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Destructive effects of Edifenphos fungicide on *Rutilus rutilus caspicus* gill

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Abstract

Caspian Sea has the valuable reserves as the largest lake in the world. Changing in ecological conditions of Caspian sea caused by agricultural, industrial pollutions, and urban sewage,producing oil and gas at the beaches, and in farer depths from the beaches, the activity of marine transportation companies and other events has changed the environmental variety, pollution of water environments and consequently has been caused to reduce the fish reserves. In this research, it was used the baby *Rutilusrutiluscapicus* with average weight 2.25 ± 0.5 gr the word fish with an average weight of 0.5 ± 2.25 g and also it was used 5 cares for the experiment and 3 repeats for each care which the cares were included the testimonial care, 0/76ppm, 1/53ppm, 2/30ppm and 3/07ppm of the Edifenphos toxinwhich the fish were under the experimental cares for two weeks and at the end of the two weeks, it was sampled from the fish and were observed by light microscope and also the damageswere reported descriptively. tissue damages such as Lamellas fracture, being mallet (being hammer), adhering the secondary lamellastogether, adhering the lamellas, epithelial lifting, shortening lamellas were observed that thee damages were increasedwith increasing the pollutant dose of theobserved damages, too.

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Introduction

Caspian Sea has valuable reserves as the largest lake in the world which it has been consideredto provide food, occupation and income by governments and beach dwellers from old times.Changing in ecological conditions of Caspian sea caused by agricultural, industrial pollutions, and urban sewage, producing oil and gas at thebeaches, and in farer depths from the beaches, the activity of marine transportation companies and other events changes the environmental variety, and reduces the fish reserves. Chemical pesticides are well identifiedas an economic approach to control pests, at thesame time such chemicals are highly toxic toother species in the environment. Most of pesticides find their way into rivers ,lakes and pond, and have been found to be highlytoxic not only to fishes but also to the organismswhich contribute to the food chain of fishes (Anees, 1975).

Laboratorial and field studies show the potential danger of toxins in aquatic ecosystems. A lot of fish toxicity studies; including histopathological and clinicopathological studies; were directed upon organophosphate pesticides. For example (Jauch, 1980) confirmed gill pathology in Hero *tilapiamultispinosa* and *Tilapia leucostica* ranging from hyperplasia and separation of the respiratory epithelium in the secondary lamellae with congestion and numerous telangiectasis, while studying the toxic effects of *Lebaycid R* on fish gills. Information driven from toxicology experiments in eco-toxicology science, show the effects of these toxins on the fish. The pesticides that are used in agriculture, each influenced on certain creatures and causes to destroy them, one of these pesticides are fungicides. fungicides are groups of chemical which are used against the fungithat causes the disease in plants to prevent and or fight with them to reduce the living creature damages on agricultural products. (Hamelink and stegeman, 1991)Toxins, in densitiesthat are not killer, can cause other biological disorders likesterilization, reduction in reproduction, lack of enough growth in creatures, or generating unhealthy generations through which extinct the animal generations. Survival of pesticides in water depends on the chemical structure of pesticides and environmental conditions. Studies have shown that the pesticides have completely different stability ,as the organochlorine have more stability than the organophosphates (Beheshti*et al.*, 2010). Potentially harmful substances-e.g. pesticides, heavy metals and hydrocarbonsare often released into the aquatic environment. When large quantities of pollutants are released there may be an immediate impact as measured by large-scale sudden mortalities of aquatic organisms, e.g. fish kills resulting from contamination of waterways with agricultural pesticides. Lower levels of discharge may result in an accumulation of the pollutants in aquatic organisms. The end results, which may occur long after the pollutants have passed through the environment, include immunosuppression, reduced metabolism, and damage to gills and epithelia.

