RESEARCH REGARDING THE INFLUENCE OF CERTAIN PESTICIDES ON SOME PHYSIOLOGICAL INDICES AT *Carrasius auratus gibelio* Bloch 1758

BACIU Claudiu Alexandru, BIŢU Diana, ZAGARDAN Magdalena Cristina, PĂUNESCU Alina, BRÂNZEA Gheorghiţa, PONEPAL Cristina Maria, DRĂGHICI Octavian, MARINESCU Alexandru Gabriel

Abstract. Many researches in the field of biological effects of pollution are directed towards detection of functional changes of the animal body amid the action of some chemical agents resulting from industrial technological processes or used in agriculture, that are then collected by inland waters. In this study we determined the variation of some physiological indices, such as oxygen consumption, respiratory rate, blood glucose and erythrocytes under the action of the insecticide Calypso 480 SC and Decis 50 EW la *Carassius auratus gibelio* Bloch. The highest variations in the physiological indices in percentage terms were reported in: oxygen consumption, which increased by 116.69% in 24 hours compared with the control value, recording the value 119.834 ml oxygen/kg/hour as compared to 55.302 ml oxygen/kg/hour at the concentration of 0.00015 ml/l Decis 50 EW and blood glucose, where the values increased significantly by 106.45%, as well as 100% at the concentrations of 0.02 and 0.025 ml/l Calypso 480 SC.

Keywords: pesticides, physiological indices, exposure time, concentration, poikilothermic.

Rezumat. Cercetări privind influenţa anumitor pesticide asupra unor indici fiziologici la *Carrasius auratus gibelio* Bloch 1758. Numeroase cercetări în domeniul efectelor biologice ale poluării mediului sunt orientate spre decelarea modificărilor funcţionale ale organismului animal pe fondul acţiunii unor agenţi chimici rezultaţi din procesele tehnologice industriale sau utilizaţi în agricultură, care sunt colectaţi apoi de către apele interioare. În prezentul studiu s-a determinat variaţia anumitor indici fiziologici, cum ar fi: consumul de oxigen, ritmul respirator, glicemia şi numărul de eritrocite, sub acţiunea insecticidului Calypso 480 SC şi Decis 50 EW la *Carassius auratus gibelio* Bloch. Cele mai mari variaţii ale indicilor fiziologici din punct de vedere procentual au fost semnalate la consumul de oxigen, care a crescut cu 116,69% la 24 de ore comparativ cu valoarea martor înregistrând valoarea de 119,834 ml oxigen/kg/oră faţă de 55,302 ml oxigen/kg/oră la concentraţia de 0,00015 ml/l Decis 50 EW şi glicemie unde valorile au crescut semnificativ cu 106,45%, respectiv 100% la concentraţiile de 0,02 şi 0,025 ml/l Calypso 480 SC.

Cuvinte cheie: pesticide, indicifiziologici, perioada de expunere, concentraţie, poikiloterme.

INTRODUCTION

Worldwide, there are used between 5 and 10 million tons of pesticides (integrated in approximately 1000 formulas) with a total value of 16.3 million dollars; of this amount, about 70% are used in agriculture and the remaining 30% in other activities (including household consumption, among others) (REPETTO, 1995; OSSUNA et al., 1997).

Despite today's technology development and emergence of the second and the third generations of pesticides, all these products present, inherently, a certain degree of toxicity to living organisms. Unfortunately, the selectivity of "target" species is not well establishedand the "non-target" species are frequently affected due to the similarity of physiological and / or biochemical systems to those of the "target" species.

It is estimated that only 0.1% of the pesticides applied on an agricultural area reach the "target" organisms, while 99.9% are dispersed in the surrounding environment, representing a potential risk to neighbouring ecosystems (REPETTO, 1995).

Because fish are good indicators of water pollution level compared to other aquatic organisms, so that most researches on the assessment of toxicity of various chemicals were conducted on different species of fish.

