THE CHANGES OF SOME PHYSIOLOGICAL PARAMETERS IN PRUSSIAN CARP UNDER THE ACTION OF THE CHAMPION 50 WP FUNGICIDE

MODIFICAREA UNOR PARAMETRI FIZIOLOGICI LA CARAS SUB ACŢIUNEA FUNGICIDULUI CHAMPION 50 WP

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Abstract. *Champion WP (copper hidroxide) is a fixed copper fungicide widely used for control of fungal and bacterial pathogens. The Cchampion WP product is toxic to fish and aquatic organisms (96-hour LC_{50} Bluegill: 180 mg/l, 96-hour LC_{50} Rainbow trout: 0.023 mg/l and 48-hour EC_{50} Daphnia: 0.065 mg/l). This study was carried out to analyze the effects of sublethal and lethal concentrations – from 0.003 to 3 mg/l - of Champion fungicide on some physiological parameters (oxygen consumption, breathing frequency, number of erythrocytes) of the prussian carp (Carassius auratus gibelio Bloch). The acute and subacute toxicity of Champion fungicide was evaluated in glass aquaria under semi- static conditions. The Champion 50 WP product, under the concentrations of 0.003 mg/l water, produces, after two weeks of exposure a significant increase of prussian carp energethic methabolism; under the concentration of 0.3 and 3 mg /l water, the fungicide produces, after one week of imersion, a significant decrease of the fish oxygen consumption. The fungicide has changed the respiratory rhythm of prussian carps in all investigated concentrations after seven days of exposure. The number of erythrocytes has significantly decrease after 14 days of immersion at fungicide concentrations of 0.3 mg Champion /l water. The acute test (96 hours) records mortality in all variants.*

Key words: Champion WP, prussian carp, oxygen consumption, breathing frequency, number of erythrocytes

Rezumat. *Champion WP (hidroxid de cupru) este un fungicid pe bază de cupru utilizat pentru controlul ciupercilor şi bacteriilor patogene. Produsul comercial Champion WP este toxic pentru peşti şi alte organisme acvatice (LC_{50} 96 ore Lepomis macrochirs: 180 mg/l, LC_{50} 96 ore Oncorhynchus mykiss: 0.023 mg/l EC_{50} 48 ore Daphnia magna: 0.065 mg/l). Această lucrare a fost realizată pentru a studia efectele concentraţiilor subletale şi letale de fungicid Champion – de la 0.003 la 3 mg/l asupra unor parametri fiziologici (consum de oxigen, frecvenţa mişcărilor respiratorii, număr de eritrocite) la caras (Carassius auratus gibelio Bloch). Toxicitatea acută şi subacută a fungicidului Champion a fost testată în condiţii semistatice, în acvarii de sticlă.* Produsul *Champion 50 WP, în concentraţia de 0.003 mg/l apă, produce, după două săptămâni de expunere, o creştere semnificativă a metabolismului energetic al caraşilor; în concentraţii de la 0.3 la 3 mg/l apă ,fungicidul produce, după o săptămână de imersie, o scădere semnificativă a consumului de oxigen al peştilor. Fungicidul a modificat ritmul respirator la caras în toate*

concentraţiile investigate, după şapte zile de la expunere. Numărul de eritrocite a scăzut semnificativ după 14 zile de imersie în fungicid la concentraţia de 0.3 mg Champion/l apă. În testul acut (96 ore) s-a înregistrat mortalitate în toate concentraţiile studiate.

Cuvinte cheie: Champion WP, caras, consum de oxigen, frecvenţa respiratorie, număr de eritrocite

INTRODUCTION

The inorganic pesticides were the most important of the early pesticides and they are still used today, primarily for the control of plant diseases and as wood preservatives. Champion WP (copper hidroxide) is a fixed copper fungicide widely used for control of fungal and bacterial pathogens.

Copper speciation is directly affected by water pH, and the free cupric ion concentration is higher in water with low pH, while a copper hydroxide complex prevails in water with high pH (Payle et al., 1992; Tao et al., 2001; Pimentel, 1971). Copper is highly toxic in aquatic environments and has effects in fish, invertebrates, and amphibians, with all three groups equally sensitive to chronic toxicity (U.S. EPA 1993; Horne and Dunson 1995).

This study was carried out to analyze the effects of sublethal and lethal concentrations – from 0.01 to 2.4 mg/l - of Champion fungicide on some physiological parameters (oxygen consumption, breathing frequency, number of erythrocytes) of the prussian carp (*Carassius auratus gibelio* Bloch). The acute and subacute toxicity of Champion fungicide was evaluated in glass aquaria under semi-static conditions.

