RESEARCH ON THE CHANGES OF SOME PHYSIOLOGICAL PARAMETERS IN SEVERAL FISH SPECIES UNDER THE ACTION OF THE FUNGURAN FUNGICIDE

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Abstract. Intensive agriculture increasingly depends on chemical fertilizers and pesticides. Considerable amounts of these substances persist in the soil and, then, they reach surface water bodies, favouring eutrophication phenomenon or adversely affecting aquatic life. The existence and evolution of aquatic organisms decisively depend on the environment they live in - water. This paper presents the results of the investigations regarding the changes of the physiological indices in fish under the action of the fungicide Funguran OH 50 WP (70% copper hydroxide, 50% metallic Cu). Copper hydroxide is toxic to aquatic organisms and it may cause long-term adverse effects in the aquatic environment. The toxic action of copper on fish manifests by the appearance of abundant mucus on gills and skin, cough, bleeding lips, stress, loss of balance and eventually death. Copper ions alter the ability of fish to distinguish toxic substances. In case of subacute concentrations (0.05 mg/l), the Prussian carp and other fish species are attracted to the ion source or tend to avoid it, according to the slope of the concentration gradient (low slope - attraction, steep slope - avoidance) and temperature, which sometimes, can reverse the reaction of fish. According to the research performed by us, it was found that the fungicide Funguran OH 50 WP has an inhibitory effect on the respiratory rate in all three investigated fish species (*Carassius auratus gibelio* Bloch, *Perca fluviatilis* L. and *Alburnus alburnus* L.); it also triggered the decrease of the number of erythrocytes and reduction of the glycaemic index. Perch proved to be the most sensitive fish species among the three studies species. The experiments performed show that the fungicide Funguran OH 50 WP is toxic to fish at very low concentrations.

Keywords: Funguran, pesticides, fish, temperature, respiratory rate, erythrocyte, glycaemia.

Rezumat. Cercetări asupra modificărilor unor parametri fiziologici la mai multe specii de peşti sub acţiunea fungicidului Funguran. Agricultura intensivă depinde tot mai mult de fertilizanţii chimici şi de pesticide. Cantităţi apreciabile din aceste substanţe persistă în sol, de unde ajung în apele de suprafaţă, favorizând fenomenul de eutrofizare a corpurilor de apă sau acţionând advers asupra vieţii acvatice. Existenţa şi evoluţia organismelor acvatice depind în mod hotărâtor de mediul în care evoluează - apa. Lucrarea de faţă prezintă rezultatele investigaţiilor referitoare la modificările unor indici fiziologici la peşti sub acţiunea fungicidului Funguran OH 50 WP (70% hidroxid de cupru, 50% Cu metalic). Hidroxidul de cupru este toxic pentru organismele acvatice, putând provoca efecte adverse pe termen lung asupra mediului acvatic. Acţiunea toxică a cuprului asupra peştilor se manifestă prin apariţia de mucus abundent pe branhii şi piele, tuse, buze hemoragice, stres, pierderea echilibrului şi în final moartea. Ionii de cupru alterează abilitatea unor peşti de a distinge substanţele toxice. La concentraţii subacute (0,05 mg/l), carasul şi alţi peşti sunt atraşi de sursa de ioni sau tind să o evite, în funcţie de panta gradientului de concentraţie (pantă mică-atracţie, pantă mare-evitare) şi de temperatură, care uneori poate inversa reacţia peştilor. În urma cercetărilor efectuate s-a constatat că fungicidul Funguran OH 50 WP are efect inhibitor asupra ritmului respirator la toate cele trei specii de peşti investigate (*Carassius auratus gibelio* Bloch, *Perca fluviatilis* L. şi *Alburnus alburnus* L.); de asemenea a determinat scăderea numărului de eritrocite şi reducerea nivelului glicemic. Dintre cele trei specii de peşti cel mai sensibil s-a dovedit a fi bibanul. Experienţele efectuate ilustrează faptul că fungicidul Funguran OH 50 WP este toxic pentru peşti în concentraţii foarte mici.

Cuvinte cheie: funguran, pesticid, peşte, temperatură, ritm respirator, eritrocite, glicemie.

