temperatures 20 and 30±2 °C, inside gastight bins. The lethal insect exposure times of 50, 90 and 99% (LT₅₀, LT₉₀ and LT₉₉) after exposure period of 1, 2, 3 and 5 days were recorded (Table 1).

1. Toxicity of carbon dioxide:

The LT₅₀, LT₉₀ and LT₉₉ (days) for *T. castaneum* stages are shown in Table 1. The LT₅₀ at temperatures of 20 and 30 °C was 6.43 and 2.63 days for eggs; 7.33 and 3.63 for the 4th instar larvae; 10.07 and 5.25 days for pupae and 6.37 and 3.68 days for adults. For LT₉₀, this variation was ranged from 32.22 to 6.81 days for eggs; 27.57 to 10.4 days for the 4th instar larvae; 37.77 to 22.48 days for pupae and 18.60 to 8.73 days for adults. Meanwhile, For LT₉₉, the reduction was ranged from 119.8 to 14.78 days for eggs; 81.21 to 22.98 days for the 4th instar larvae; 111.01 to 73.59 days for pupae and 44.56 to 17.68 days for adults.

On the basis of LT₅₀ values and the degree of temperatures inside gastight bins the sensitivity order of the developmental stages of *T. castaneum* to carbon dioxide was measured as: egg > adult > 4th instar larvae > pupae at 30% CO₂ under the two degree of temperatures 20 and 30±2 °C, and 60±5% R.H. The higher temp. 30° C was more effective on the toxicity of the different stages of *T. castaneum* than at 20° C.

2. Toxicity of phosphine

The LT₅₀ values at 30° C of eggs, larvae, pupae and adults were 1.61, 1.27, 1.54 and 1.32 days, respectively to achieve 99% kill. These values increased to 6.68, 4.76, 7.40 and 4.36 days for the different stages, respectively. The adult stage of *T. castaneum* was more susceptible to phosphine treatment than other insect stages. Nearly the same trend was recorded for the different stages of *T. castaneum* at 20° C. Data in table (1) showed the time needed to obtain 50% and 99% kill for the various stages at the two degrees of temperatures (20 and 30±2°C), and 60±5% R.H. The 4th instar larvae of *T. castaneum* were more susceptible to phosphine treatment, while the pupae was the most tolerant to the treatment of 100 ppm PH₃ at 20±2°C. because the nature of pupae which need to low amount of respiration.

3. Toxicity of combined action of carbon dioxide and phosphine

Data in Table (1) showed the effect of 30% carbon dioxide conc. in an atmosphere of 100 ppm phosphine under the two tested degrees of temperatures 20 and 30±2 °C, and 60±5% R.H.

LT₅₀ for egg, larva, pupa and adult stages, were 1.15, 0.90, 1.23 and 0.94 days at 30°C, respectively. The time to achieve 99% kill were 3.88, 4.67, 4.87 and 4.78 days for various stages, respectively. This result clearly indicates that pupae stage was the least sensitive to the combined action efficacy of 30 % CO₂ plus 100 ppm phosphine at 30°C. Meanwhile, the results showed that the time needed to obtain 50% mortality for the different stages were 1.24, 1.20, 1.02 and 1.68 days for adult, egg, larvae and pupa, respectively. To achieve 99% kill, the days increased to 10.07, 36.60, 5.44 and 14.11 days for various stages, respectively. This result clearly indicates that the egg and pupae stages were the least sensitive to the combined of 30 % CO₂ with 100 ppm phosphine at 20°C

The results showed that the mortality percentage of the insects exposed to combination was higher than those obtained from each treatment alone and was more effective at 30°C than at 20°C against the various insect stages. Data achieved are in harmony with the results of Valizadegan (2011); Raimundo *et al.*, (2010); Ebrahime (2002) and El-lakwah *et al.*, (1989, 1991 and 1992).

As the main route of entry of the fumigants in insects is the respiratory system, factors associated with its activity, such as temperature, may affect uptake fumigants (Chaudhry *et al.*, 2004; Mittham *et al.* 2006). Thus, the increased toxicity of carbon dioxide and phosphine combination with increasing temperature may occurred due to the increased rate of insects respiration having as a consequence a catchment most of the insecticide molecules. The major toxicity of the combination of carbon dioxide phosphine for the 4th instar larvae can also be associated with increasing respiratory rate. Emekci *et al.* (2002) observed that the respiratory rate of eggs, young and old larvae, pupae and adults of *T. castaneum* at 30°C, under normal atmospheric air was 0.32; 29.08; 3.33; 0.59 and 2.37 mg insect µl CO₂ h⁻¹, respectively.