When we survey the side effects of pesticides on the environment, we must know that by spraying the agricultural products actually three types hydrosphere, troposphere, and atmosphere, can be polluted in contact with the toxins (Filazi, 2003). Fish are the most important aquatic creatures who have special importance due to economic value and sensitivity against pollutants and so they areused extensively to do the biological-measuring experiments (Velmurugan, 2009). *Rutilusrutiluscapicus* is considered as One of the most important and the most valuable economic fish in south area of the Caspian Sea because its reserves amount has been reduced for various reasons like overfishing, different pollutions (industrial, agricultural, heavy metals, etc.) in recent years .The laboratorial data are used to evaluate the effects potential of pesticides on the environment and also such experiments should be done to limit the use of toxic materials and the probability of surveying the existence of pollutants.

few studies are conducted using edifenphos, (El-Gendy*et al*,1996) stated the depressive effect of edifenphos on the activities of acetyl cholinesterase

(AChE), adenosine triphosphates (ATPase) and glutathione-S-transferals (GST) with the elevation of catalase activity in tissues of O. niloticus. Also (Aly,1996) found the same results in addition to decrease of serum protein and reduced response of splenocytes to mitogens. In the same year (Ramadhan, 1996) investigated the probable genotoxic effects of edifenphos on common carp fish and found a significant changes in the relative proportions of protein fractions in the electrophoretic patterns of hepatopancreas and brain esterase isozyme of the fish.So, the motivation and aim of experiments to evaluate the pollutant toxicity, is to achieve reliable criteria for the protection of aquatic resources. In this research, the effects of Edifenphos toxin on histopathology of *Rutilusrutiluscaspicus*gill *(Rutilusrutiluscaspicus*) has been surveyed as one of the most important economic and ecological fish of the zone.

Material and methods

The *Rutilusrutiluscaspicus*with an average weight of 2.25±0.5gr was supplied from the reproduction center of *Rutilusrutiluscaspicus* in Syjual and they were kept for adjustment for 1 week in 200-liters. In this experiment, it was used 15 aquariums in volumes of 20 liters and also it was used the Edifenphos fungicide with EC 50%, which was produced byresearch alchemy company of Kerman. And empirical formula: $C_{14}H_{15}O_2PS_2$.The material shall consist of edifenphos together with related manufacturing impurities and shall be a light to dark yellow liquid free from visible extraneous matter and added modifying agents. The organophosphate fungicide edifenphos (Hinosan) is cutinase inhibitors and displays a specific antipenetrant action, but in practice its therapeutic activity may also involve direct fungitoxicity (Sisler, 1986). As organophosphate pesticide, it causes irreversible inhibition of the cholinesterase enzymes (Haddad and Winchester, 1983).To do this experiment, it was used 5 cases and 3 repeat for each case which the cases were included the testimonial case, 0/76ppm, 1/53ppm, 2/30ppm and 3/07ppm of the Edifenphos toxin which and it was placed 20aquariums in each case. During the experiments, physical and chemical parameters of water were kept almost constant. Water temperature 20-22 ° C, the solution oxygen 2/7-8 mg per liter, pH between 7/6-8/5 and the optical was period 12 hours dark, 12 hours light. During the adjustment period with the fish conditions in24 hours, was given two business meals. Also, 48 hours before the test fish were not fed and during the experimental period, the fish were not feeding. It was sampled from the fish at the end of the two weeks as at first the fish were stupefied by clove powder and next it was got a tissue from the second left gill bow of the red fish and then it was fixed in 10% formalin and it was colored in haematoxylin- eosin Method and finally the lams were observed by optical microscope.

Results

Based on the obtained results, the type and theamount of the damages in different cases are various.

Mucus

mucus secretion amount increased with increasing the Endosulfan pesticide. As in case 1, we had the lowest amount of mucus and it was secreted average mucus in cases 2 and 3, But the most mucus secretion amount was observed in case 4 that means the case was with the most amount of Endosulfanpesticide. the adhesion of the secondary blades was observed in all experimental cases and it was increased with increasing the dose of cadmium. The adhesion of secondary blades also increased as mucus with increasing the amount of Endosulfan pesticide In water, as we had the lowest amount in case 1 and the average amount in cases 2 and 3, and the highest amount of secretion damage of the secondary blades in case 4.

Hyperplasia

Being mallet (hyperplasia) was observed in all cases with Endosulfanpesticide which the amount of the damage was observed equally in cases 1 and in case 2 and also in case 3, as there was the damage in all three cases in little amount and the amount of being

mallet in case 4 was more than two other cases, but not so much. Being mallet of the gills expands less rapidly and intensely than increasing mucus and adhesion of the secondary blades.

