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STUDY ON WATER TEMPERATURE EFFECT IN EXPERIMENTAL INTOXICATIONS WITH TALSTAR ONE INSECTICIDE ON PERCH

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Abstract
This paper aims was to investigate the changes of some important physiological indices (energy metabolism, respiratory rate, number of red blood cells, blood glucose level, survival) for perch (Perca fluviatilis L.), exposed to the action of Talstar One insecticide under different temperature conditions (18-20ºC and 6-8°C). Talstar One insecticide had a similar effect on the oxygen consumption, translated into a stimulating phase, particularly for samples adapted to room temperature, usually in inverse proportion to the concentration, followed by a slower reduction period or stabilization of the physiological indices. The average number of red blood cells decreased after 14 days from the fish immersion in insecticide. The fish adapted to temperatures of 6-8°C recorded significant decreases in the number of red blood cells and increases in blood glucose levels. Talstar One increased the blood glucose level after two weeks of exposure. The insecticide had a higher toxicity at low temperature.

*Keywords: erythrocytes, glycaemia, insecticide, oxygen consumption, perch, respiratory rate, survival, Talstar One***.**

1. INTRODUCTION

Insecticides are most often associated with environmental degradation. Their purpose is to kill insects and, consequently, may have sublethal and lethal effects on non-target organisms. The characteristic of these pesticides is their increased affinity for adipose tissue and high stability.

Talstar One (7.9% active bifenthrin) is part of pyrethroid pesticides. It is an insecticide-acaricide, with a wide spectrum of control for vineyards, fruit trees, vegetables and ornamental plants. Bifenthrin, [2-methylbiphenyl-3-ylmethyl] (Z)-(1RS, 3RS) -3- (2-chloro-3,3,3-trifluoroprop-1-enyl) -2,2-dimethylcyclopropanecarboxylate] is a contact insecticide which is part of the third generation of pyrethroid insecticides characterized by a strong persistence in the environment and intense insecticidal action (Mokry and Hoagland, 1989). Similar to most pyrethroid pesticides, bifenthrin affects the central and peripheral nervous system of insects, causing paralysis (Miller and Salgado, 1985).

Bifenthrin is a type I-non-cyano pyrethroid (Shan et al., 1997) that acts on the central and peripheral nervous system of invertebrates and vertebrates, Na channels at the level of the nerve endings which it closes and opens, finally causing depolarization of presynaptic membranes and cell death (Lund and Narahashi, 1981; Hayes 1994). It also inhibits the production of ATPase (Clark and Matsumura, 1982; Roberts and Hutson, 1999), which explains the much stronger effect

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of bifenthrin on aquatic organisms compared to terrestrial ones (maintaining the critical level of ion concentration against the concentration gradient in the much diluted aquatic environment requires intensive ionic transport processes, the required energy being supplied by ATPase; the decrease in production of this enzyme leads to the death of organisms).

Due to their extremely strong lipophilicity, pyrethroid insecticides have rapid access to the bronchial epithelium, reducing accessibility of oxygen to internal organ tissues (Evans, 1987). Increased sensitivity of fish to bifenthrin can also be explained by its slower metabolism and elimination (Bradbury and Coats, 1989b). Siegfried (1993) explains the strong toxic effect of bifenthrin on the aquatic organisms by deionizing a large area (gills) after the decrease of ATPase.

Specialized studies indicates LC_{50} value in fish less than 30 µg bifenthrin/l water (Dobsikova et al., 2006; Velisek et al., 2006, 2007). The reported values are quite different: Velisek et al. (2009) found a value of 57.5 µg l^{-} for carp (*Cyprinus carpio L.*), while Liu et al. (2005) found a value of 2.08 µg l^{-1}.

The thermal factor is one of the most important eco-factors to which living organisms have to adapt. Most physiological processes of poikiloterm organisms change when changing temperature of the environment; in general, the rate of physiological processes increases with increasing temperature to a maximum, then decreases faster or slower (Precht et al., 1973). Among the species belonging to Percidae family, the perch - *Perca fluviatilis* was identified as a potential candidate for aquaculture (Kestemont and Dabrowski, 1996). The effect of various pesticides on this species became topical.

2. MATERIALS AND METHODS

Determinations were performed on samples of perch (*Perca fluviatilis*) caught in the lakes bordering Pitesti. Fish were acclimatized for 2 weeks before the completion of experiments in aquariums with a capacity of 100 l, under conditions of natural photoperiods, a period in which they were fed once a day (*ad libitum*), at around 10 am. In the case of experimental variations, acclimatization was done in a refrigerator (6-8°C) in aquariums with a capacity of 50 l under conditions of artificial illumination (10 hours per day).