In case of fish exposure to pesticides, GRAY & SÖDERLUND (1985) reports a series of physiological and biochemical responses, such as cardio-respiratory changes, as well as blood chemical parameter changes (increased glucose, lactate, epinephrine, norepinephrine, oxygen consumption).

Fish change their energy metabolism in the meaning of consuming a higher amount of energy to alleviate toxic stress (FERRANDO & MOLINER, 1992), which results in the improvement of oxygen use on hypoxia and even anoxia conditions.

The information in the specialized literature shows that fish were the most researched in terms of haematology (MOTELICĂ et al., 1965).

Regarding haematological indices, we find out from the specialized literature that the erythrocytes at the trout poisoned for 96 hours with cypermethrin in concentration of 3.14 μg/l did not change significantly (VELISEK et al., 2006).

Of a particular interest in the field of biological effects of water pollution are, during the past few years, the researches made to reflect the physiological changes in the aquatic organisms under the action of various chemical organic and inorganic agents (PORA & NIŢU, 1952; WOHLSCHLAG et al., 1968; McLEOD & PRESSAH, 1973).

The lack of results on the change of certain physiological indices at the crucian under the action of insecticides Calypso 480 SC and Decis 50 EW motivated us to make different researches on some physiological parameters such as oxygen consumption, respiratory rate, erythrocytes and blood glucose.

MATERIAL AND METHODS

In the researches, there were used specimens of the *Carassius auratus gibelio* originating from Oești, Cerbureni, Budeasa and Cătească lakes, weighing 3-20 g.

The preparation of the experimental animals was made in such a way that, prior to experimentation, an "acclimation" will be achieved (FRY, 1967) for each lot at such temperature (for 1 week) (AT = ET).

During the performance of the experiments, temperature was 18-20 ^{O}C and lighting was 8-12 hours.

Thus, in all cases, there have been avoided possible influences of some factors indifferent for the goals of such an experiment. It was particularly avoided the "negative" influence (in the sense of a "hypometabolic" effect) of low concentrations of oxygen dissolved in water, the oxygen consumption being provided (in preliminary "optimization" determinations) not to exceed 25-30% of the total amount existing at the beginning of the experiment.

The specimens used in different experimental variants were selected and sorted by weight categories, in order to avoid, or, on the contrary to highlight the effect of individual factor of body weight. Choosing specimens and establishment of experimental groups were made with great care and were used only healthy and proper-looking fish.

The specimens were divided into the following lots:

- Control group consisting of 10 specimens
- Group 1 consisting of 10 specimens treated with the insecticide Calypso 480 SC with a concentration of 0.025 ml/l;
- Group 2 consisting of 10 specimens treated with the insecticide Calypso 480 SC with a concentration of 0.02 ml/l;
- Group 3 consisting of 10 specimens treated with the insecticide Calypso 480 SC with a concentration of 0.15 ml/l;
- Group 4 consisting of 10 specimens treated with the insecticide Decis 50 EW with a concentration of 0.00015 ml/l;
- Group 5 consisting of 10 specimens treated with the insecticide Decis 50 EW with a concentration of 0.00012 ml/l;
- Group 6 consisting of 10 specimens treated with insecticide Decis 50 EW with a concentration of 0.0001 ml/l;

For each specimen of the 7 groups there were determined the oxygen consumption and respiratory rate at 24, 48, 72, 96, 168 and 336 hours and, after that, erythrocytes were counted and blood glucose was determined.

The determination of oxygen consumption was performed through Winkler classical method or confined space method (PICOȘ & NĂSTĂSESCU, 1988).

The measurement of the respiratory rate was achieved by a procedure indicated by PORA & NIȚU (1952) during the restraint of fish for carrying out the Winkler method (PICOȘ & NĂSTĂSESCU, 1988); successive determinations of this index were performed (using a timer), until three similar values have been obtained (their arithmetic average representing their respiratory rate at that time).

The determination of the blood glucose was done using an Accutrend GCT device, allowing the measurement of its value in blood drop sampled from the caudal artery (PICOȘ & NĂSTĂSESCU, 1988), in a very short period of time.