INTRODUCTION

The contamination of freshwater with a wide range of pollutants has become a matter of concern in recent decades (VINODHINI & NARAYANAN, 2008). Increased human activities, especially the rapid development of agriculture and industry, have led to a considerable increase in the level of pollution, such as heavy metal pollution, which is the main anthropogenic pollutant that causes serious and long-term negative effects for all living organisms (SASTRY & SUKLA, 1993; MURUGAN et al., 2008).

Some metals such as copper, which is also essential for cell metabolism, become highly toxic to aquatic animals if their concentration in water increases (CARVALHO & FERNANDES, 2006).

Fish have the ability to accumulate heavy metals in tissues, gills being the main target of pollutants, and in case they are affected, there can be noticed an immediate impact on the entire body (AL-YACOOB et al., 1994). The human body can be also exposed at a high risk due to the contamination of the food chain (COSTA & HARTZ, 2009).

Many researchers have reported nocive effects of copper on aquatic life (OLAIFA et al., 2004; MUTHUKUMARVEL et al., 2007).

The negative effect of heavy metals on fish is linked to disturbances in the biochemical and physiological processes (VIELLA et al., 1999).

Biochemically, copper interferes in a series of enzymatic and redox processes, which are vital to living organisms as an activator or inhibitor. In case of the rainbow trout, *Salmo gairdneri*, the oxidation of the lactic acid in the gills has been inhibited in 53% of the survivors exposed to lethal concentrations of copper (DIUDEA et al., 1986).

The main cause of death in the fish exposed to heavy metals is hypoxia, as there occur changes in the brachial epithelium, disturbances in the osmoregulation process, decrease of the oxygen consumption and, finally, death (ALBASTER & LIOYD, 1982; PEURANEN et al., 1994; HASSAN, 2005). The gills are very sensitive to metals often

appearing various metal-induced lesions. This leads not only to osmotic imbalance, but may also cause a deterioration of the function of the respiratory system of fish, which varies depending on the type of metal and the site of action (JEZIERSKI & SARNOWSKI, 2002; DOBREV et al., 2008).

Funguran OH 50 WP (77% copper hydroxide and 50% metallic Cu) is a contact fungicide characterized by a very good capacity of penetration in plants and high protection. These features confer the product the ability to combat pathogenic fungi, especially those belonging to Phycomycete group (which produce "mana"), as well as the fire blight in the Rose family. Funguran OH 50 WP has great preventive action by inhibiting spore germination and blocking the development of the mycelium in plants. The period of protection depends on the local conditions and varies from 7 to 14 days; treated plants: potato, tomato, cucumber, beans, onion, vine, apple tree, pear tree, cherry tree, sugar beets, hop. It is classified in group III of toxicity. Copper hydroxide is toxic to aquatic organisms and it may cause long-term adverse effects in the aquatic environment.

MATERIAL AND METHOD

The research was conducted on specimens belonging to three species of fish from the Olt river: *Carassius auratus gibelio* (Bloch), *Perca fluviatilis* L. and *Alburnus alburnus* L. (Figs. 1, 2, 3). The biological material was chosen taking into consideration the physiological state, body integrity and size of the specimens, sensitivity to toxic substances, as well as the fact that they easily adapt to the 'retention' conditions in the aquarium, tolerate high temperature variations and show resistance to oxygen deficiency, displaying a low lethal hypoxic limit, especially the Prussian carp.

Figure 1. *Carassius auratus gibelio* (Bloch 1783).

Figure 2. *Perca fluviatilis* (Linné 1758).

Figure 3. *Alburnus alburnus* (Linné 1758).

The transfer of fish in aquariums was done about a month before the start of the experiments; thus, they have adapted to the new conditions.

The aquariums used during the experiments had a capacity of poisoning of 20-30 litres and were equipped with lighting, stirring and aeration systems. These containers have been cleaned and sanitized.

In case of the variants made at a temperature of 5-7ºC, fish were placed in refrigerators, lighting being artificial.

The solutions of toxic substances from the aquariums were renewed at intervals of 24 hours.

The fish specimens used in different experimental variants were selected and sorted by weight categories in order to avoid or, on the contrary, to emphasize the effect of the individual weight factor. We used groups of fish of ten specimens.

