INTRODUCTION

Fish and aquatic animals are exposed to pesticides in three primary ways, dermally by direct absorption through the skin by swimming in pesticide contaminated water. Breathing by direct uptake of pesticides through the gills during respiration. Orally by drinking pesticide contaminated water or feeding on pesticide contaminated prey. Acephate is an organophosphate foliar spray insecticide of moderate persistence with residual systemic activity of about 10-15 days at the recommended use rate. It is used for control of a wide range of biting and sucking insects, especially aphids, including resistant species, in fruit, vegetables e.g. potatoes and sugar beets, vine, and hop cultivation and in horticulture e.g. on roses and chrysanthemums grown outdoors [1]. It also controls leaf miners, lepidopterous larvae, sawflies and thrips in the previously stated crops as well as turf, mint and forestry. It is considered non-phytotoxic on many crop plants [2].

Acephate is primary metabolite, methamidophos, are toxic to Heliothis spp. that are considered resistant to other organophosphate insecticides [3]. Acephate emits toxic fumes of phosphorus, nitrogen, and sulfur oxides when heated to decomposition. Symptoms of exposure to Acephate include a slight irritation of eyes and skin. Acephate comes in soluble powder, pressurized spray and granular formulations.

Objective of the study

Histopathological study of an organophosphorus pesticide Acephate on the ornamental fish Poecilia sphenops. From the above literature, it is evident that no elaborate works on the histopathological aspects of Acephate on fishes are available. Therefore, an attempt is made to study the effects of Acephate in Poecilia sphenops.

The study include includes

1. Assessment of LC50.
2. Animal behavior.
3. Histopathological changes in the organs such as intestine, liver, muscle, gills.

MATERIALS AND METHODS

Animal selection

Poecilia sphenops belongs to the family Poeciliidae of order Cyprinodontiformes. They are native to fresh, brackish and saltwater and some species in the genus are eurhaline. Poecilia sphenops is a species of fish, of the genus Poecilia, known under the common name molly, to distinguish it from its congeners, it is sometimes called short finned molly or common molly. The wild type fish are a dull silvery colour, often sprinkled black all over (fig.1).

Pesticide selection

The Acephate is taken as our study material.

Structure of Acephate

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Abstract

Toxic effects of Acephate in the tissue of Poecilia sphenops have been reported. The laboratory animal was treated with Acephate, the LC50 was found. The tissues were fixed in formalin for histopathological studies. The study revealed remarkable changes in the vital tissues like liver, intestine, muscle and skin. Hence this pesticide is recommended to be used in agriculture at low ratio for its toxic effect.

Key words: Acephate, Pesticides, Toxic effect, Poecilia sphenops.
**Appearance:** Colourless to white solid.

**Chemical Name:** O,S-dimethyl acetylphosphorimidothioate (IUPAC), N-[methoxy (methylthio)phosphinoyl] acetamide

**CAS Number:** 30560-19-1 () OHS Database.

**Molecular Weight:** 183.

**Water Solubility:** Readily soluble in water (79 g/100 ml at 20 degrees C). 650 g/l at 20 degrees C

**Solubility in Other Solvents:** In acetone 15.1, ethyl acetate 3.5, benzene 1.6, hexane 0.01 (all in g/100 ml at 20 degrees C) (Kidd, H. 199113) ethanol < 50 g/l at 20 degrees C.

**Melting Point:** 93 degrees C technical grade Acephate (purity 80 - 90%) is 82 - 89 degrees C. 72 - 80 degrees C (Spencer, E. Y. 1981.), 64 - 68 degrees C for impure.

**Vapour Pressure:** 2.3 x 10^-6 mbar at 24 degrees C, 0.226 mPa (24 degrees C)

**Assessment of LC50**
The assessment of toxicity was based upon the percentage mortality of fishes. In the toxicological study, the 72hrs LC50 bio-assay method involves placing groups of organisms (10 fishes per group) and the mortality rate was observed and recorded at time intervals of 24hrs, 48hrs and 72hrs. The concentration which produces 50% mortality at 72 hrs was taken as the LC50 value. The percentage mortality was converted into probit values and plotted against the log dose values.