The determination of erythrocytes was performed using a Thoma counting chamber through the method described by PICOȘ & NĂSTĂSESCU (1988), from blood taken from the caudal artery.

RESULTS AND DISCUSSION

The analysis results registered at oxygen consumption amid the action of insecticide Calypso 480 SC in concentrations of 0.025, 0.02 and 0.015 ml/l towards the control are shown in figure 1.

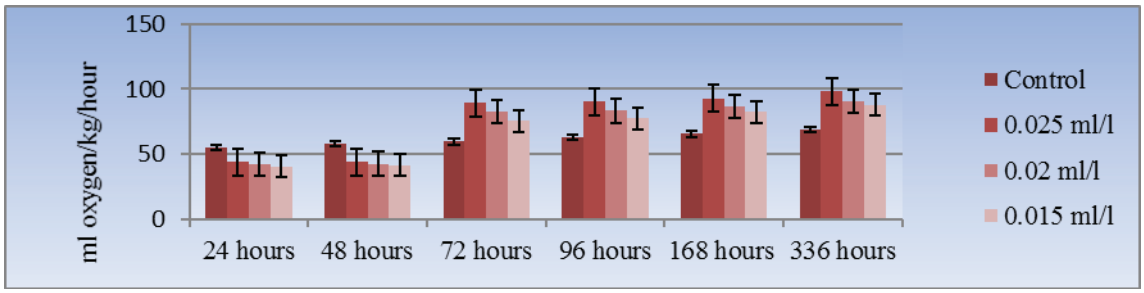

Figure 1. The influence of Calypso insecticide on the oxygen consumption at *Carassius auratus gibelio* Bloch.

At the concentration of 0.025 ml/l Calypso, oxygen consumption increased by 49.88% for 72 hours compared to the control value recording the value of 89.332 ml oxygen/kg/hour compared to 59.601 ml oxygen/kg/hour.

Studying the variation of oxygen consumption at the concentration of 0.02 ml/l Calypso, it is noted an increase in the oxygen consumption value of 31.85% to 168 hours compared with the control value of 107.778 ml oxygen/kg/hour compared to 69.16 ml oxygen/kg/hour.

Increases in oxygen consumption under the influence of insecticides are highlighted in the reference literature at the crucians exposed to the action of insecticide Talstar One on the first three concentrations tested (0.000625, 0.00125 and 0.0025 ml/l water); thus, there were identified increases of oxygen consumption in the first step

(PONEPAL et al., 2010) – its duration ranging between 96 and 24 hours after exposure, the most important intensification of energy metabolism being registered 24 hours after the exposure to the insecticide in the concentration of 0.0025 ml/l water (45.2% higher compared to the value registered prior to the introduction of fish in the toxic area) .

Reviewing the results registered in case of another index - respiratory rate, we find out that under the action of Calypso insecticide towards the control group, it decreases significantly at all concentrations of the insecticide researched (Fig. 2).

The lowest value of the respiratory rate was 48 breaths/minute in 48 hours, at the concentration of 0.015 ml/l towards the control value of 77.66 breaths/minute (Fig. 2).

The respiratory rate decreased by: 38.2% (48 breaths/minute towards the control value of 77.66 breaths/minute) at 0.015 ml/l after 48 hours, 34.33% (51 breaths/minute towards the control value of 77.66 breaths/minute) at 0.025 ml/l after 48 hours and 33.05% (52 breaths/minute towards the control value of 77.66 breaths/minute) at 0.02 ml/l after 48 hours.

These decreases of the respiratory rate are considered to be caused by the mucus placed on the gill, as a reaction to a toxic substance (SCHAUMBURG et al., 1967).