The measurement of the respiratory rate was performed by successive determinations by means of a timer (their arithmetic mean representing the respiratory rate at the respective moment).

For the experiments carried out to underline the change in the number of erythrocytes and blood glucose levels under the action of the fungicide Funguran OH 50 WP, there were taken blood samples from the analysed fish. Blood was sampled from the caudal artery. Erythrocytes were determined using Thoma counting chamber (PICOŞ & NĂSTĂSESCU, 1988). Glucose determination was performed using Accutrend GCT device that allows the measurement of its value in a drop of blood using strips in a short time.

All the performed determinations were carried out under strict control.

The experiments that required series conducted at different dates were always made within a nyctemer as narrow as possible (same time each day) to avoid any influence of the circadian variations in the respiratory rate.

There were 12 experimental variants designed to determine the respiratory rate and 6 experimental variants to determine the number of erythrocytes and blood glucose.

The measurements were made after 14 days of treatment at two thermal levels: 5-7ºC and 20-22ºC.

The concentrations of Funguran OH 50 WP used in this work are 0.25 and 0.5 mg/l, concentrations corresponding to quantities of 0.2 respectively 0.4 mg copper hydroxide/l.

RESULTS AND DISCUSSIONS

1. The action of the fungicide Funguran OH 50 WP upon the respiratory rate

The variation of the respiratory rate in case of the Prussian carp specimens exposed to the fungicide Funguran OH 50 WP is rendered in figure 4.

At a temperature of 20-22°C, the fungicide has an inhibitory effect in case of both concentrations used. The concentration of 0.25 mg/l has a weak effect on the respiratory rate. The inhibitory effect increases at the concentration of 0.5 mg/l. After completing the experiment, the reductions are 6.25% and 12.3% compared to control group (according to the increase of the concentration).

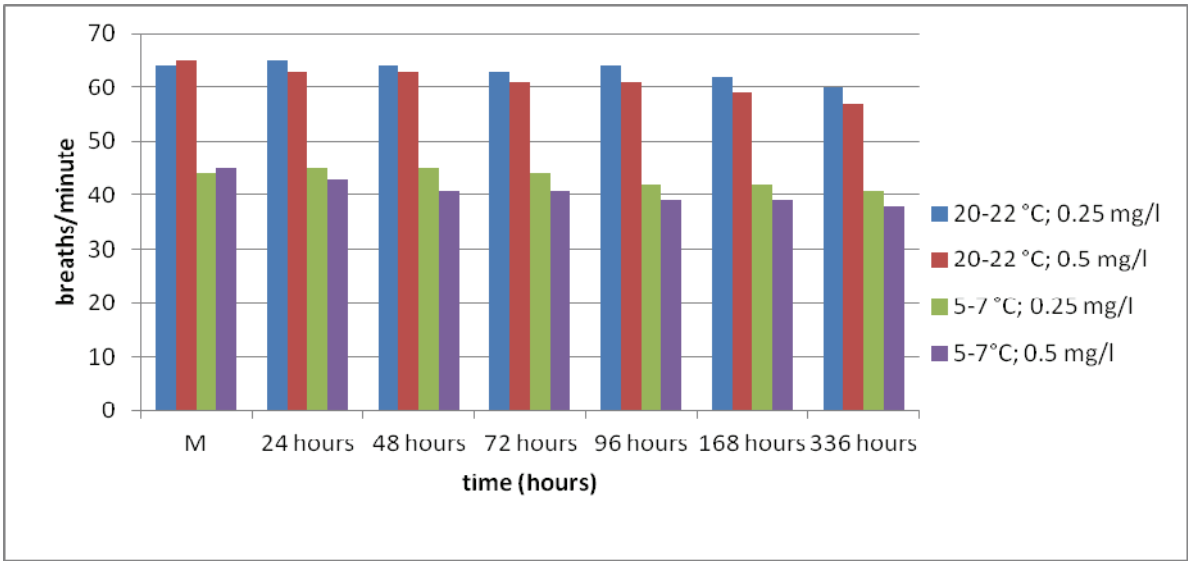

Figure 4. Variation of the average respiratory rate at the Prussian carp specimens exposed to the fungicide Funguran OH 50 WP at two thermal levels.