Epithelial lifting

Rising the coating tissue (Epithelial lifting) was observed in all cases except in case 1. The Epithelial lifting in case 1 was not observed and the damage amount was less in cases 2 and 3 and it was observed average in case 4.

Table 5. Found Complications in the gill of Rutilusrutiluscaspicus exposed to different doses of Endosulfan pesticide.

Damage	Density of Endosulfan Pesticide (ppm)				
	0	0.76	1.53	2.30	3.07
Mucus	–	+	++	++	+++
Lamella Fracture	–	++	+	++	++
Being mallet (to hammer)	–	+	+	+	++
The adhesion of secondary lamellas	–	+	++	++	+++
The adhesion of lamellas	–	++	++	++	+++
Epithelial lifting	–	–	+	+	++
Shortening lamella	–	–	+	++	+++

No phenomenon observed (-),low(+),average(+ +), high (+ + +).

Epithelial hypertrophy

Inflation of cobblestone cells (Epithelial hypertrophy): was observed in all cases of Endosulfan pesticide in average, except in case 2 which there was less amount of this phenomenon.

Lamella

Shortening of Lamellas were not observed in case 1, but the shortening of the lamellas were also increased by increasing doses of Endosulfan pesticide in the water,as it was observed the highest damages in case 4.

Discussion

Based on the obtained results, the type and the amount of the damages in different cases are various.mucus secretion amount increased with increasing the Endosulfan pesticide. mucus secretion is one of the defending ways of the different spices of fish against environmental pollutions like agricultural pesticides, heavy metals and etc.In this performance mucus can helps regulate body functions, including protection against external parasites. Also, some studies showed that mucus will help the fish swim around faster, slowing water movement around the fish.As a result of exposure to the toxin causes mucus to be produced for defense performance against the conditions are unsuitable. Whatever More Inappropriate conditions, mucus production is increased.Concerning the fishes, the exposure of Edifenphos skin and gill membranes of fishes, Cause in response produce copious amounts of mucus to protect tissues.If disease organisms and pollution condition are present on the gills and skin, the mucus produced engulfs the organisms. When the mucus is sloughed off, the disease organisms are lost as well. Under high dosages or prolonged treatment with such medications, a loss of fish livestock results from direct uptake of medicants and mucoid production so great as to impede gaseous exchange by the gills and skin.

Gills are the most sensitivebody against the environmental pollutants (agricultural pesticides, heavy metals, oil, etc.) (Shahsavani, 2007).The gills, which be involved in many important functions in the fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment and particularly sensitive to changes in the quality of the water are considered the primary target of the contaminants and toxins (Fernandes*et al.*, 2003, Camargo and Martinez, 2007).Being

sensitive of the gills against pollutions can be due to the metabolic activity of most of them than muscular tissues (Najiet *al.*, 2006).Moreover, gills are important not only for gaseous exchange but also for osmoregulation and excretion of toxic waste products (Robert, 2001), thus any harm in the gills leads to impairment of such vital functions revealing respiratory distress, impaired osmoregulation and retention of toxic wastes. Hyperplasia may in some situations represent an adaptation by the organism to protect underlying tissues from any irritant. However, increased thickness of the epithelial layers including mucous cell hyperplasia and fusion of adjacent secondary lamellae as the result of hyperplasia will not only decrease the surface area available for oxygen extraction but also will increase the oxygen diffusion distance between water and blood (Kumaraguruet *al.*, 1982).

