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Effects of Pesticide Temephos on the Liver of *Aphanius dispar* (*Rüppell* 1828) (Pisces: Cyprinodontidae): A Microscopic Study

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ABSTRACT: The effects of the pesticide temephos, at different concentrations, on the liver of *Aphanius dispar*, (Rüppell 1828) a cyprinodont fish, have been described. The changes in the liver tissue after exposure to 1 ppm, 5 ppm, 10 ppm and 25 ppm concentrations of temephos have been presented. Light and transmission electron microscopy showed that the degeneration of the liver cells commenced after exposure to 1 ppm and steadily progressed to show maximum changes at 25 ppm. Histopathological changes included the dilation of sinusoids, an increase in the number of lipid droplets and cytoplasmic vacuolation, pyknosis of the nuclei and focal necrosis. These results falsify the claim that temephos is a non-systemic poison.

Keywords: Pesticide; *Aphanius dispar*; Liver; Temephos; Fish; Ultrastructure; Hepatocytes.

تأثير المبيد الحشري تيموفوس على كبد سمك الصد: دراسة مجهرية

طاهر باعمر، ايمان الخروصي و ريجنالد فيكتور

ملخص: توضح هذه الدراسة تأثير المبيد الحشري تيموفوس بتركيزات مختلفة (1 و 10 و 25 جزء من المليون) على كبد سمك الصد، وهذه التأثيرات تتمثل في التغيرات في خلايا الكبد وتتفاوت هذه التغيرات حسب التركيز المستخدم في هذه الدراسة. وتشمل التغيرات موت بعض الخلايا بدراجات متفاوته وذلك حسب التركيز وتزداد مع زيادة التركيز. وتشمل التغيرات ايضا توسع السينوسودات وزيادة القطرات الدهنية والحويصلات السيتوبلازمية وتدهور نوى الخلايا وموت الخلايا. ويمكن القول ان هذا النوع من المبيدات الحشرية من المواد السامة.

مفتاح الكلمات : المبيد الحشري ، سمك الصد ، الكبد ، السمك ، خلايا الكبد ، دراسة مجهرية.

1. Introduction

Temephos (Abate® 4-E, Cyanamid, USA), an allegedly non-systemic organophosphorus pesticide, is used for the control of mosquito larvae. In Oman, it is sprayed in both fresh and brackish water pools in concentrations and frequencies recommended by the manufacturer. Temephos is considered to be a safe pesticide because it is non-persistent in water and has low toxicity to mammals and is recommended by the US Environmental Protection Agency (EPA) (Aiub *et al*., 2002; Edward and Sogbesan, 2007). It is cheap and can easily be stored in tropical conditions; hence it is used widely in developing countries for the eradication of insect vectors of pathogens transmitting human diseases. However, this pesticide shows a wide range of toxicity to non-target aquatic organisms such as fish and insects (Ansari and Kumar, 1988; Aiub *et al*., 2002; Ba-Omar *et al*., 2011).

Aphanius dispar (Rüppell 1828), a cyprinodont fish known as killifish or *sud* fish, is common and widely distributed in fresh and brackish waters of Oman. It is also found in the coastal and inland waters of the Arabian Peninsula, the Arabian Gulf, the Red Sea Region and the eastern Mediterranean. In Oman, it is one of the three species of freshwater fish found on the coastal plains, the others being the cyprinids *Garra barreimiae* and *Cyprinion micropthalmum*. Apart from its contribution to the poor biodiversity of freshwater fish, its economic value has been underestimated. It is an effective predator of mosquito larvae in shallow waters and can be used as a biological control agent of mosquito larvae (Fletcher *et al*., 2002). Since it is a hardy fish capable of withstanding higher salinities, it also serves as an indicator in coastal freshwaters impacted by salinization. *A. dispar* is also at present threatened in nature by the invasive exotic, *Oreochromis niloticia*, which ironically was introduced to control mosquito larvae. Any additional impact on this species due to toxic substances like pesticides may lead to its local extinction.

Recently we studied the effect of temephos on the gills of *A. dispar* (Ba-Omar *et al.*, 2011). Despite the damage caused to the gills, many of these fish were able to survive temephos concentrations up to 25 ppm for at least seven days. *A. dispar* certainly ingests the pesticide treated water through the digestive system and temephos is also picked up through blood flow in the gills and is likely to be distributed to other organ systems far removed from the areas of direct contact.

