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THE ACTION OF CORAGEN INSECTICIDE ON CERTAIN PHYSIOLOGICAL BIOMARKERS ON *CARASSIUS AURATUS GIBELIO* BLOCH L. 1758

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Abstract
In our researches we have determined the variation of certain physiological indexes, such as the oxygen consume, the breathing rhythm, the glycaemia and the number of red blood cells under the action of Coragen insecticide on Carassius auratus gibelio Bloch. Under the action of Coragen, we have registered significant changes in the oxygen consume, the breathing rhythm, the number of red blood cells and glycemia at the Carassius auratus gibelio Bloch items, considered as answers to the stress provoked by emissions.
The highest variations of the physiological indexes, from the perspective of the percentage, were noticed at the glycemia, which at the mark was 28 mg/dl, and in the treated sample, with 0.1 ml/l Coragen is 42 mg/dl, representing a 50% growth and at the breathing rhythm in 24 hours, where values significantly decreased with 41.18% at the concentration of 0.07 ml/l and with 39.33% at the concentrations of 0.05 and 0.1 ml/l Coragen.
The slightest variations of the physiological indexes, from the perspective of percentage, were noticed at the oxygen consumption, which, at the mark is of 55.302 ml oxygen/kg/hour, and for the treated sample, with 0.1 ml/l Coragen is 34.81 ml oxygen/kg/hour, representing a decrease of 37.06% in 24 hours and the number of red blood cells, where the values have significantly decrease with 9.58%, 13.48%, respectively 18.44% for the concentrations of 0.05, 0.07 and 0.1 ml/l Coragen.

Keywords: physiological biomarkers, fishes, insecticides

1. INTRODUCTION

The contamination of freshwater bodies with a wide range of pollutants has become a matter of concern in recent decades (Vinodhini & Narayanan, 2008).
The production and use of pesticides represents a serious threat to the hydrosphere, whom they reach due to the residual flows from the anti-parasite factories or due to the fact that these substances are washed away by rain from treated agricultural fields (Mohan & Ardelean, 1993).
Insect and snail pests are extensively controlled by using organic pesticides which are a serious environmental hazard (Singhand & Agarwal, 1993). Common use of these pesticides by methods like crop dusting, orchard and forest spraying or mosquito control means that some inevitable enter aquatic ecosystems. The effects of insecticide pollution on non-target organisms in the environment can be studied by detecting changes in organisms at the physiological, biochemical, or molecular

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levels, providing "early warning" tools in monitoring environment quality (Crane et al., 1991; Miren et al., 2000).
These sensitive early warning biomarkers can measure interaction between environmental xenobiotics and biological effects. Inhibition and induction of these biomarkers is a good approach to measure potential impacts of pollutants on environmental organisms (Rendo´N-von Osten et al., 2005). It is necessary to know the effects of these broad spectrum pesticides on aquatic organisms (Elliot, 1977; Casida et al., 1983).
The lack of results regarding the changes of some certain physiological indexes in the case of *Carassius* under the action of Calypso 480 SC and Decis 50 EW insecticides motivated us to make different researches on some physiological parameters, such as the oxygen consumption, the breathing frequency, the number of red blood cells and the glycemia.