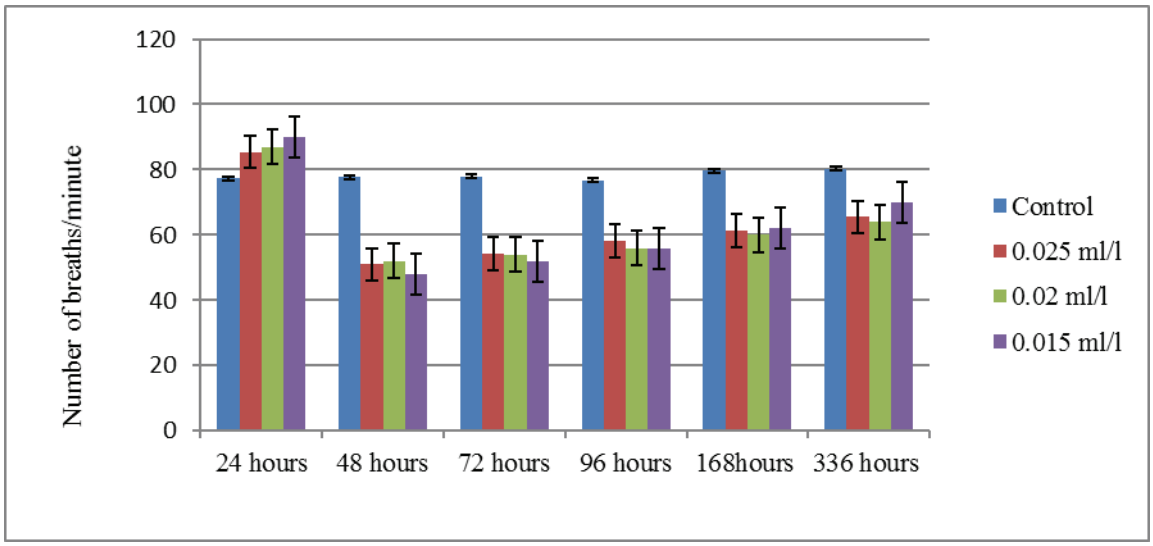

Figure 2. The influence of Calypso 480 SC insecticide on the respiratory rate at *Carassius auratus gibelio* Bloch.

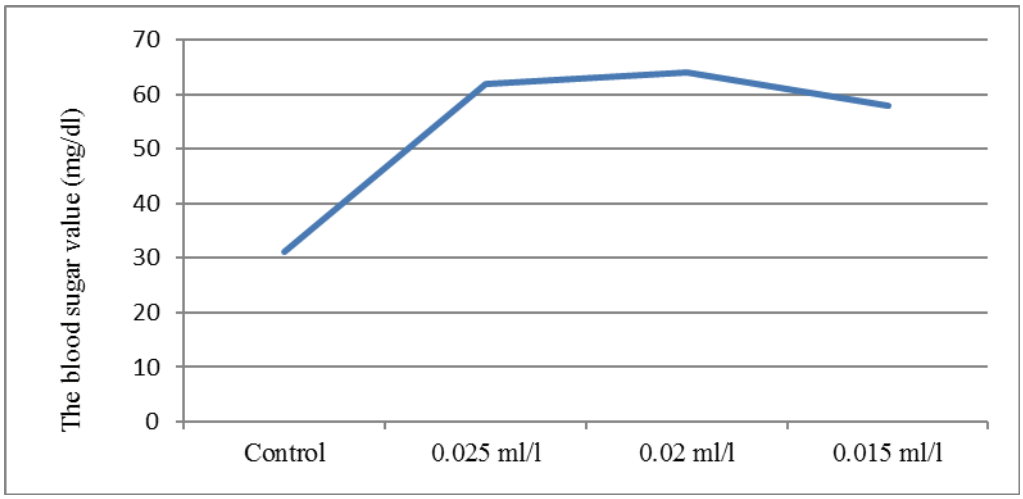

Figure 3. The influence of Calypso 480 SC insecticide on the blood sugar at *Carassius auratus gibelio* Bloch.

The blood glucose values shown in figure 3 reveal some interesting facts; we find out that, under the action of the insecticide Calypso 480 SC, the blood glucose values are significantly increased at the concentrations of 0.02 and 0.025 ml/l by 106.45%, as well as 100%.