Temephos inhibits the action of cholinesterases, and these enzymes are found throughout the body including the nervous system and the blood stream (Aiub *et al*., 2002). The liver, the largest gland in the body, is highly vascular. It stores digested and assimilated materials. It also detoxifies waste and harmful substances (Robertson and Bradley, 1991). Only a few studies have been conducted to evaluate the effects of pesticides on fish liver. For example, hepatic lesions in the liver tissues of fish exposed to deltamethrin were characterized by hypertrophy of hepatocytes, significant increase in Kupffer cells, focal necrosis, fat degeneration, nuclear pyknosis and narrowing of sinusoids (Cengiz and Unlu, 2006). Similarly, histopathological changes like vacuolation of hepatocytes and nuclear pyknosis were seen in *Oreochromis niloticia L.* exposed to Roundup, a glyphosate herbicide (Jiraungkoorskul *et al*., 2002). This study examines the effects of temephos in different concentrations on the liver of *A. dispar* to detect histopathological changes, if any.

2. Materials and methods

Thirty healthy *A. dispar* (Rüppell 1828) were collected with nets from Al Zepri Falaj, Siya village, Quriyat, Oman, and were brought to the laboratory at Sultan Qaboos University, Muscat. The fish were acclimatized to the laboratory conditions for two weeks in two glass aquaria filled with aerated tap water. They were fed twice a day with Tetramin flakes, a commercial fish food. Water in the aquaria was changed daily to avoid a decrease in pH caused by fish excreta.

Commercial Temephos 50 EC (Abate® 4-E, Cyanamid, USA) was used as the stock solution. It is (0,0'-thiodi-4,1-phenylene)bis(0,0'-dimethyl phosphopthioate) a concentrate that has 50.0% w/vol + emulsifier and aromatic solvents making it up to 100%. It is a straw coloured mobile liquid with a flashpoint of 50 °C Pensky-Martens Closed-Cup (PMCC) and **(**specific gravity of 1.1 at 20 °C**)**. From this stock solution four different concentrations of temephos, 1, 5, 10 and 25 ppm, were prepared. Two replications were used for each concentration and three fish were exposed in each replicate. Control fish, not exposed to temephos, were simultaneously maintained in a separate aquarium for the duration of each experiment. Changing of the test solution, cleaning the aquaria and feeding were done on a daily basis. Fish that survived the exposure for seven days and the controls were killed by placing them in the freezer for a few minutes. The livers were dissected out and were cut into small pieces and immediately fixed in Karnovsky fixative buffered with sodium cacodylate at pH of 7.4 for four hours at 4°C. The tissues were washed twice with sodium cacodylate and then stored in this buffer at 4 °C. The tissues were post-fixed in 1% aqueous solution of osmium tetroxide for 1 hour, dehydrated in a series of alcohol and embedded in Agar 100 resin.

Semi-thin and ultra-thin sections were cut using ultramicrotome with glass knives. Semi-thin sections were stained with 1% toluidine blue and the ultra-thin sections were stained with super saturated uranyl acetate and lead citrate and were examined under JEOL JEM-1230 Transmission Electron Microscopy (TEM).

3. Results

3.1 Light Microscopy

The liver of the control fish revealed no abnormalities. The hepatocytes were polygonal in shape with a central spherical nucleus. The cells were arranged as irregular cord-like structures in the section separated by sinusoids with usually one nucleolus (Figure 1A).

Histological changes of the liver started to appear at the temephos concentration of 1 ppm. At this concentration, large lipid droplets were seen and some of the cells looked abnormal (Figure 1B, C, E). Liver tissues of fish exposed to 5 ppm, 10 ppm and 25 ppm showed diffuse changes in the hepatic parenchyma. Significant changes were the dilation of sinusoids, high numbers of large lipid droplets and an increase in cytoplasmic vacuolation (Figure 1B-E). Additionally, the liver showed hepatocellular necrosis, this being more obvious in fish exposed to 25 ppm (Figure 1E). In general, the histological aberrations in the liver increased with the increase in temephos concentration.

3.2 Transmission Electron Microscopy (TEM)

The hepatocyte ultrastructure showed the following common features: polyhedral cells containing one large round or ovoid nucleus with a visible nucleolus (Figure 2A). The rough endoplasmic reticulum (RER) was arranged in parallel stacks of cisternae and distributed around the plasma membrane and the nucleus (Figure 2B). The mitochondria were usually associated with RER; their shape differed from circular to elongate (Figure 2B).

In the treated group, the hepatocytes showed changes after different durations of exposure. There were certain changes in the structures of some cell organelles, such as in the cristae of the mitochondria and in the appearance of some lysosomes (Figure 3).