2. MATERIALS AND METHODS

During this research, we used items of *Carassius auratus gibelio* Bloch, originating from Oesti, Cerbureni, Budeasa and Cateasca lakes, with a weight between 5-20 g.
The preparation of the experimental items was made such as to, previously to the experiment, an "acclimatization" (FRY, 1967) had been made for each sample apart, at the respective temperature (for a week) (AT=ET).
During the experiments, the temperature has varied between 18-20□C, and the illumination has been between 8 and 12 hours.
Thus, in all cases, we avoided possible influences of some factors that were not significant for the experiment. We especially avoided the "negative" influence (to the extent of a "hypometabolic" effect) of low concentration of oxygen dissolved in water, the oxygen consumption being set (during the preliminary "optimization" experiments) such as not to overpass 25-30% of the total existing volume in the beginning of the experiment.
The items used during different experimental values were selected and classified depending on the weight category, in order to avoid, or contrary, to emphasize the effect of the individual factor of the weight. The choosing of the items and the formation of the experimental samples were carefully made, using only healthy, good looking fishes.
To carry out the research, we created samples of 10 items as follows:
The witness sample of 10 items
Sample 1, consisting of 10 items treated with Coragen, at a concentration of 0.05 ml/l;
Sample 2, consisting of 10 items treated with Coragen, at a concentration of 0.07 ml/l;
Sample 3, consisting of 10 items treated with Coragen, at a concentration of 0.1 ml/l;
For each item in the 4 samples, we have determined the oxygen consumption and the breathing frequency at 24, 48, 72, 96 168 and 336 hours, and we then numbered and summed the red blood cells and we have evaluated the glycemia.
The evaluation of the oxygen consumption was made through the classical Winkler method or the confined space method (Picoş & Năstăsescu, 1988).
The evaluation of the breathing rhythm was made corresponding to a process recommended by E.A. Pora & Nițu (1952), during the fishes' restraint, in order to use Winkler method (Picoş & Năstăsescu, 1988); we removed the successive determinations of this index (using a chronometer), until we have attained 3 close values (their arithmetical average representing the breathing rhythm at the moment).
The glycemia evaluation was made using an Accutrend GCT device, which allows the evaluation of its measure in the blood drop that is collected from the caudal artery (Picoş & Năstăsescu, 1988), in a very short time.

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The evaluation of the red blood cells was made using a Thoma numbering camera through the method described by Picoş & Năstăsescu,(1988), from the blood collected from the caudal artery.

3. RESULTS AND DISCUSSIONS

Carefully analyzing Figure 1, we can see that Coragen insecticide greatly reduces the oxygen consumption between 24 and 168 hours, compared to 336 hours, where the influence of Coragen causes a lower decrease of the oxygen consumption at the concentrations of 0.07 and 0.1 ml/l, producing in turn a slow increase compared to the witnesses at the concentrations of 0.05 ml/l.

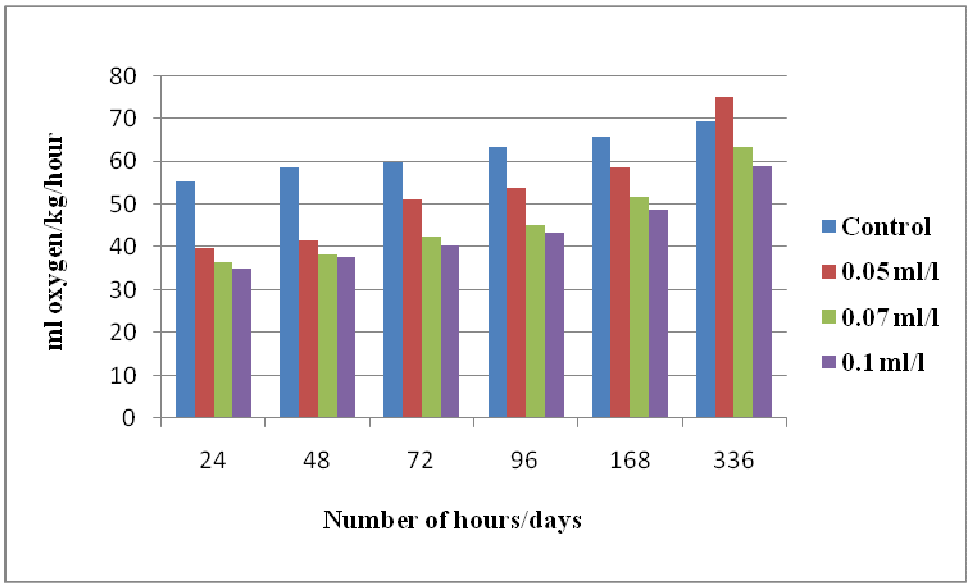

Figure 1. The influence of Coragen on the oxygen consumption at Carassius auratus gibelio items

The slightest reductions happened in 24 hours, when the lowest value of oxygen consumption (34.81 ml oxygen/kg/hour) reported for a concentration of 0.1 ml/l is 37.06% lower than the witness value of oxygen consumption (55.302 ml oxygen/kg/hour). This is followed by the value of the oxygen consumption (36.5 ml oxygen registered for the concentration on 0.07 ml/l, which is 34% lower than the before mentioned value of the upper mentioned witness value, so that it finally follows the value of 39.79 ml oxygen/kg/hour at a concentration of 0.05 ml/l, 28.05% lower than the witness value of the oxygen consumption (55.302 ml/oxygen/kg/hour).