**Experiment Design For Sub Lethal Study**
The sub lethal study was carried out by taking ten fishes in a tank. The organophosphate was weighted and mixed directly in water, in which the fishes were introduced and maintained.
Experimentation was carried out for duration of 10 days.

**General Observation**

The control and the experimental fishes were closely monitored for their,
1. General behavior.
2. The mortality of fishes was recorded.

All sign of ill health and reactions to the toxicant were observed and recorded under the control medium and also with the treated medium with Acephate. The experiment was done for a period of 10 days. The observations were recorded. Photos were taken during the period of study. After the exposure of insecticide for 10 days, the animals were sacrificed and the tissues were subjected to histopathological studies.

Histopathological examination of selected tissues was carried out on the tissue samples of the control and experimental groups of fishes, exposed to the sub lethal concentration of Acephate. Tissues were fixed in 10% neutral buffered formalin (10 times of its size). Thin sections of 5 u thickness were prepared, stained with haemotoxylin and eosin and observed under the light microscope.

**Histopathological studies**

Histopathological studies were carried out on control fishes and on experimental fishes, which were sacrificed after a period of 10 days. The gill, liver, muscle and intestine were removed and preserved separately in neutral buffered formalin. They were processed by the routine histological techniques as follows.
1. Fixation in neutral buffered formalin for 48hrs.
2. Tissue dehydrated by placing in graded series of alcohol (30%, 50%, 70%, 90% and 100%) for about 2hrs each.
3. Two changes in absolute alcohol, 12hrs each.
4. Clearing in xylene- two changes of 3 hours each.
5. Wash in water.
6. Blocks were prepared by embedding the tissues in paraffin wax.
7. Sections of 5u thickness were obtained using microtome.
8. Stripes of sections each containing four were mounted onto a glass slide.
9. Glass slide were placed in xylene to remove the wax.
10. Hydration was done by passing through graded series of alcohol (100%, 90%, 80%, 70%, 50% and 30%).
11. Washed in water.
12. Staining was done using haemotoxylin stain and counter stained with eosin and coverslip was placed using DPX and slides were made permanent and examined for histopathological changes.

**RESULTS**

**Assessment of LC$_{50}$**

The LC$_{50}$ of Acephate in the *Poecilia sphenops* was found to be 25 mg/lt. The LC$_{50}$ value was calculated by constructing the regression line, taking test doses and their corresponding mortalities in logarithmic values using Finney's probit analysis. The one tenth value of LC$_{50}$ that is 25mg/lt was choosen for sub lethal toxicity study.

**General behaviour**

The control groups of the fishes were very normal (Fig. 2) whereas, *Poecilia sphenops* treated with sub lethal dose of Acephate showed signs of intoxication characterized by irregular, erratic and sometimes jerky movements. The fish exhibited a peculiar behavior of trying to jump out from the treatment medium, to avoid the toxicant medium. The colour change in the skin from white to little yellow was observed in the treatment group exposed for 10 days. Bulging of the abdomen, haemorrhage in the operculum region, reddening of eyes and oozing in the anal region was noticed during the period of experimentation (Fig. 3).

**Body weight**

The body weight of the tissue recorded while the study indicates that the control fishes show significant increase in body weight whereas the treated fishes showing decrease in the body weight. (Table 1 and Graph 1)

<table>
<thead>
<tr>
<th>DAY</th>
<th>CONTROL FISH</th>
<th>TREATED FISH</th>
</tr>
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<tbody>
<tr>
<td>1st</td>
<td>2.30 gms</td>
<td>2.30 gms</td>
</tr>
<tr>
<td>5th</td>
<td>2.35 gms</td>
<td>2.16 gms</td>
</tr>
<tr>
<td>10th</td>
<td>2.40 gms</td>
<td>2.0 gms</td>
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**HISTOPATHOLOGY**

**Gill**

The control section of gill showed a normal histological appearance, (fig. 5). Whereas the gill section of *Poecilia sphenops* treated with sub lethal dosage of Acephate showed rupture of gill lamellae, hyperplasia, epithelial necrosis, enlargement of the secondary gill lamellae and distorted hemorrhage is seen in the gills (Fig.6).