The most prominent feature was the enlargement of lipid droplets (Figure 4 A, B). Necrosis of the cells started at 5 ppm (Figure 4B). Pyknosis of the nuclei and focal necrosis were observed more at 5 ppm and greater concentrations of temephos (Figure 4 A, B and 6 A, B). In addition, quite a number of lysosomes and cell death were also seen (Figure 5 and 6 A, B).

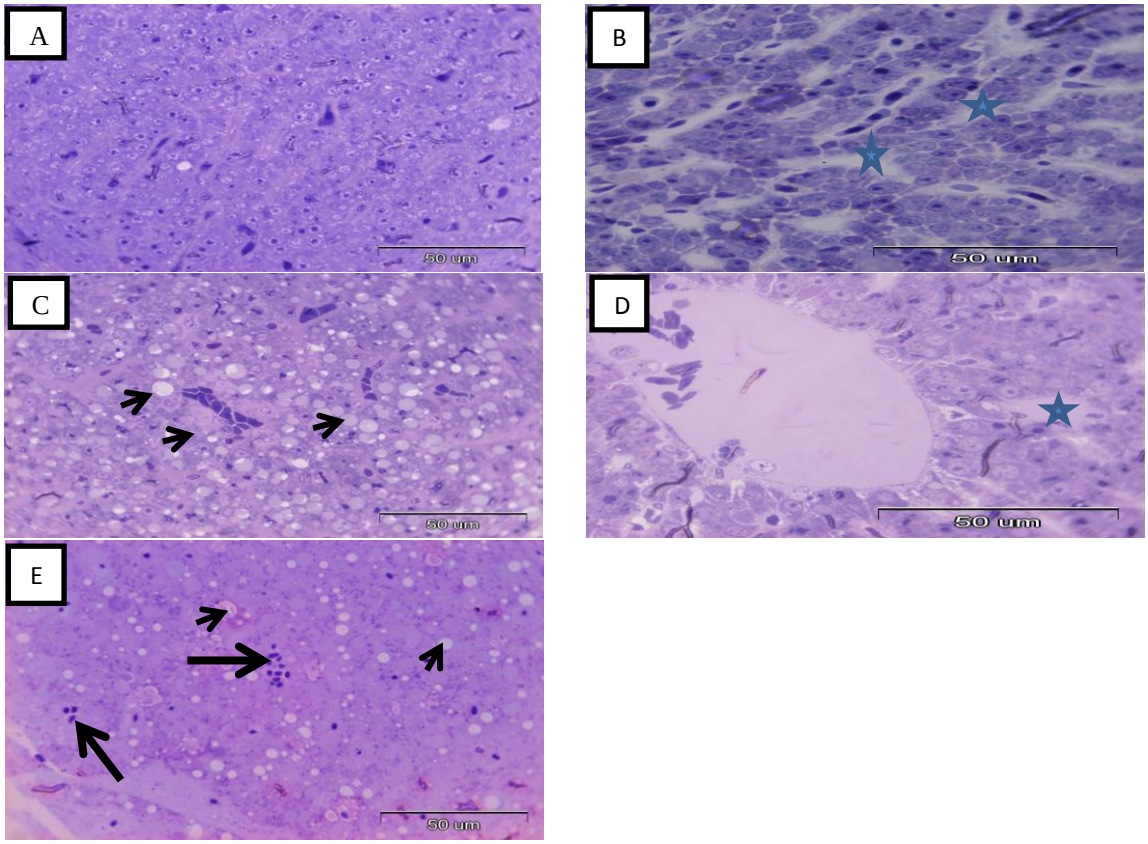
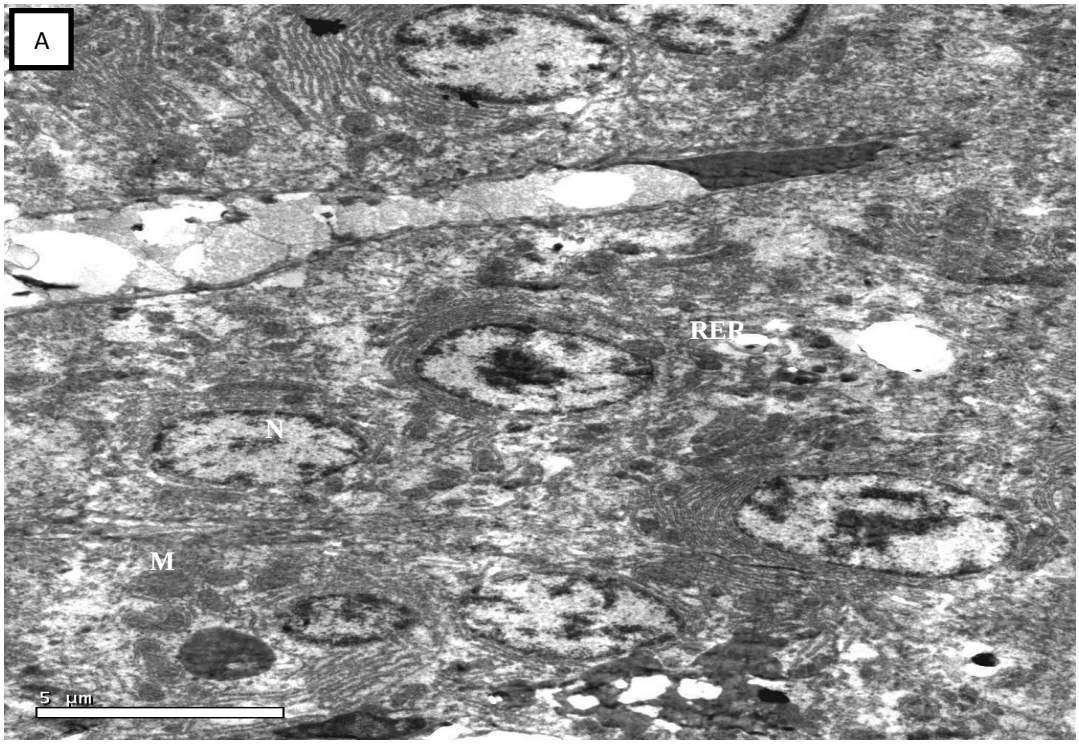