4. Histological studies of the effect of 30 % carbon dioxide with 100 ppm phosphine combination on rat organs.

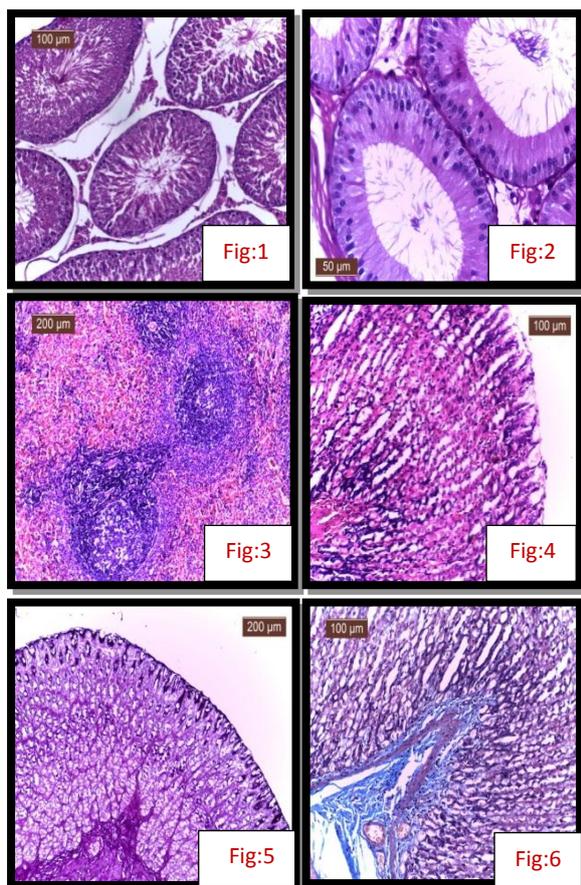
Results showed that testis, epididymis, stomach and spleen of all rats fed on exposed grains to the combination were not affected and showed normal histological structures (Figs. 1-6). While, liver, kidney and lung were affected and showed histological changes in comparison to control rats.

Table 1. lethal time values (days) and parameters of probit regression line estimated for different stages of *T. castaneum* exposed to 30% carbon dioxide; 100 ppm phosphine and their combination at 30 and 20±2° C; 60±5% R.H.

Stage	Temp. (°C)	LT ₅₀	LT ₉₀	LT ₉₉	Slop ±SD	R .
30% carbon dioxide						
Egg	20	6.43 5.51-7.51	32.22 22.92-45.29	119.80 68.87-208.39	1.83 ± 0.31	0.967
	30	2.63 2.35-2.95	6.81 5.80-7.99	14.78 11.53-19.18	3.10 ± 1.52	0.928
Larva	20	7.33 6.42-8.37	27.57 20.99-36.23	81.21 52.49-125.65	2.22 ± 0.07	0.994
	30	3.63 3.27-4.04	10.04 8.59-11.73	22.98 17.89-30.53	2.90 ± 1.33	0.946
Pupa	20	10.07 8.73-11.61	37.77 27.55-51.79	111.01 67.74-181.90	2.23 ± 0.04	0.996
	30	5.25 4.58-6.02	22.48 16.99-29.74	73.59 46.14-117.14	2.02 ± 0.14	0.986
Adult	20	6.37 5.71-7.10	18.60 15.29-22.62	44.56 32.54-62.62	2.75 ± 0.08	0.995
	30	3.68 3.34-4.05	8.73 7.5-10.04	17.68 14.21-22.0	3.41 ± 1.02	0.969
100 ppm phosphine						
Egg	20	1.72 1.49-1.98	3.80 3.16-4.58	7.27 5.32-9.93	3.71 ± 1.51	0.895
	30	1.61 1.39-1.87	3.53 2.94-4.25	6.68 4.89-9.31	3.77 ± 1.11	0.921
Larva	20	1.33 1.11-1.60	3.11 2.56-3.77	6.19 4.40-8.72	3.49 ± 0.72	0.938
	30	1.27 1.01-1.59	2.63 2.06-3.37	4.76 3.06-7.40	4.06 ± 1.40	0.868
Pupa	20	1.80 1.56-2.08	4.03 3.35-4.85	7.75 5.69-10.55	3.67 ± 1.85	0.873
	30	1.54 1.30-1.82	3.65 3.01-4.44	7.40 5.27-10.39	3.41 ± 1.53	0.877
Adult	20	1.68 1.45-1.96	3.81 3.17-4.58	7.41 5.42-10.13	3.62 ± 1.54	0.888
	30	1.32 1.08-1.60	2.55 2.02-3.21	4.36 2.93-6.49	4.48 ± 0.77	0.933
Combination of 30% carbon dioxide and 100 ppm phosphine						
Egg	20	1.20 0.66-2.19	7.89 2.78-22.41	36.60 4.37-305.92	1.56±0.04	0.969
	30	1.15 0.88-1.50	2.49 1.94-3.19	4.67 2.93-7.44	3.82±1.27	0.865
Larva	20	1.02 0.72-1.46	2.57 1.84-3.60	5.44 2.84-10.45	3.21±0.10	0.981
	30	0.90 0.58-1.40	2.29 1.73-3.03	4.87 2.70-8.79	3.18±1.59	0.790
Pupa	20	1.68 1.25-2.26	5.43 3.22-9.17	14.11 5.47-36.39	2.52±0.01	0.995
	30	1.23 0.97-1.56	2.60 2.03-3.33	4.78 3.05-7.47	3.96±1.41	0.861
Adult	20	1.24 0.86-1.78	3.93 2.49-6.02	10.07 4.15-24.39	2.55±0.06	0.981
	30	0.94 0.66-1.33	2.05 1.58-2.67	3.88 2.33-6.45	3.79± 0.39	0.951