Hyperglycaemia at fish in stress conditions was reported by many authors to different species (BLANCHARD et al., 1993; WINBERG & NILSSON, 1993).

Increases of blood glucose levels were also reported by VELISEK et al. (2009) to trout, as a response to metabolic stress induced by the action of the insecticide Talstar 10 EC.

Returning to the erythrocytes, we notice in (Fig. 4) that, under the action of the insecticide Calypso 480 SC, a significant decrease in the erythrocytes to all concentrations takes place, so that the lowest value is 255,000 erythrocytes /ml erythrocytes, 36.25% lower than the control value (400,000 erythrocytes/ml) at the concentration of 0.025 ml/l.

Decrease in the erythrocytes at the carps intoxicated with pyrethroid insecticides (permethrin and cypermethrin) was also developed by SVOBODOVA et al. (2003) and DORUCU & GIRGIN (2001), being attributed to the dysfunction of haematopoiesis.

Further, the same physiological indices will be established to the specimens exposed to the action of the insecticide Decis 50 EW, at the concentrations mentioned in the experimental protocol.

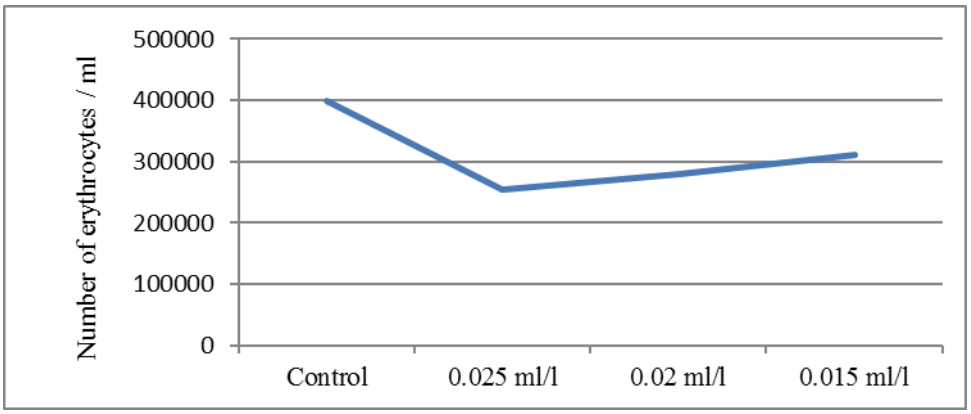

Figure 4. The influence of Calypso 480 SC insecticide on the number of erythrocytes at *Carassius auratus gibelio* Bloch.

Taking into discussion the results registered in the oxygen consumption against the action of the insecticide Decis 50 EW in concentrations of 0.00015, 0.00012 and 0.0001 ml/l compared to the control group, it is found a significant increase in the oxygen consumption at the concentration of 0.00015 ml / l and 0.00012 ml / shown in figure 5).

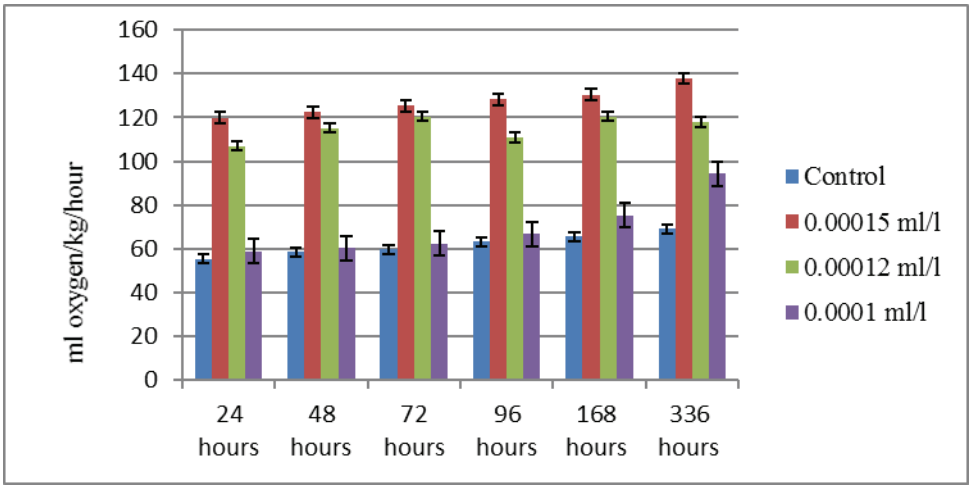

Figure 5. The influence of Decis 50 EW insecticide on the oxygen consumption at *Carassius auratus gibelio* Bloch.