Figure 1. Light micrographs (A-E) showing the liver of the *A. dispar*: (A) control, (B) 1 ppm, (C) 5 ppm, (D) 10 ppm, and (E) 25 ppm, lipid droplets (small arrows), dilation of sinusoid (stars) and dying cells (large arrows).



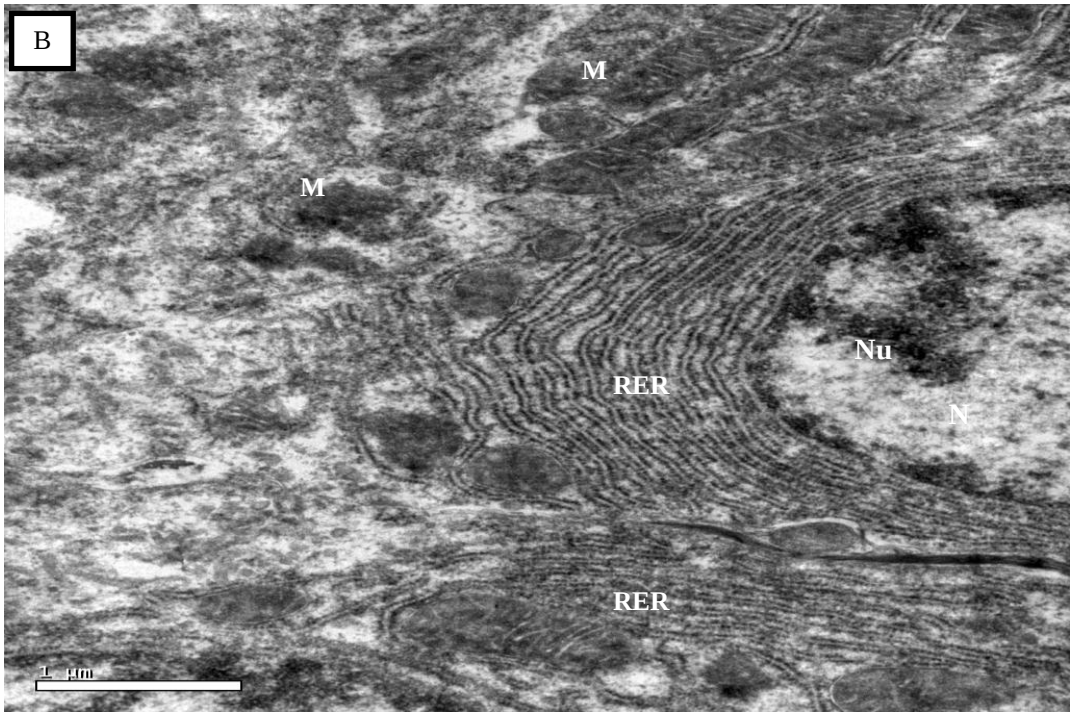


Figure 2. Electron micrographs (A & B) of *A. dispar* of control showing: normal structure of the liver, nucleus (N), endoplasmic reticulum (RER), nucleolus (Nu) and mitochondria (M).