At a temperature of 5-7°C, the inhibitory effect of the fungicide is stronger as compared with that registered at the temperature of 20-22°C for both concentrations, but especially at the concentration of 0.5 mg/l (reduction of 7% and 15.5% respectively compared to the control group).

The action of the fungicide Funguran OH 50 WP on the respiratory rate at perch and bleak is rendered in figures 5 and 6.

The inhibitory effect of the fungicide on the respiratory rate occurs in the first 24 hours after exposure and continues to increase until the end of the experiment (14 days).

The respiratory rate decreased at the perch specimens poisoned with Funguran OH 50 WP by 10% at a temperature of 20-22°C and 12% at 5-7°C at a concentration of 0.25 mg/l and by 17% at a temperature of 20-22°C and 19% at a temperature of 5-7°C at a concentration of 0.5 mg/l as compared to the control group.

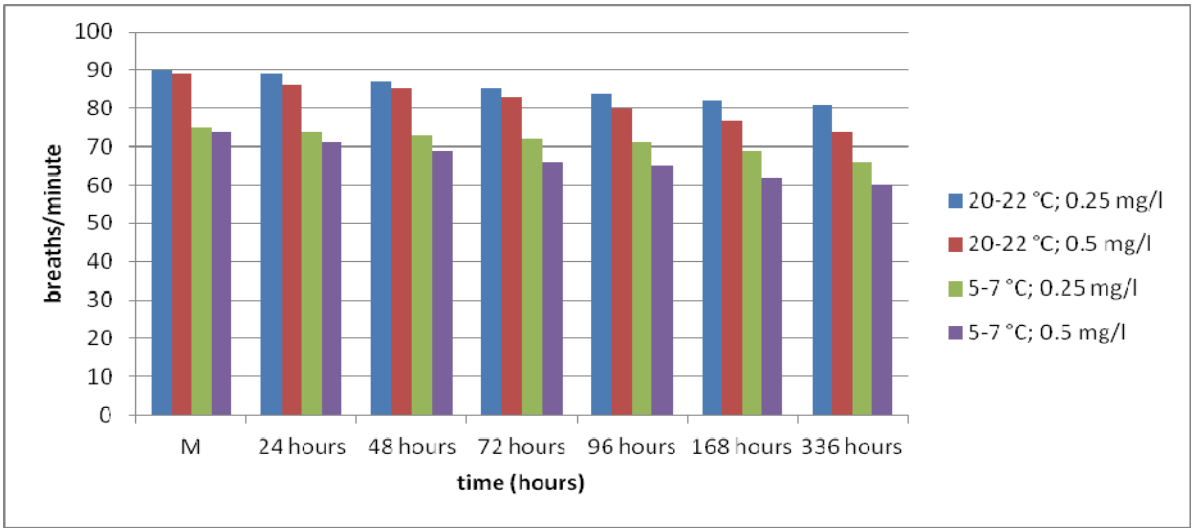

Figure 5. Variation of the average respiratory rate at the perch specimens exposed to the fungicide Funguran OH 50 WP at two thermal levels.

The respiratory rate decreased at the bleak specimens poisoned with Funguran OH 50 WP by 8% at a temperature of 20-22°C and 9% at the temperature of 5-7°C at a concentration of 0.25 mg/l and by 13.5% at a temperature of 20-22°C and 15.5% at a temperature of 5-7°C at a concentration of 0.5 mg/l as compared to the control group (Fig. 6).