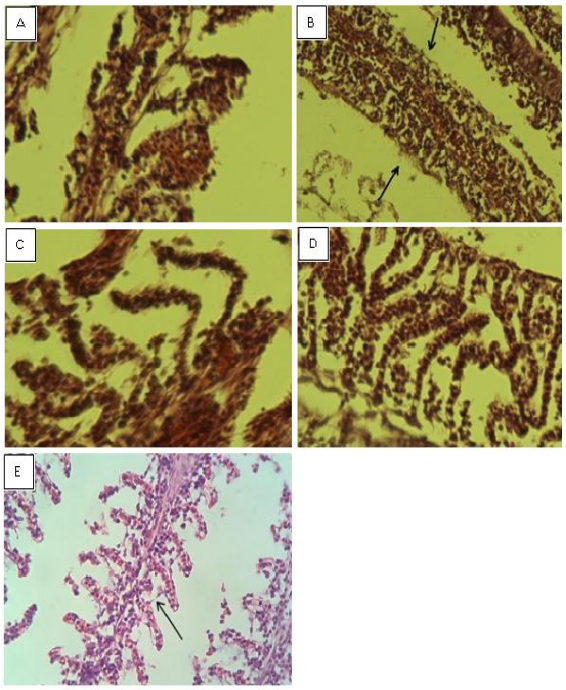

Fig. 1. A) being mallet, B) secondary lamellar fusion, C) lamellar fusion, D) secondary lamella Adhesion , E) epithelial lifting.

In Pond fish *(Carassiusauratus*) in cases with pheny-Tueidinsodium reported shows that mucus secretion is probably one of the first reactions of the fish to the presence of heavy metals or other pollutions in the aquatic environment.Shahsavani and co-workers examined the effect of phenytoin sodium on the pond fish (*Carassiusauratus*) in 2007,and they referred to the presence of blades adhesion damage in their observations. Also, Velmurugan and his co-workers reported the presence of lamellas adhesion in fish Cirrhinusmrigala against densities of lethal organo-phosphate, Dichlorvos, for 10 days ,in 2009.

the Results of the present research confirm Shahsavani and colleagues' research results(2007), Najiand co-workers(2006), Atabatiand co-workers (2009), and ghovati and co-workers(2011),Yildrim*et al,* 2006.Atabatiand his co-workers examined the different effects of zinc and copper metals on the fish gill tissue Cyprinuscarpioand they observedthe Hyperplasiain fish gill which increasing in hyperplasia amount has been increased whish were consistent with the obtained findings and also with Shahsavany and his co-workers research results In 2006, Velmurugan and his co-workers in 2009, Mishra and co-workers in 2008, Velmuruganet, *al.* in 2007 and Congas in 2006.It can be said that the intensity of the Epithelial lifting phenomenon increasesin higher doses. of the Epithelial lifting phenomenon generates due to some pesticides in water (Olurinet *al.*, 2006). Mishra and her co-workers in 2008,Velmuruganand his co-workers in 2007 was observed in fish tissue under their cases Epithelial lifting.

Also exposure to pollutants, including pesticides can cause rupture of the retaining pillar, or pilaster cells, which normally join the dorsal surface of secondary lamellae to the ventral one. The result will be dilation of the lamellar capillary and pooling of the blood, thrombosis and eventually fibrosis. Fusion with adjacent lamella, leads to the telangiectasis which is a characteristic pathological change of the gill associated with physical or chemical trauma (Robert, 2001). Pollution of the aquatic environment is a serious growing problem. Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment led to various deleterious effects on the aquatic organisms (McGlashan and Hughies, 2001). Aquatic organisms, including fish, accumulate pollutants directly from contaminated water and indirectly via

the food chain (Sasaki *et al*., 1997). Pathologic changes have been reported in the gills of many fish as a result of exposure to different toxicants (Olurin*et al*., 2006,Triebskorn*et al*., 2008). These results supports that edifenphos has the same nervous toxic effect of its chemical group the organophosphate as described by Jauch (1980) and Joshi and Desai (1981) with unique anemic action leading to cachexia with prominent paleness of whole body surface as observed by El-Aulaimi*et al.* (1994) while investigating imethoate.

In general, according to the results fromthis experiment can say since the *Rutilusrutiluscaspicus* fish is one of the economic and valuable fish of the Caspian sea and the rivers around the Caspian sea are considered the reproduction region of the fish, it seems that based on the recent information about the pollution rate of Endosulfan in this areas in near future reproduces and survival of the fish faces with serious threat. Therefore, by making the new ways of controlling the environmental pollutants for constant development, actually rises the return rate of the fish to the river and change these places into healthy and safe environment for reproduction, so finally the fish reserves are protected greatly.

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