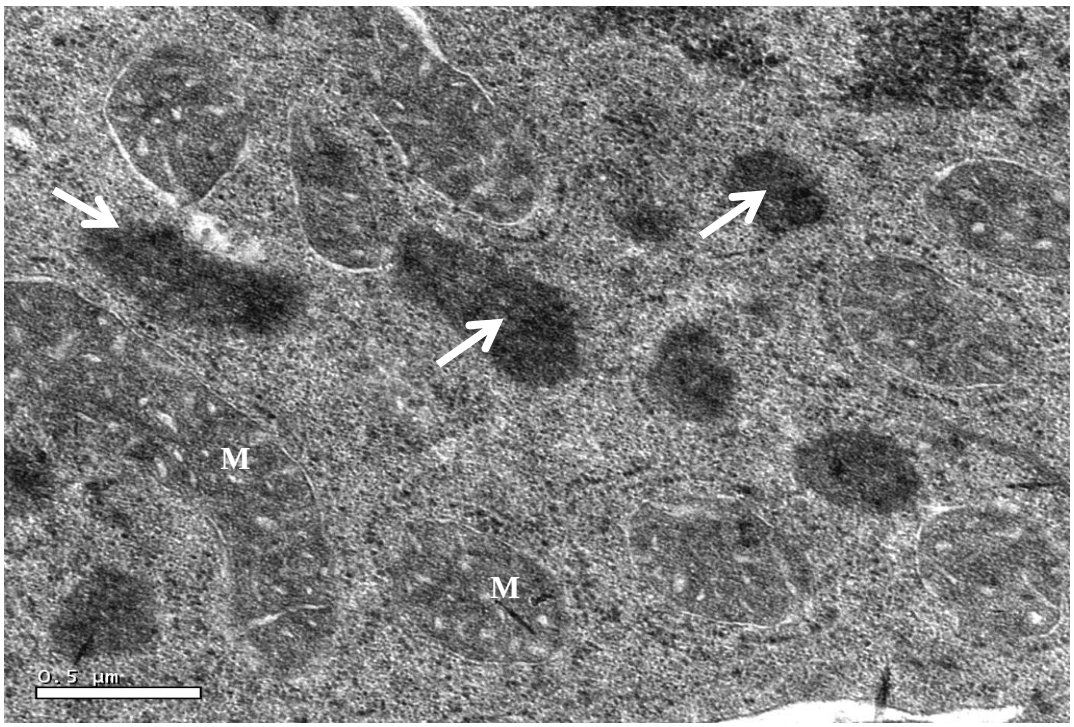


Figure 3. Electron micrograph of *A. dispar* exposed to 1 ppm showing: mitochondria some of which show certain changes in the cristae (M) and the presence of lysosomes (arrows).