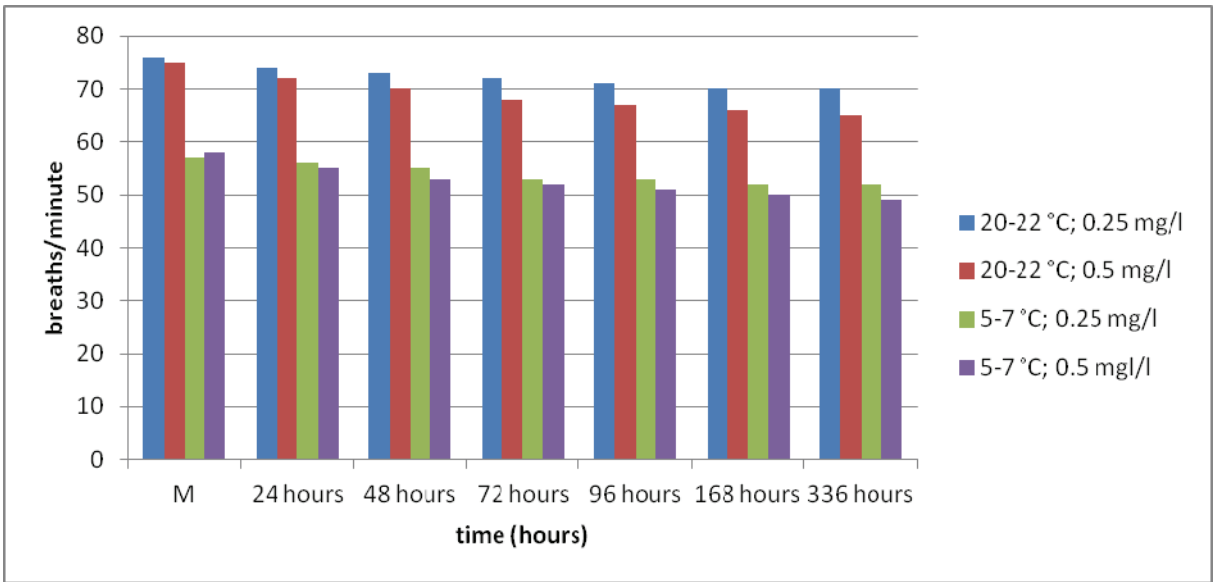

Figure 6. Variation of the average respiratory rate at the bleak specimens exposed to the fungicide Funguran OH 50 WP at two thermal levels.

The decrease of the respiratory rate is more pronounced at perch specimens than at bleak specimens. The toxic action of the fungicide should be correlated with the respiratory lesions.

Copper is concentrated in gills and liver (DURVE 1980, quoted by DIUDEA et al. 1986) at the species *Ictalurus nebulosus* after 30 days of exposure to 0.027 mg / l copper.

Metals can affect the respiratory function by reducing the respiratory surface, by atrophy and fusion of secondary lamellae, as well as by the internal action of the metal, which enhances the action of the respiratory inhibiting factors (MUTHUKUMARVEL et al., 2007; SHEREEN & LOGSWAMY, 2008).

Copper exposure causes histopathological changes leading to the separation of the epithelium from the secondary lamellae, hyperplasia, fusion of the secondary lamellae and necrosis (HASSAN, 2011).

The same effects were reported by MUHVICH et al. (1995) after exposing the fish to sub-lethal concentration of copper sulphate for 96 h, PANDEY et al. (1997) at sub-lethal concentrations of lead for 15 days, VUTUKURU (2005) and HASSAN (2005) after exposing the species *Carassius Carassius* to sub-lethal concentrations of cadmium.

2. The action of the fungicide Funguran OH 50 WP upon the number of erythrocytes

By analysing figure 7, where it is rendered the action of the fungicide Funguran OH 50 WP at a concentration of 0.25 mg / l at carp, perch and bleak at a temperature of 20-22°C, it can be noticed that the reduction in the number of red blood cells is higher in case of perch specimens (20%). At bleak, the reduction in the number of erythrocytes is 16.5% compared to the control group, while at the Prussian carp the reduction is 15%.