R= Correlation Coefficient of regression line

SD= Standard deviation of the mortality regression line.

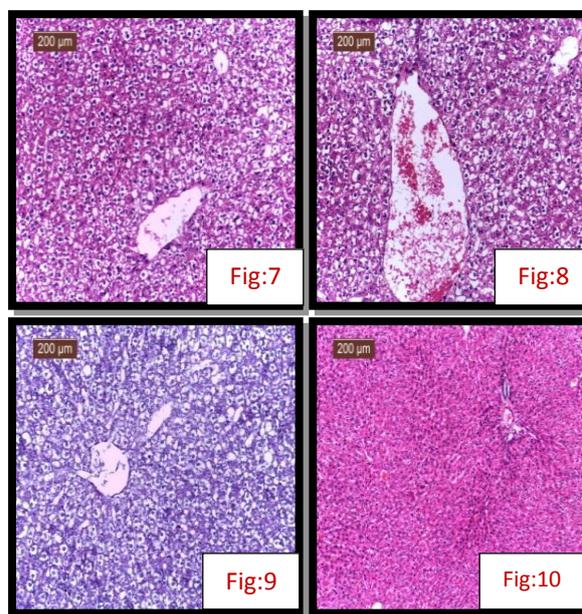


- Fig. 1. Photomicrograph of treated rats with combination of CO₂ and PH₃ showing normal histological structure of testis. H&E stain.
- Fig. 2. Photomicrograph of treated rats with combination of CO₂ and PH₃ showing normal histological structure of epididymis. H&E stain
- Fig. 3. Photomicrograph of treated rats with combination of CO₂ and PH₃ showing normal histological structure of spleen. H&E stain
- Fig. 4. Photomicrograph of treated rats with combination of CO₂ and PH₃ showing normal histological structure of stomach. H&E stain
- Fig. 5. Photomicrograph of treated rats with combination of CO₂ and PH₃ showing normal distribution of PAS positivity in stomach in comparison to control stomach. PAS technique.
- Fig. 6. Photomicrograph of treated rats with combination of CO₂ and PH₃ showing normal distribution of collagen fibers in lamina propria of stomach in comparison to control stomach. Masson's trichrome stain

Liver:-

At 1st day after treatment, most of hepatocytes were enlarged with pale cytoplasm and darkly stained nuclei indicating hydropic degeneration (Fig. 7) but some normal hepatocytes were seen. At 3rd and 5th days after treatment, hepatocytes were still showing hydropic degeneration and central vein appeared dilated and congested (Fig. 8). These Livers showed no evidence for collagen fibers deposition (Fig. 9).

These histological changes in livers were recorded in comparison to normal histological structure of control liver where the hepatocytes arranged in cords radiating from central veins, blood sinusoids and portal area are seen (Fig. 10).