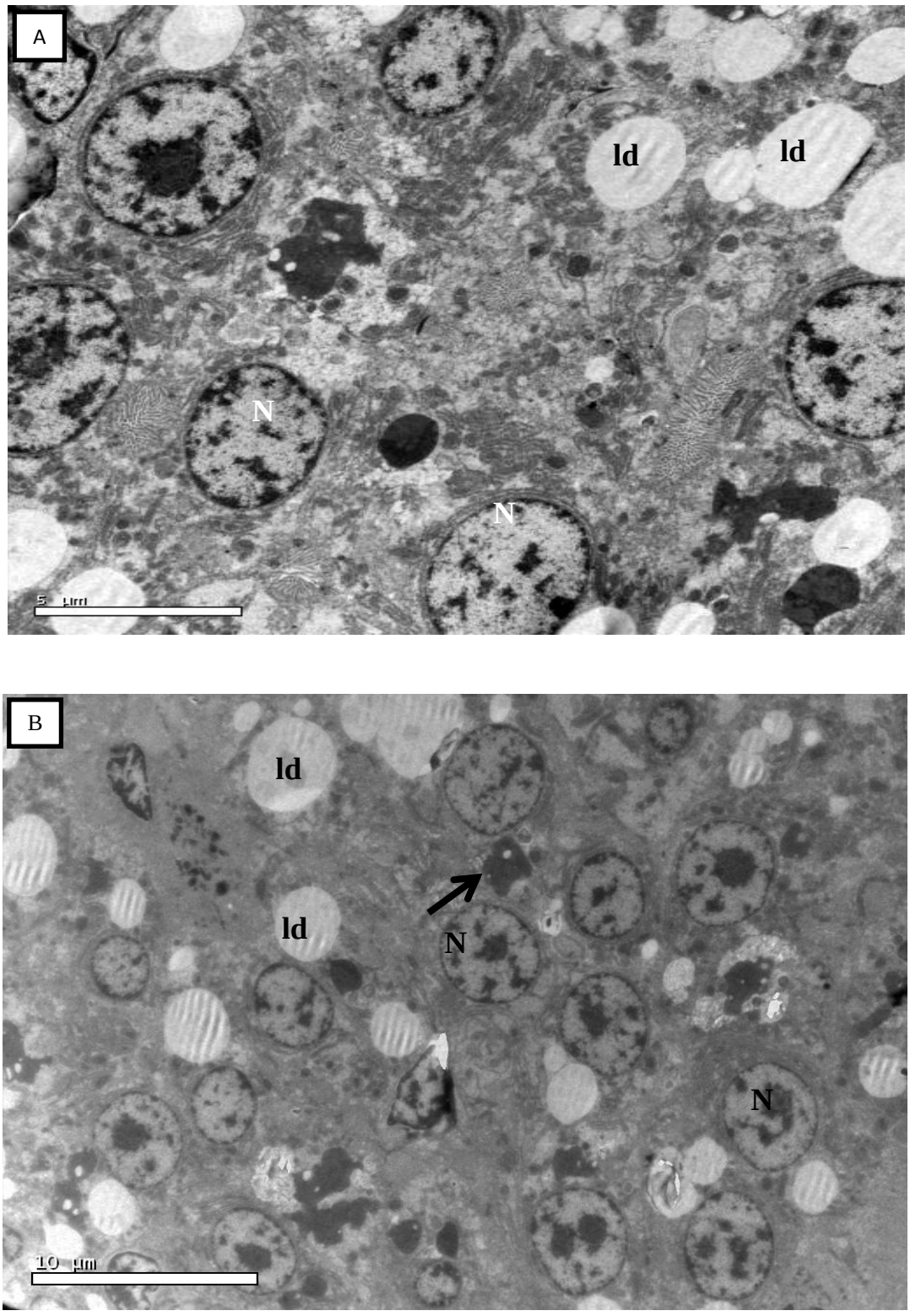


Figure 4. Electron micrographs (A & B) of *A. dispar* exposed to 5 ppm showing: nucleus (N) and lipid droplets (Ld) dying cells (arrows).