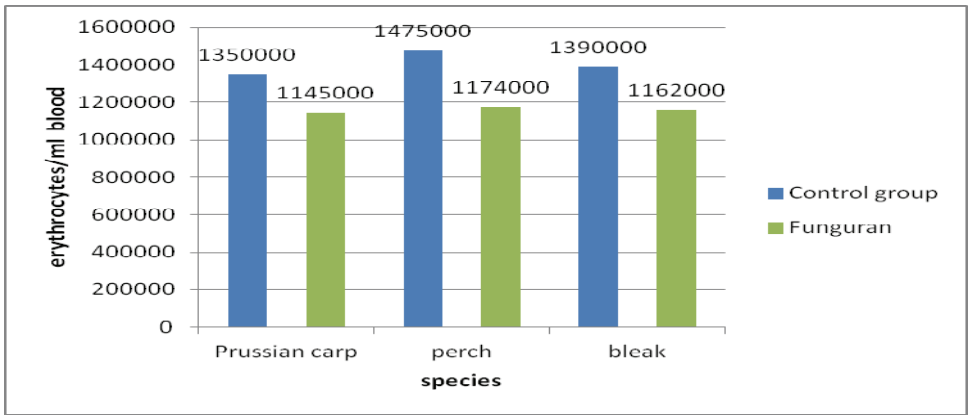

Figure 7. Action of the fungicide Funguran OH 50 WP at a concentration of 0.25 mg/l upon the number of erythrocytes at Prussian carp, perch and bleak at 20-22°C.

At a low temperature (5-7°C), the reduction in the number of erythrocytes is 19% for carp, 17% for perch and 14% for bleak. Low temperature influences the reduction in the number of erythrocytes only at perch and bleak (Fig. 8).

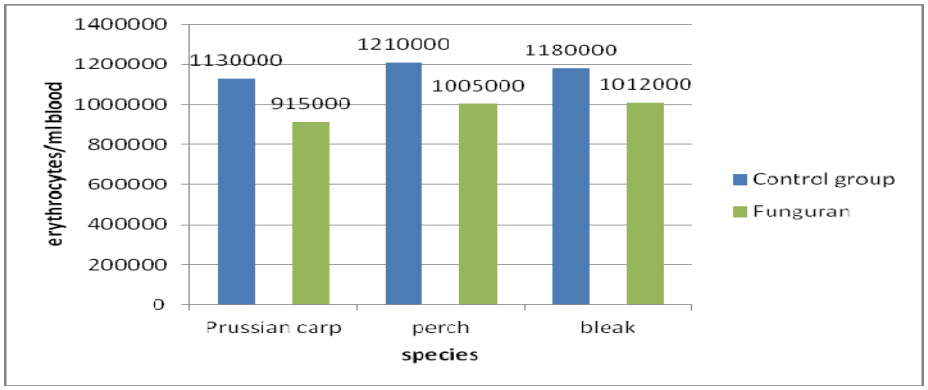

Figure 8. Action of the fungicide Funguran OH 50 WP at a concentration of 0.25 mg/l upon the number of erythrocytes at Prussian carp, perch and bleak at 5-7°C.

The reduction in the number of erythrocytes was reported in the species *Carassius auratus gibelio* Bloch by MIHAI (2013) under the action of the fungicides Funguran Champion, Ridomil and Curzate manox (which contain metallic copper).

At the same time, a decrease in the number of erythrocytes, haemoglobin and haematocrit levels along with a severe anaemia was reported in the species *Channa punctatus* subjected to a treatment with copper sulphate (0.36 mg/l) by SINGH et al. (2008) and copper and chromium (SINGH, 1995). The metal penetrates the fish body and is eliminated slowly (NEWMAN & MITZ, 1988; JAMES & MITZ, 1996; JAMES et al., 1996); therefore, the haematological parameters are affected by the toxicity of the metal. Oxygen transport in the blood depends on the amount of haemoglobin.

Acute exposure of the species *Colisa fasciatus*, *Oreochromis mossambicus* to sub-lethal concentrations of lead, copper and zinc showed that haemolytic anaemia occurs due to the lysis of erythrocytes with concomitant decrease in haemoglobin and haematocrit (SOIVEO & NIKINMAA, 1981; SAMPATH et al., 1998).

Anaemia under the stress induced by copper may also be due to cell injury and disruption of the blood haemoglobin synthesis (McKIM et al., 1970; GROSS et al., 1975). Similar results, rendering significant reduction of erythrocytes and haemoglobin levels in fish exposed to different heavy metals have been previously reported by GOEL et al. (1985) and GOEL & SHARMA (1987). According to DE BOECK (1995) and (SINGH et al. (2008), the reduction of the haemoglobin level in the fish exposed to toxic substances could also be a result of the inhibitory effect of the toxic substance on the enzyme system responsible for the synthesis of haemoglobin. JOSHI et al. (2002) suggested that the exposure to heavy metals also decreased the number of erythrocytes, haemoglobin and haematocrit due to the dysfunction of iron intestinal absorption. Anaemia is an early manifestation of an acute and chronic intoxication with heavy metals.