At the concentration of 0.00015 ml/l Decis 50 EW, the oxygen consumption increased by 116.69% at 24 hours compared to the control value of 119.834 ml oxygen/kg/hour compared to 55.302 ml oxygen/kg/hour.

A pretty significant increase in the oxygen consumption, but lower than that in the concentration of 0.00015 ml/l was also detected at the concentration of 0.00012 ml/l Decis 50 EW where the percentage increase was 97.25% at 48 hours (oxygen consumption value registered was 15.242 ml oxygen/kg/hour compared to the control value of 58.423 ml oxygen/kg/hour).

The concentration of 0.0001 ml/l Decis 50 EW has not significantly influenced the consumption of oxygen.

Increases in oxygen consumption under the influence of insecticides are highlighted in the reference literature at the specimens exposed to the action of the insecticide Talstar One on the first three concentrations tested (0.000625, 0.00125 and 0.0025 ml/l water); there were revealed increases of oxygen consumption in the first step (PONEPAL et al., 2010) – its duration ranging between 96 and 24 hours after the exposure, the most important intensification of energy metabolism being registered 24 hours after the exposure to the insecticide in the concentration of 0.0025 ml/l water(45.2% higher compared to the value registered prior to the introduction of fish in the toxic area).

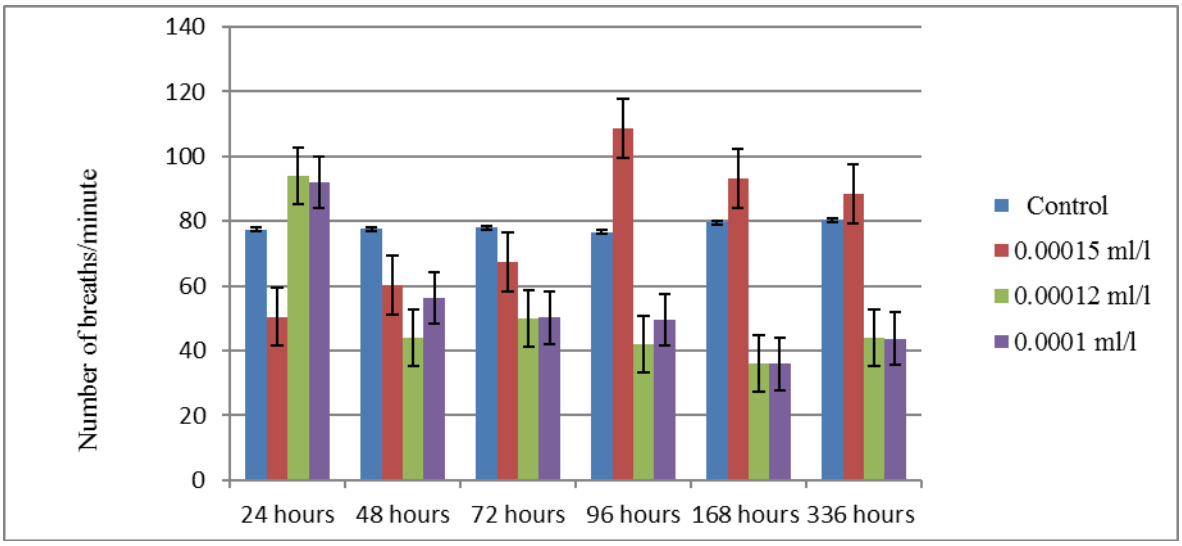

Figure 6. The influence of Decis 50 EW insecticide on the respiratory rate at *Carassius auratus gibelio* Bloch.