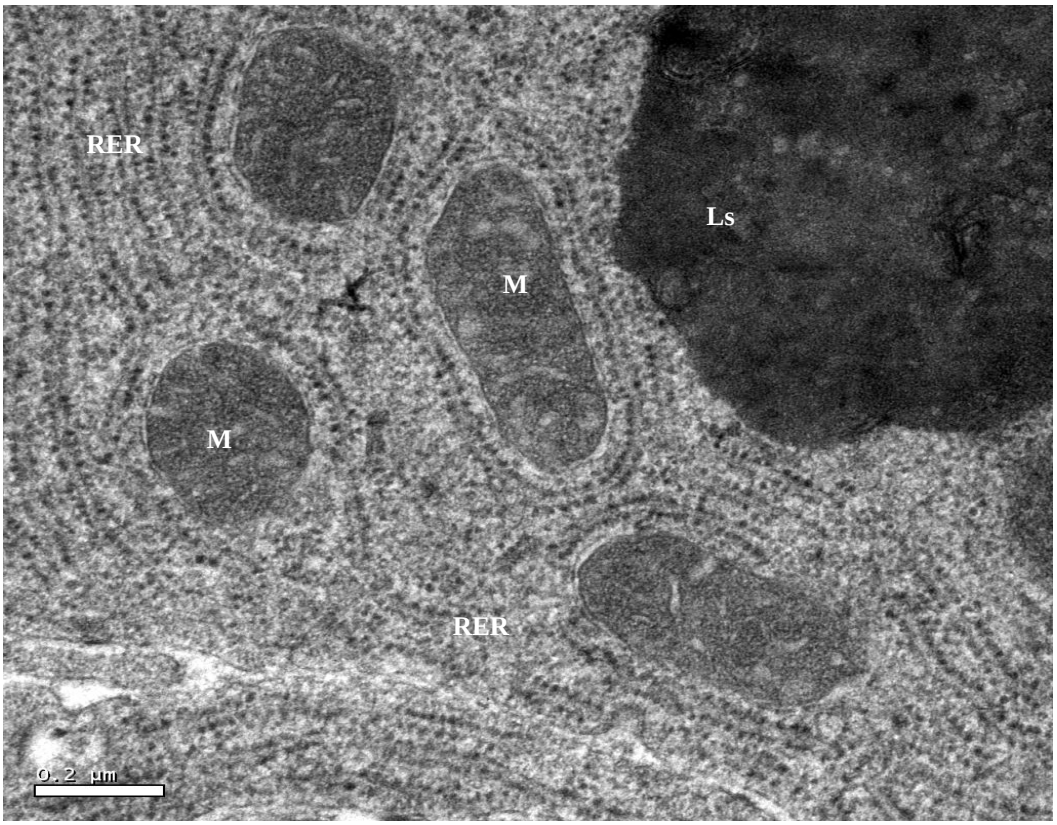
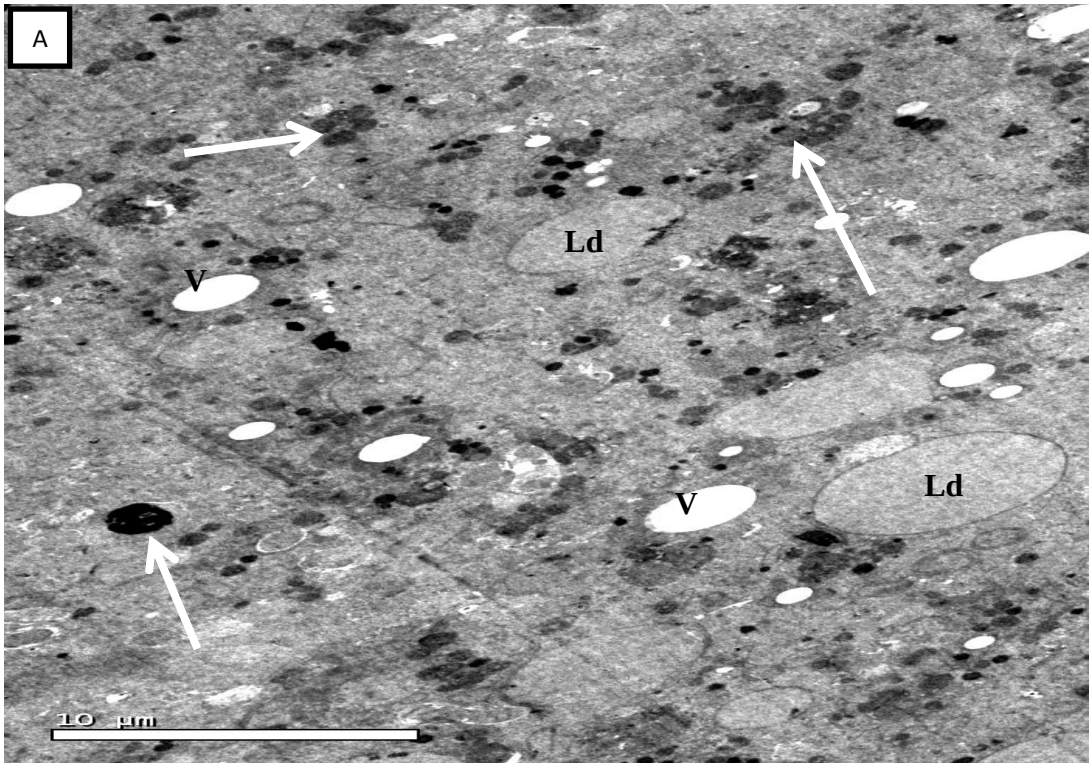


Figure 5. Electron micrograph of *A. dispar* exposed to 10 ppm showing: mitochondria (M), lysosomes (Ls) and rough endoplasmic reticulum (RER).