- Fig. 7. Photomicrograph of liver of treated rats with combination of CO₂ and PH₃ at 1st day showing enlarged hepatocytes with pale cytoplasm and darkly stained nuclei (hydropic degeneration). H&E stain.
- Fig. 8. Photomicrograph of liver of treated rats with combination of CO₂ and PH₃ at 3rd and 5th days showing hydropic degeneration in hepatocytes and dilated and congested central vein. H&E stain.
- Fig. 9. Photomicrograph of liver of treated rats with combination of CO₂ and PH₃ showing no evidence for collagen fibers deposition (no cirrhosis). Masson's trichrome stain.
- Fig. 10. Photomicrograph of liver of control rats showing normal histological structure of liver where the hepatocytes arranged in cords radiating from central veins. H&E stain.

Kidney:-

At 1st day after treatment, renal tubules showed mild degenerative changes with clear lumen but intertubular hemorrhage were seen clearly (Fig. 11). At 3rd and 5th days after feeding, renal tubules showed moderate degenerative changes with degenerated materials in the lumen and the glomeruli showed small sizes (Fig. 12) in comparison to control kidney. Renal tubules in medulla of the kidney showed intertubular hemorrhage (Fig. 13). These histological changes in kidneys were recorded in comparison to normal histological structure of control kidney where the glomeruli showed normal size and feature, in addition, renal tubules showed intact epithelium and with clear lumen (Fig. 14).

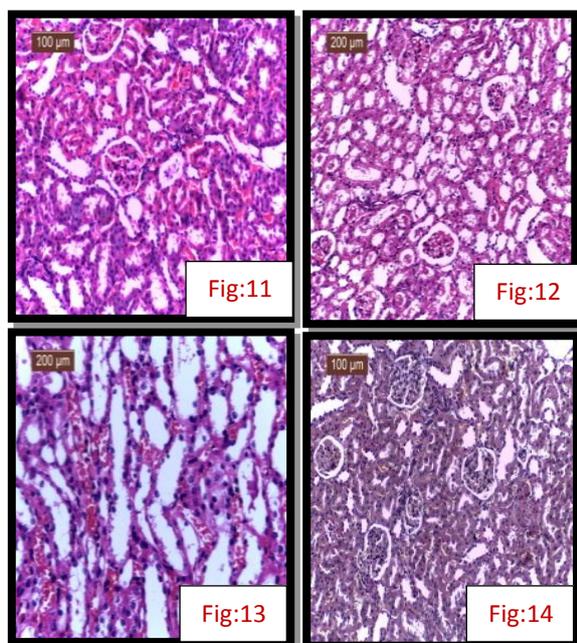


Fig. 11. Photomicrograph of kidney of treated rats with combination of CO₂ and PH₃ at 1st day showing, mild degenerative changes in renal tubules with clear lumen in addition to severe intertubular hemorrhage. H&E stain.

Fig. 12. Photomicrograph of kidney of treated rats with combination of CO₂ and PH₃ at 3rd and 5th days showing moderate degenerative changes in renal

Fig. 13. Photomicrograph of kidney of treated rats with combination of CO₂ and PH₃ at 1st, 2nd, and 3rd days showing intertubular hemorrhage in medulla of the kidney. H&E stain

Fig. 14. Photomicrograph of kidney of control rats showing normal histological structure of kidney where the glomeruli showed normal size and feature, in addition, renal tubules showed intact epithelium and with clear lumen. Masson's trichrome stain

Lung :-

The histological changes in lung were clearly seen at 1st day only. Peribronchial and perivascular mononuclear cellular aggregations were seen (Figs. 15,16), dilated and congested blood vessels were seen (Fig. 17). Such findings were in comparison to control lung where it showed normal alveoli, bronchioles and blood vessels (Fig. 18).

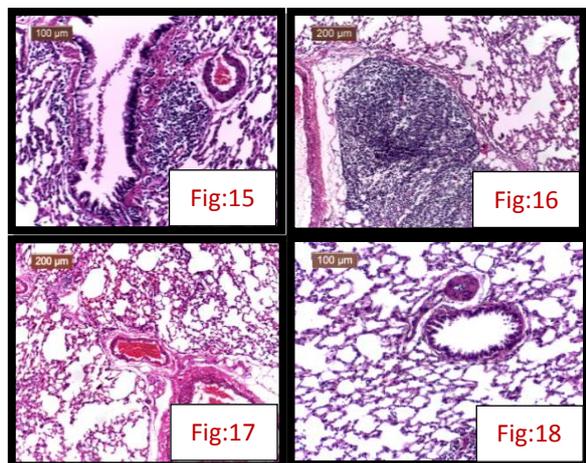


Fig. 15. Photomicrograph of lung of treated rats with combination of CO₂ and PH₃ at 1st day showing peri-bronchial mononuclear cellular aggregations. H&E stain.