After acclimatization in the laboratory, fish were separated into lots and placed in the experiments. Variant I - includes two lots of 10 fish (average weight 32 ± 1.57 g) subjected to Talstar One insecticide at a temperature of 18-20°C in concentrations of 0.625, and 1.25 µl / l water (respectively 0.05 şi 0.1 µl bifentrin/l) and the control lot. Variant II - includes experiments in the cold (6-8°C) and consists of two lots of 10 fish (average weight 34 ± 0.62 g) subjected to insecticide in a same concentrations and the control lot. The introduction of fish in solutions was done after their mixing and aeration for 5 minutes.

The concentrations used were determined after investigations of the relevant literature works, taking into account the insecticide concentrations that can be accidentally produced in water and preliminary tests of survival.

The testing method was systematic with refreshing solution at 24 hours after the calculations of the day, in aquariums of 100 l (50 l, respectively) for each experimental lot.

There have been made determinations of oxygen consumption and frequency of respiratory movements at intervals of 24, 48, 72, 96, 168 and 336 hours on all samples of these lots (depending on survival). After two weeks of exposure to the insecticide, samples of 0.625 µl insecticide/l apă were sacrificed to achieve intakes of blood necessary to haematological calculations (glycaemia and number of erythrocytes).

Determination of oxygen consumption was done by means of the oximeter and Winkler method, glycaemia was determined using the glucometer and erythrocytes were counted with Thoma

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chamber, using a small amount of blood from the caudal artery on the optic microscope (Picoş and Năstăsescu, 1988; Şerban et al, 1993).
For all experimental batches, survival was monitored daily, the dead specimens being periodically removed. Values are given as arithmetic means ± standard error of the mean (SEM). The data was statistically analyzed using multiple comparison tests (LSD - SPSS / PC program version 10.0 for Windows).

3. RESULTS AND DISCUSSIONS

A poikiloterm organism passage from higher to lower temperature results in a decrease of its oxygen consumption according to the so-called "Krogh curve" as a consequence of the direct kinetic effect of the new temperature on the reactions involved; after stabilization, which can take hours or days (Kinne, 1964, quoted by Marinescu, 2000), the oxygen consumption reaches the value corresponding to the new temperature.
Perch acclimatization to low temperatures (6-8°C) includes a series of changes: decreased oxygen consumption (by 19.29% compared to the values in control group L_m), decresed breathing rate (by 36.99% compared to L_m), decreased average number of erythrocytes (by 13.79% compared to L_m) and increased plasma glucose (by 15.7% compared to L_m) - the values were recorded two weeks after the experiment.
Decreased number of erythrocytes in fish exposed to low temperatures has been reported by numerous researchers (Frankel et al., 1966, De Wilde and Houston, 1967, Houston and De Wilde 1968, Huggurgs and De Wilde 1969, Cameron, 1970 Precht et al., 1973).
Decreases in the metabolic rate as a result of the temperature decrease were also found by Prosser (1973) quoted by Marinescu, 2000 - to the so-called "thermal shocks". Brenda Moffit and Larry Crawshaw (1983) - quoted by Marinescu, 2000, also reported a decrease in the metabolism and frequency of breathing movements in carp under conditions of gradual decrease in temperature.
Increases of plasma glucose levels in fish as a result of lowering the acclimatization temperature have been reported by a number of researchers, including Kuo and Hsieh (2006) in *Chanos chanos* and *Ctenopharyngodon idella* species from 25 to 15°C and Staurnes (2001) in *Scophthalmus maximus* species from 8 to 1°C.
At the temperature of 18-20°C in the experimental variants, the metabolic values at the end of the test were significantly lower compared to control values (more than 30% lower in the perches exposed to 0.00125 ml/l Talstar One concentration). The perches exposed to a concentration of 0.000625 ml/l Talstar One (Figure 1) had an interesting evolution. A strong metabolic stimulation occurred 72 hours after exposure followed by a sharp decrease of this index, eventually reaching an average metabolism, 44.71% lower than control group. This value was lower than the one recorded after the same exposure period to a double amount of insecticide.
The metabolic effect in variants performed at low temperatures was stimulating in the first phase (24 hours after exposure) followed by high decrease of the average metabolic values (by 20.8% and 23.28% after 14 days, compared to the average control values).
Changes in oxygen consumption recorded in experimental variants can also be explained by histological changes in the gills resulting from the toxic action of insecticides in the same group as Talstar One which affect their ability to take oxygen from water (Gill et al., 1988). Cengiz (2006) found histopathological changes in carp gills following acute exposure to deltamethrin in concentrations of 0.029 and 0.041 mg l^{-1} (exfoliation, necrosis, edema, hyperplasia, fusion of secondary lamellae, etc.). Similar changes caused by the action of deltamethrin have been identified by Cengiz and Unlu (2006) in *Gambusia affinis*. Costin et al. (2007) found morphological changes