**Muscle**

In the control fish, normal architecture of the muscle was seen (Fig.7). Muscle tissue of *Poecilia sphenops* exposed to Acephate for 10 days, showed intercellular space, rupture of cell wall, disruption of cells and the muscle bundles are separated. The vacuolation of cells are seen all over the focal area (Fig.8).

**Liver**

The section of liver showed normal architecture in the case of control group. (Fig.9). The section of liver tissue of *Poecilia sphenops* exposed to Acephate for 10 days, displayed haemorrhage, edema, necrosis, disruption of hepatocytes, distortion and damage in the focal area (Fig.10).

**Intestine**

The section of intestine showed normal architecture in the case of control group (Fig.11) whereas in the treated group the animal showed rupture in the lining cells and clotting in the mucous cells. Widening of cells is seen in the focal area (Fig.12).

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**Graph 1. Showing the difference in the body weight of both control and treated fish**

![Graph showing body weight difference](image1)

**Fig 5. Photomicrograph showing CS of Gill control stained in haematoxylin and eosin**

![Image of control gill](image2)

**Fig 6. Photomicrograph showing CS of Gill treated stained in haematoxylin and eosin**

![Image of treated gill](image3)
DISCUSSION

Histopathological studies have been carried out [4]. Histological examination of the tissues is an important part of toxicity studies of chemical substances. [5]. In the present study the histopathology of gill, liver, muscle and intestine were studied. Whatever be the cause, if the pesticide are allowed direct conduct with the human being or food articles, later could lead to various diseases [6]. Lifting of the basement membrane was observed. Extensive damage of the gill lamella was recorded complete dissociation of acidophilic cells was also observed. Changes may be due to the result of the chemical reaching the through the circulatory pathway and inflicting damage. Behavioural changes, such as erratic swimming may be due to the damage of gill lamellae. In the present study the gill section of Poecilia sphenops treated with sub lethal dosage of Acephate showed disruption of lamellae, hyperplasia, and epithelial necrosis, enlargement of
secondary lamellae and fusion of secondary lamellae. Edema in the gill epithelium is also seen. [7] and [8] also reported that the toxic substances causes damage to gill tissues, thereby reducing the oxygen consumption and disrupting the osmoregulatory function of aquatic organisms. Inflammatory changes, such as swelling, lifting of lamellar epithelium and hyperplasia have also been noted in a various species of fish following exposure to insecticides by [9]. The gills represent the print of closet proximity of the internal and external environments in terms of the diffusion distance from the water to the blood and large surface area exposed to the water. Consequently, histopathological changes in the respiratory epithelium of gill would ultimately affect the rate of oxygen uptake. Thus, direct contact of the respiratory surface area with polluted water may lead to its alteration as well as its diffusing capacity. Liver is an important organ actively involved in metabolic functions and is a frequent target for a number of toxicants. In toxicological studies, a proper evaluation of the function of the liver and kidney, a major detoxification and excretory organs that are exposed to foreign substances, are of prime importance. The section of tissue treated with Acephate for 10days, displayed vacuolation, disruption of hepatocyte and necrosis in focal area. Muscle tissue of *Poecilia sphenops* exposed to sub lethal dose of Acephate showed fragments of muscle bundles, inter cellular space, vacuolation, rupture of cell wall and disruption of cell. The doses of Acephate used in the present study are insignificant when compared to the doses recommended for crop application a fields [10]. The observations of the present study provides information about the hazards of Acephate on the aquatic organisms, whereby suggesting that it is harmful to humankind when it is used for long run. This study is a small piece of work which strongly says that Acephate is a harmful pesticide when used for long years. It residue make hazardous effects to the vital organs of fish, amphibians, mammals etc., hence use of this pesticide can be controlled to a maximum level to safe guard our eco system and provide this land a eco friendly land to the next generation.

**REFERENCES**