Fig. 16. Photomicrograph of lung of treated rats with combination of CO₂ and PH₃ showing at 1st day showing perivascular mononuclear cellular aggregations. H&E stain.

Fig. 17. Photomicrograph of lung of treated rats with combination of CO₂ and PH₃ at 1st day showing dilated and congested blood vessels. H&E stain.

Fig. 18. Photomicrograph of lung of control rats showing normal lung structure. H&E stain.

Our histological findings in liver, kidney and lung were in accordance with **Sudakin and Power (2007)**; **Turkez and Togar (2013)** where they concluded that inhalation of phostoxin may cause severe pulmonary irritation leading to acute pulmonary edema, renal and hepatic damage. Such findings were appeared due to the ability of phostoxin to activate signals that increase necrosis factors that causes inflammation, malignancy and cell death **Arora *et al.*,(1995)**; **Sinha *et al.*,(2005)**; **Saleki *et al.*,(2007)**.

In addition, **Ozmen *et al.*, (2009)** and **Mogilner *et al.*, (2009)** conducted the histological changes after exposure to CO₂ as a result of hypoxia and/or ischemia that lead to acute cellular swelling, cytoplasmic vacuoles, hydropic degeneration, focal hemorrhage and epithelial cells within the tubular lumens.

Conclusions

1. The temperature affected toxicity of combination phosphine and carbon dioxide on all stages of *T. castaneum* development assessed.
2. The toxicity of the combination of carbon dioxide and phosphine was higher than those obtained from each gas alone.
3. The carbon dioxide is combined with the phosphine potential alternative to the use of conventional insecticides in tropical regions;
4. The carbon dioxide is combined with the phosphine was effective and safe to control *T. castaneum* in the stored grains and recommended feeding of animals on these fumigated grains after 3 days from fumigation.
5. The histological findings are reversible.

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سمية مخلوط غاز ثاني اكسيد الكربون والفسفين ضد حشرة خنفساء الدقيق الصدفية في الصوامع محكمة الغلق وتأثيره الهستولوجي على الفئران

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اجرى هذا البحث بغرض دراسة سمية غاز ثاني اكسيد الكربون وغاز الفوسفين وكذلك مخلوط الغازين معا ضد الاطوار المختلفة لحشرة خنفساء الدقيق الصدفية واجريت التجارب داخل صوامع من الصلب محكمة الغلق ومملوءة بحوالي 450 كيلو جرام من القمح. وصممت هذه الصوامع بالمصنع الحربي ببلوان، ووضعت على سطح مبنى قسم وقاية النبات بكلية الزراعة بمشتهر واجريت المعاملات المختلفة على درجتي حرارة 30، 2±20 م° ورطوبة نسبية 5±60%. علاوة على ذلك تم تقييم التأثير الباقي لمخلوط مكون من 30% غاز ثاني اكسيد الكربون و100 جزء بالمليون من غاز الفوسفين على التغيرات الهستولوجية للأجهزة المختلفة للفئران. ويتقدير الوقت اللازم لقتل 50، 90، 99 % من الحشرات بعد فترة تعريض 1، 2، 3، 5 ايام اشارت النتائج الى ان فاعلية كل من غاز ثاني اكسيد الكربون وغاز الفوسفين وكذلك مخلوط الغازين كانت اكثر فاعلية على درجة الحرارة المرتفعة عنه في درجة الحرارة المنخفضة وان طور العذراء هو الاقل حساسية بينما طور اليرقة هو الاكثر حساسية لمخلوط الغازين، بالإضافة الى ان نسبة الموت المتحصل عليها لمخلوط الغاز في المراحل المختلفة لحشرة خنفساء الدقيق الصدفية كان اعلى من تلك التي تم الحصول عليها من كل غاز على حده. كما اظهرت نتائج تقييم التأثيرات الهستولوجية للفئران التي غذيت على حبوب القمح المعاملة بمخلوط الغازين الى ان كل من الخصية والبربخ والمعدة والطحال لم تتأثر بشكل عام في حين ظهرت بعض التغيرات الهستولوجية الخفيفة على كل من الكبد والكلية والرئة. وتوصلت هذه الدراسة الى ان استخدام مخلوط غازي ثاني اكسيد الكربون والفسفين لمكافحة حشرة خنفساء الدقيق الصدفية بالحبوب المخزونة هي طريقة فعالة وامنة كما توصى هذه الدراسة بتغذية الحيوانات على الحبوب المبخرة بذلك المخلوط بعد 3 ايام من المعاملة.