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of gills in crucian carp exposed to 2 µg deltamethrin/l water (pyrethroid insecticide) 48 hours after exposure to toxic. These changes were more prominent after 14 days of exposure (the longest interval tested); the author reported hyperaemia, fusion of secondary lamellae, epithelial layer damage and chlorogenic cell proliferation. Fish poisoned with pyrethroid products suffered from vital organ hypoxia (Sastry and Shukla, 1994).

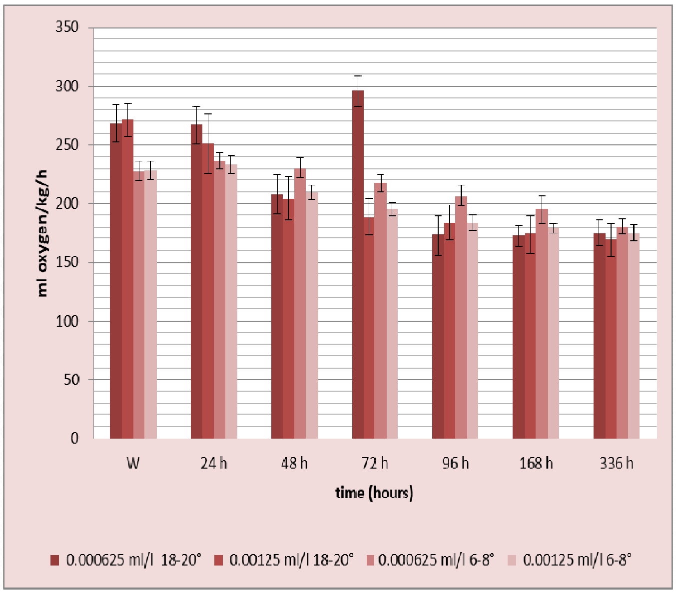

Figure 1. Variation in the average oxygen consumption and standard deviation to Talstar One intoxicated perches at two thermal levels

The breathing rhythm of perches poisoned with Talstar One recorded a slight stimulation in the first 24 hours, except for the cold version in a concentration of 0.00125 ml insecticide/l followed by stabilization of this index 72 hours after exposure (Figure 2).

Decreased breathing rate may be the result of gill disorders. Decreased oxygen uptake was also found by Bradbury et al.(1986) in the rainbow trout exposed to the action of fenvalerate and cypermethrin. A similar phenomenon was observed by Mushigeri and David (2002) in *Cirrhinus mrigal* and *Labeo rohita* exposed to fenvalerate. After 14 days of perch exposure to the action of Talstar One in a concentration of 0.000625 ml / l at 18-20°C, the number of erythrocytes decreased by 17.51% compared to the control; in the low temperature version the decrease of this index was lower compared to the previous variant (by 8.85% compared to the control value).

The decreased number of carp erythrocytes after poisoning with pyrethroid insecticides (permethrin and cypermethrin) was also found by Svobodova et al. (2003), Doruncu and Girgin (2001) and was due to hematopoiesis dysfunction. However, exposure to Talstar EC 10 in a concentration of 57.5 µg l^{-1} had no effect on the number of erythrocytes in common carp (*Cyprinus carpio*), the values being not significantly different compared to the control group (Velisek et al., 2009).

After two weeks of immersion in the insecticide at room temperature, blood glucose levels increased significantly by 21.01% compared to control values); significant increases in blood glucose also occured in low temperature variants, 29.59% more than the average value in the control group). Increases in the amount of energy associated with lactate production were reported in fish poisoned with pyrethroids (Rojik şi colab., 1983).

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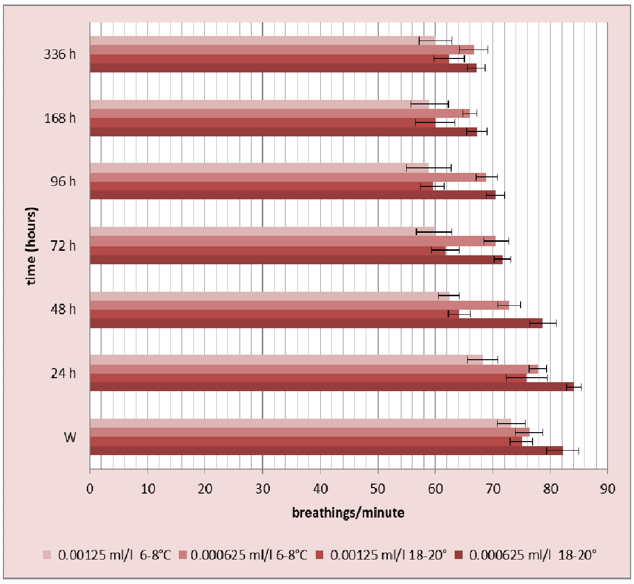