Marinescu et al., (2004) and Ponepal et al., (2009a, 2009b) also noticed decreased oxygen consumption under the action of some pesticides and changes in respiratory rate.

Between 24 and 336 hours, all the oxygen consumption values, at the concentrations of 0.05, 0.07 and 0.1 ml/l Coragen were lower than the values of the oxygen consumption of the witness, excepting the oxygen consumption of 74.864 ml oxygen/kg/hour at 336 hours reported at a concentration of 0.05 ml/l, 8.24% higher than the witness value of the oxygen consumption (69.16 oxygen ml/kg/hour).

Carefully noticing Figure no. 2, we can see that Coragen insecticide determines the reduction of the breathing rhythm between 24 and 168 hours, whereas for 336 hours the breathing rhythm is not influenced compared to the breathing rhythm of the witness. The most significant breathing rhythm reduction are emphasized for 24 hours, so that the highest decrease is reported for the concentration of 0.07 ml/l, where the value of the breathing rhythm (60 breaths/minute) is 41.18% lower than the

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witness value of the breathing rhythm (102 breaths/minute). Finally, the 62 breaths/minute follow, reported at 0.05 and 0.1 ml/l concentrations, 39.22% lower than the breathing rhythm of the witness (102 breaths/minute).

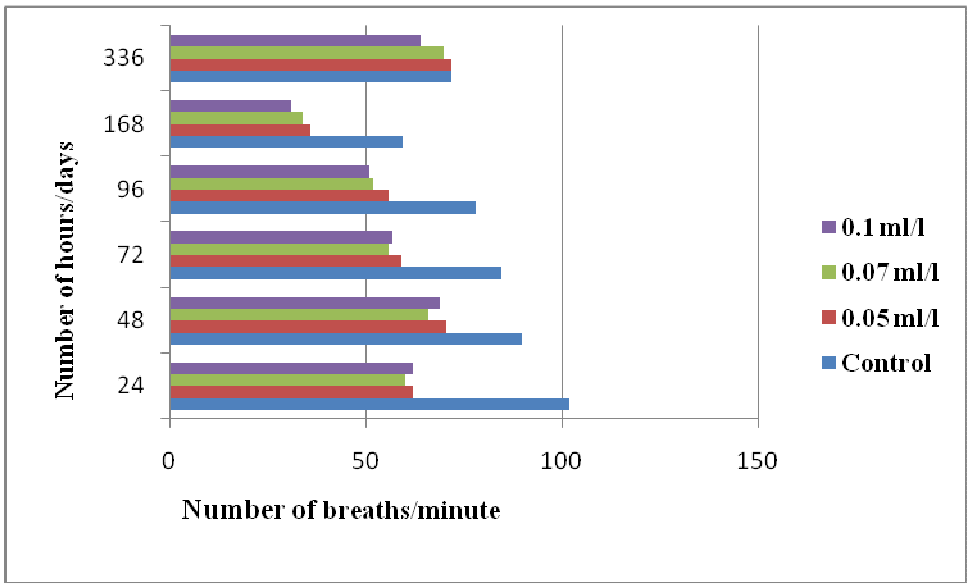

Figure 2. The influence of Coragen insecticide on the breathing rhythm by Carassius auratus gibelio