3. The action of the fungicide Funguran OH 50 WP upon glycaemia

The fungicide Funguran OH 50 WP lowers the blood glucose at 20-22°C in all three studied fish species. At the end of the experiment (Fig. 9), higher reductions in the glucose levels were recorded at perch (33%) and bleak (27.6%); in the case of the Prussian carp, the reduction was 21.2%.

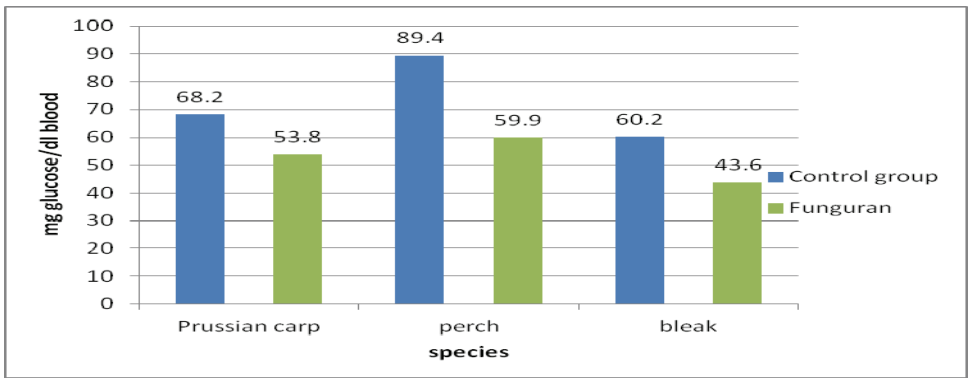

Figure 9. Action of the fungicide Funguran OH 50 WP at a concentration of 0.25 mg/l upon glycaemia at Prussian carp, perch and bleak at 20-22°C.

The temperature of 5-7°C determines the decrease of glycaemia by 19% in the Prussian carp, 30.9% in perch and 26.75% in bleak (Fig. 10).

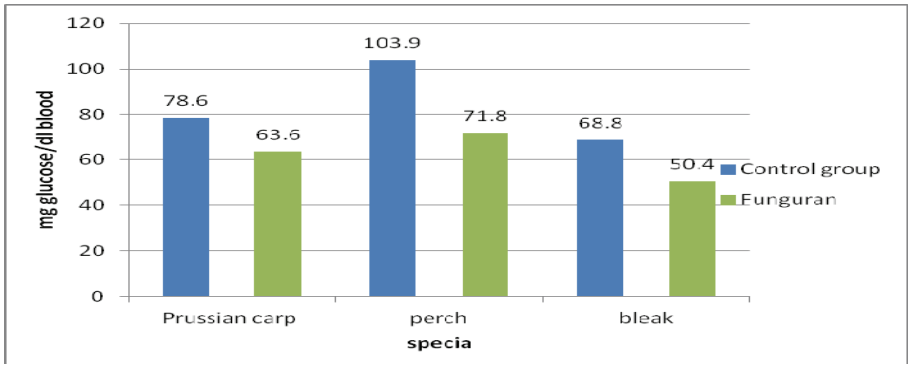

Figure 10. Action of the fungicide Funguran OH 50 WP at a concentration of 0.25 mg/l upon glycaemia at Prussian carp, perch and bleak at 5-7°C.

CONCLUSIONS

Low temperatures (5-7°C) lead to the decrease of the respiratory rate, low number of erythrocytes and to the increase of glucose levels compared to the values recorded at 20-22°C. The fungicide Funguran OH 50 WP reduces the respiratory rate in the three studied species of fish (*Carassius auratus gibelio* Bloch, *Perca fluviatilis* L. and *Alburnus alburnus* L.). The number of erythrocytes reduces after the exposure to Funguran OH 50 WP. The glucose values decrease in the fish exposed to Funguran OH 50 WP, this fungicide having hypoglycaemic effect. Of all the three studied fish species, perch registered greater reductions in all the investigated physiological indices, this species being the most sensitive, followed by bleak and the Prussian carp.

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