Following the analysis of figure 6, we find that, under the action of the insecticide Decis 50 EW at the concentration of 0.00015 ml/l, there was a decrease in the respiratory rate in the first 72 hours, the lowest value (50.5 breaths/minute) being recorded within 24 hours of immersion (34.7% lower than the control value of 77.33 breaths/minute). Then, from 96 to 336 hours, the respiratory rate values were much higher than the control value (the highest value being recorded to 96 hours, 41.89% higher). At the other concentrations of 0.00012 and 0.0001 ml/l, the respiratory rate increased significantly at 24 hours and then from 48 to 336 hours, it significantly decreased; the lowest value is 35.88 breaths/minute at 0.0001 ml/l Decis 50 EW, approximately 54.96% lower compared to the control value of 79.66 breaths/minute.

Such decreases of the respiratory rates are considered to be caused by the mucus placed on the gill, as a reaction to a toxic substance (SCHAUMBURG et al., 1967).

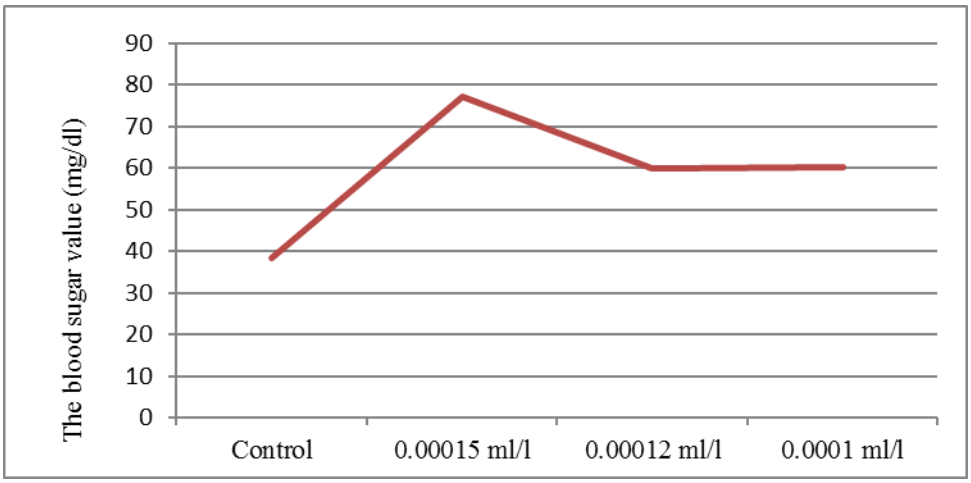

Figure 7. The influence of Decis 50 EW insecticide on the blood sugar at *Carassius auratus gibelio* Bloch.

From the data collected and arranged in the diagram shown in figure 7, it results that he insecticide Decis 50 EW in all concentrations tested, significantly increase the blood glucose.

Thus, DAVIES et al. (1994) reports increases in the plasma glucose at the Galaxias maculates species exposed to a concentration of 4.4 mg/l acephate and at the *Oncorhynchus mykiss* species exposed to a concentration of 0.2 mg/l of the same compound.

The highest value compared to the control one (38.33 mg/dl) was registered to the concentration of 0.00015 ml/l, of 77.16 mg/dl, approximately 101.30% higher.