The slightest reductions of the breathing rhythm happen at 336 hours, so that its slightest reported value for the concentration of 0.1 ml/l is 64 breaths/minute, 11.12% lower than the witness value of the breathing value (72 breaths/minute). Close to it, we find the value of the breathing rhythm of 70 breaths/minute reported for a concentration of 0.07 ml/l, 2.78% lower than the breathing rhythm, and on the last position we find the breathing rhythm (72 breaths/rhythm), reported for a concentration of 0.05 mg/l, equal to the breathing rhythm of the witness (72 breaths/minute).
Respiratory irregularities are thought to be caused by mucus precipitation on the gill epithelium in response to a toxicant (Schaumburg et al., 1967).
At the final of the 14 days test, the *Carassius auratus gibelio* items that were the subject of Coragen concentrations were sacrificed in order to determine the number of red blood cells and the value of the glycemia represented in the figures below.
Analyzing the number of red blood cells in figure 3, we can see that under the action of Coragen, a significant decrease in the number of red blood cells happens for all concentrations, so that the lowest decrease is emphasized at the concentration of 0.05 ml/l, where the reported number of red blood cells (1275000 red blood cells/blood ml) is 9.58% lower than the number of red blood cells of the witness (1410000 red blood cells/ blood ml).
Furthermore, the concentration of 0.07 ml/l follows, where the reported red blood cells number (1220000 red blood cells/blood ml) is 13.48% lower, compared to the number of red blood cells of the above mentioned witness, so that, in the end, the 0.1 ml/l concentration follows, where the number of red blood cells (1150000 red blood cells/ blood ml) is approximately 18.44% lower compared to the witness value.

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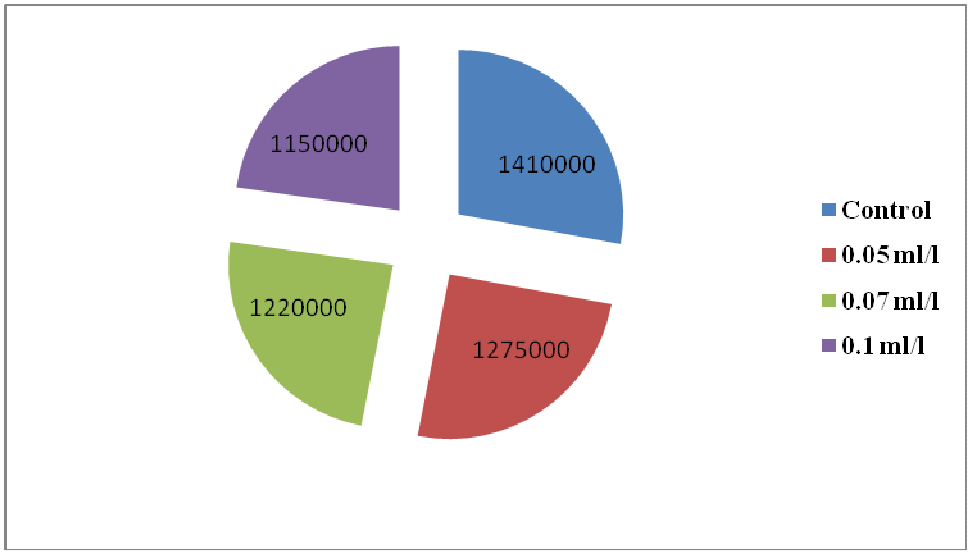

Figure 3 The influence of Coragen insecticide on the number of red blood cells at Carassius auratus gibelio items

Reports on a decrease in Red Blood Cells number during exposure to various pesticides revealed by Anees (1978), Krishan & Garg (1981) were similar to the findings in this study.
Soivio and Oikari (1976) and Adedeji et al. (2009) opined that a decrease in RBC number in the test fish could be ascribed to reduction in erythrocyte lifespan and/or a suppressive effect of the active substance of the insecticide on the erythropoietic tissues, resulting in the failure of erythrocyte production. Narain and Srivastava (1979) and Dutta et al. (1992) suggested that decline in RBC count in biocide-exposed H. fossilis might be due to microcytic or normocytic anaemia (anaemia either due to decrease in RBC size or proportionate decrease in HB content, Hct, MCV and TEC).
From the collected data, exposed in Figure 4, we can see that Coragen insecticide, for all the experimental concentrations, significantly increases the glycaemia of all *Carassius auratus gibelio* items.
Discussing the results exposed in the above graphic, depending on the witness value of the glycemia (28 mg/dl), reported at the *carassius* items, we can see that the approximately 25% increase of the glycemia at the concentration of 0.05 ml/l, with its value of 35 mg/dl, followed by a higher increase of approximately 39.28%, at the concentration of 0.07 ml/l, where the value of glycemia is 39 mg/dl, and finally the highest increase appears at the concentration of 0.1 ml/dl, where the value of glycemia is 42 mg/dl, is 50% higher compared to the witness' glycemia (28 mg/dl).
A significantly higher level of blood glucose level was reported at the species Korean rockfish and Sebastes schlegeli by Jee et al. (2005) due to the long-term exposure to high concentrations of cypermethrin.