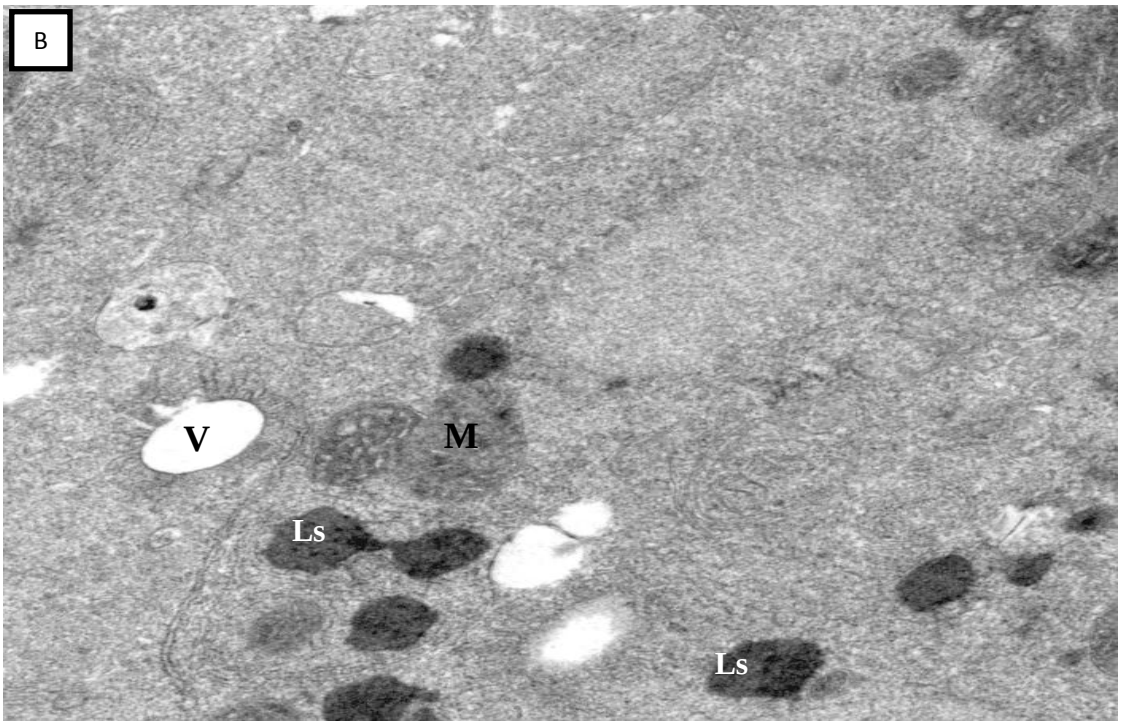


Figure 6. Electron micrographs (A & B) of *A. dispar* exposed to 25 ppm showing: mitochondria (M), lipid droplets (Ld), vacoules (V), lysosomes (Ls) and cell death (arrows).

4. Discussion

The liver is a vital organ of detoxification. The present study clearly elucidates the effects of temephos on the liver tissue of *A. dispar*. The alterations in the liver due to the impact of toxicity are often associated with a degenerative necrotic condition (Arellano *et al*., 1999; Olojo *et al*., 2005; Figueiredo-Fernandes *et al*., 2007). The histopathological changes of the liver following temephos exposure were similar to those reported by previous studies. Matthiessen and Roberts (1982) observed toxic necrosis, focal necrosis, and lipid accumulation in other species of fish exposed to the insecticide endosulfan**.** The changes induced by temephos in the hepatocytes of *A. dispar* have also been reported in *Puntius conchonius* exposed to carbaryl and dimethoate (Gill *et al.*, 1988), *Oreochromis niloticia Ham* exposed to waterborne copper (Figueiredo-Fernandes *et al.*, 2007), and *Parachanna punctatus* exposed to hexavalent chromium (Mishra and Mohanty, 2008). In general, the effects of pesticides like endosulfan, phosphamidon, aldicarp and deltamethrin, and of glyophosate herbicides on the liver of fish appear to be vacuolation of cytoplasm, nuclear pyknosis, sinusoid enlargement and focal necrosis (Gill *et al.*,1990; Cengiz *et al*., 2001, Jiraungkoorskul *et al.*, 2002; Cengiz and Unlu, 2006).

Our studies, including those on the histopathological changes of organ systems in *A. dispar* exposed to different pesticides (Ba-Omar *et al*., 2011; Al-Ghanbousi *et al*., 2012) lead us to believe that the pathological responses elicited are very similar irrespective of the pesticide used. Our review of literature leads us to believe that several unrelated species of freshwater fish also show similar responses when exposed to the same pesticide (Matthiessen and Roberts, 1982; Arellano *et al*., 1999; Olojo *et al*., 2005; Figueiredo-Fernandes *et al*., 2007).

As a follow-up to this study, we would attempt a cause-effect response summary listing pesticides, histopathological responses and fish species responding, and this could be of use in the risk evaluation of pesticides currently in use in Oman.

It is often argued that the experimental conditions do not realistically reflect the impacts of pesticide exposure in nature. However, there is a need to demonstrate the acute toxicity of the so called 'safe pesticides' like temephos and this could only be done easily under experimental conditions. The present study shows that histopathological changes occur in organs like the liver, far removed from the sites of direct contact during the application of temephos. This seriously contests the claim that temephos is non-systemic. This study also demonstrates that even at low levels, temephos induces pathology as seen in the liver. This finding again questions the claim that application of temephos in low concentration like 1 ppm is harmless to non-target organisms like fish. Our study exposed the fish to the pesticide only for a period of seven days and histopathological studies on fish subjected to the chronic exposure of temephos in field conditions are likely to provide further data on this 'safe pesticide'. Finally, from the view point of biodiversity conservation in Oman, temephos is an additional threat to the existence of *A. dispar*.

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