Figura 2. Variation in breathing rhythm and standard deviation to Talstar One intoxicated perches at two thermal levels

Velisek et al. (2009) examined the biochemical profile of carp (*Cyprinus carpio L.*) 96 hours after exposure to bifenthrin in a concentration of 57.5 μg l^{-1}, and found significant increases in glucose levels. Bálint et al. (1995) observed an increase in carp blood glucose levels after exposure to deltamethrin. Increased blood glucose was also recorded in trout and crucian carp - Velisek et al., (2009) in response to metabolic stress caused by Talstar 10 EC insecticide.
Increased blood glucose in fish was due to their exposure to cypermethrin (pyrethroid insecticide) as reported by Jee et al. (2005) in *Sebastes schlegeli* species. Researchers also reported a decrease in cholesterol and plasma proteins. Datta and Kaviraj (2003) recorded increased plasma glucose and decreased liver glycogen for *Clarias gariepinus* in a concentration of 0.005 mg/l. On the contrary, Davies et al. (1994) found a decrease of plasma glucose in *Onchorynchus mykiss* as a result of fish exposure to a concentration of 0.49 μl/l.

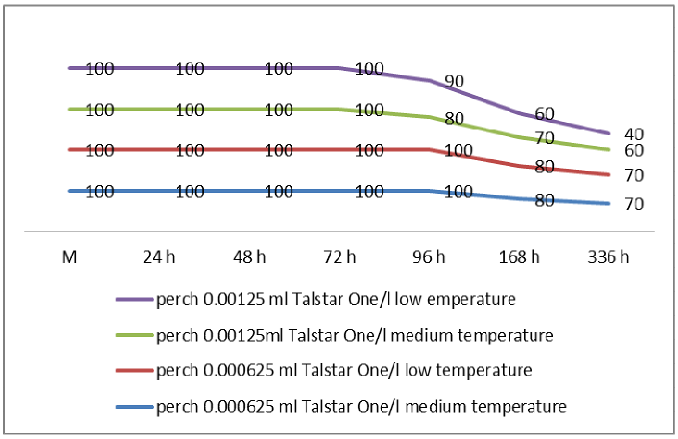

Figure 3. Survival curves in perches poisoned with Talstar One

The survival analysis of perches poisoned with Talstar One at different temperatures shows higher toxicity of the insecticide at low temperatures (Figure 3). This conclusion is not related to the

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evolution of oxygen consumption and breathing rate. Mauck et al. (1976) also found that bifenthrin is more toxic at low temperatures and its toxicity is only slightly influenced by water hardness.

During perch intoxication with Talstar One, we found irritability, muscle twitching, convulsions, ataxia and even death. Paralysis was preceded by muscle spasms due to hyperactivity of the nerve endings (produced by repeated polarization and depolarization of the sodium channels); there was a decrease in the swimming intensity before the paralysis occurred. Symptoms found by other authors in case of fish exposure to pyrethroid pesticides are similar to the above-mentioned ones: swimming near the surface of the water, hyperactivity, loss of balance, increased secretion of branchial mucus, etc. (Edwards et al., 1986, Bradbury and Coats, 1989, Prashanth et al., 2005, Velisek et al., 2006, 2009 and Ponepal et al., 2010).

4. CONCLUSIONS

The Talstar One insecticide produces a decrease in the oxygen consumption and respiratory rhythm of *perch* in both concentrations investigated.

The hematological chart indicates an decrease in mean erythrocyte count at concentrations of 0.625, µl insecticide/ l water after 14 days of insecticide exposure, effect more pronounced at a higher temperature. The response of the perch to Talstar One aggression is also highlighted by increases in plasma glucose levels at concentration of 0.625 µl insecticide/ l water.

Lethal effects as a result of exposure to the Talstar One insecticide were noted at both concentration of insecticide - 0.625, and 1.25 µl / l water (respectively 0.05 şi 0.1 µl bifentrin/l).

In case of exposure to Talstar One in 0.625 and 1.25 µl/l water concentrations, we found behavioural changes such as: irritability, muscle twitching, convulsions, ataxia - higher in 18-20°C variants.

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