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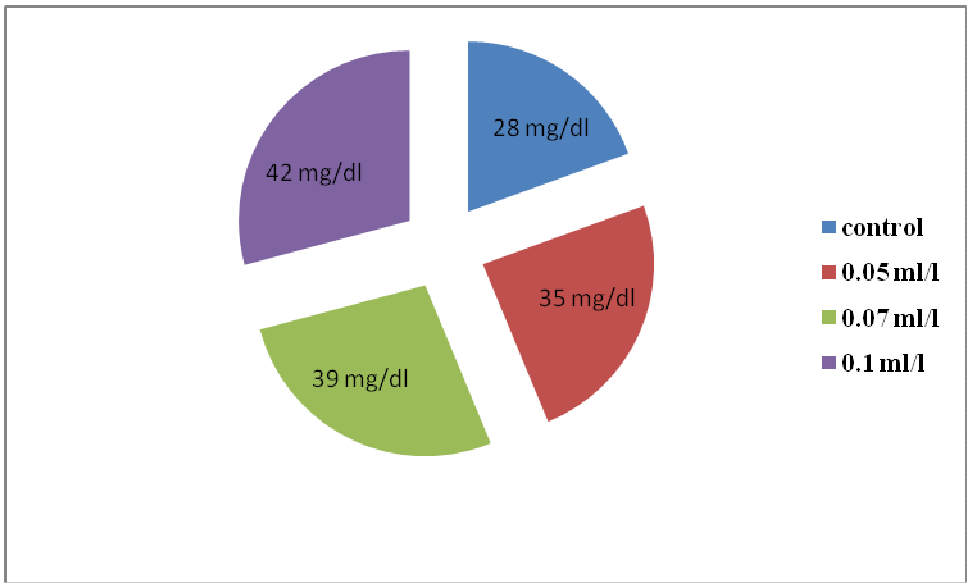

Figure 4 The influence of Coragen on the glycaemia of all Carassius auratus gibelio items

4. CONCLUSIONS

The physiologic index with the highest variation was the glycaemia, which, for the witness is 28 mg/dl, and at the treated sample with 0.1 ml/ l Coragen is 42 mg/dl, which represents a 50% increase.

The growth of glycemia can be associated to an answer to the metabolic stress induced by Coragen insecticide.

The highest decreases of the breathing rhythm are emphasized in 24 hours, so that the highest decrease is reported at the concentration of 0.07 ml/l, where the value of the breathing rhythm (60 breaths/minute), is 41.18% lower than the witness value of the breathing rhythm (102 breaths/minute). Finally comes the 62 breaths/minute rhythm reported at the concentrations of 0.05 and 0.1 ml/l, 39.22% lower than the breathing rhythm of the witness (102 breaths/minute).

The decrease in the breathing rhythm is caused by the mucus surplus on the gill, as an answer to the action of Coragen and can also be caused by the emergence of the injuries provoked by the insecticide at the level of the gill epithelium.

The lowest variations of the physiological indexes, from the percentage perspective, were noticed in the oxygen consumption, which is 55.302 ml/oxygen/hour for the witness, and, for the sample treated with 0.1 ml/l Coragen is 34.81 ml oxygen/kg/hour, representing a 37.06% decrease in 24 hours and the number of red blood cells, where values significantly decreased by 9.58%, 13.48%, respectively 18.44% for the concentrations of 0.05, 0.07 and 0.1 ml/l Coragen.

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