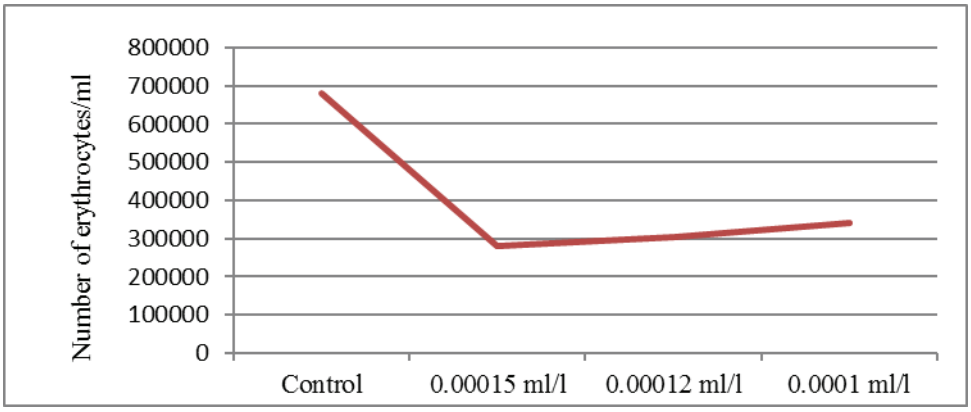

Figure 8. The influence of Decis 50 EW insecticide on the number of erythrocytes at *Carassius auratus gibelio* Bloch.

As sown in figure 8, Decis 50 EW significantly decreased the erythrocytes towards the control value (680,000 erythrocytes/ml) at all experimented concentrations, so that the lowest registered value was 280,000 erythrocytes/ml of blood, about 58.83 % lower at the concentration of 0.00015 ml/l.

After 14 days of exposure of *Perca fluviatilis* and *Alburnus alburnus* specimens at the action of Talstar One insecticide in concentration of 0.000625 mg/l, the erythrocytes decreased significantly compared to the control groups (PONEPAL et al., 2010; PONEPAL, 2011).

Correlating the results registered at the oxygen consumption against the action of investigated chemicals, it is found that, in the experiments performed, the increase in the oxygen consumption is finally correlated to the decrease in the respiratory rate.

Correlating the results to the erythrocytes against the action of Calypso 480 and Decis 50 EW, it is found that, in the experiments made, the insignificant changes and decrease in the erythrocytes is finally correlated to the increase in the oxygen consumption.

Studying the results recorded for blood glucose against the action of investigated chemicals, it is found, in the experiments performed, the increase in the blood glucose especially for Calypso and Decis.

CONCLUSIONS

Increase in the oxygen consumption under the action of the insecticides Decis 50 EW and Calypso 480 SC indicates a direct action on the nerve centres, so that, at the concentration of 0.00015 ml/l Decis 50 EW, oxygen consumption increased by 116.69% in 24 hours compared to the control value of 119.834 ml oxygen/kg/hour as compared to the value of 55.302 ml oxygen/kg/hour and it can also be associated to the decrease in the erythrocytes due to the stress caused by them, where it can be noticed that Decis 50 EW significantly decreased the erythrocytes compared to the control value (680,000 erythrocytes/ml) at all experienced concentrations, so that the lowest value registered was 280,000 erythrocytes/ml blood, approximately 58.83% lower at the concentration of 0.00015 ml/l.

Decrease in the respiratory rate may be due to the direct action on the nerve centres and excess of mucus located on the gill, as a reaction to the action of insecticides. The lower respiratory rates were caused by the insecticide Decis 50 EW where, during the interval of 48-336 hours, they significantly decreased, the lowest value being 35.88 breaths/minutes at 0.0001 ml/l of Decis 50 EW, approximately 54.96% lower compared to the control value 79.66 breaths/minutes.

Increased blood glucose under the action of both insecticides can be considered a reaction to the metabolic stress induced by Calypso 480 SC and Decis 50 EW, where values increased significantly by 106.45% and 100% at concentrations of 0.02 and 0.025 ml/l Calypso 480 SC and, at the concentration of 0.00015 ml/l of Decis 50 EW, the blood glucose value increased by approximately 105.26%.

ACKNOWLEDGEMENTS

This study was conducted and supported by POSDRU/159/1.5/S/138963 – PERFORM project, co-financed by the European Social Foundation for Human Investment, through the Sectoral Operational Programme of Human Resources Development.

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Baciu Claudiu Alexandru
University of Pitești, Faculty of Sciences, Department of Natural Sciences, Pitești,
Târgu din Vale Street, No. 1, Romania.
E-mail: claudiu_alexandru88@yahoo.com

Received: March 9, 2015
Accepted: April 27, 2015