MATERIAL AND METHOD

Determinations were made between January and April 2009 on prussian carp samples (*Carassius auratus gibelio* Bloch), captured from the surrounding rivers of Piteşti. Animals were acclimatized for 2 weeks before the completion of experiments in aquariums with a capacity of 100 l and 50 l (Picoş and Năstăsescu, 1988), under conditions of natural photoperiodism, a period in which they were fed once a day (*ad libitum*), at around 10 am. After acclimatization in the laboratory, the fish were separated in six lots of 10 fish (average weight 10.089, 9.3, 7.36, 9.45 and 9.75 g) subjected to Champion 50 WP fungicide at a temperature of 16-18°C in concentrations of 0.003, 0.03, 0.3 and 3 mg/l water (variants I-IV) and the control lot (variant V).

The fungicide concentrations were determined by preliminary tests of survival. The introduction of fish in solutions was done after their mixing and aeration for 5 minutes. The water temperature was 16-80°C, the "immersion" solution was changed every 24 hours, and aeration of water was continuous; the fish were not fed during experiments to avoid further intervention of this factor (Picoş and Năstăsescu, 1988). 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

fungicide, samples of variant I (0.003 mg fungicide) and V (the control lot) were sacrificed to achieve intakes of blood necessary to hematological calculations (number of erythrocytes).

Determination of oxygen consumption was done by means of the oximetre and Winkler method and erythrocytes were counted with Thoma chamber, using a small amount of blood from the caudal artery on the optic microscope (Picoş and Năstăsescu, 1988; Şerban et al, 1993).

The statistical interpretation of the results was performed with ANOVA (LSD) test.

RESULTS AND DISCUSSIONS

The figure 1 shows the average frequency of the respiratory movements of prussian carps exposed to the action of Champion fungicide at temperatures of 16-18ºC (0.003, 0.03, 0.3 and 3 mg/).

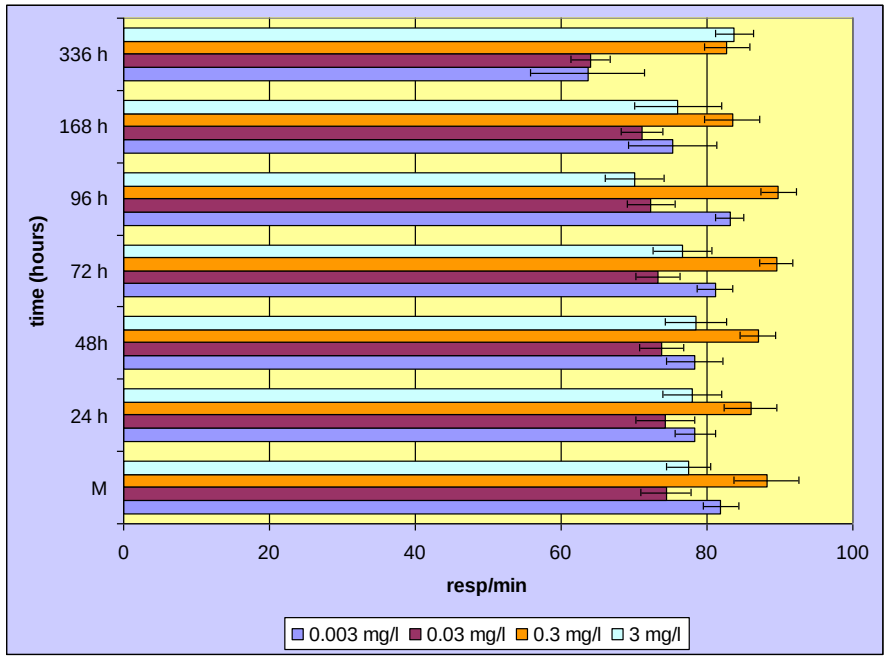

Fig.1. The influence of Champion fungicide upon breathing frequency on prussian carp

In all studied concentration, the Champion modified the values of breathing frequency; for the concentrations of 3 mg/l water, the fungicide effect, after two weeks of exposure, is stimulating (significantly difference for $p<0,05$).

At the concentration of 0.003 and 0.03 mg Champion/l water, effect of breathing frequency has been inhibiting. The values recorded at the end of the experiments with fungicide, except variant III, were significantly difference comparing to the control values (established before the immersion).

In all experimental variants have been applied have only been observed three stages of the sympthomatologicycal scheme described by Schäperclaus for the intoxicated fish (Năstăsescu, 1986; Picoş and Năstăsescu, 1988).

Common symptoms of initial acute toxicity are apparent fish hypoxia, disoriented (ataxic) at the surface, and mucus-producing effects.

Changes in oxygen consumption of prussian carps exposed to the fungicide Champion in different concentrations are shown in figure 2.

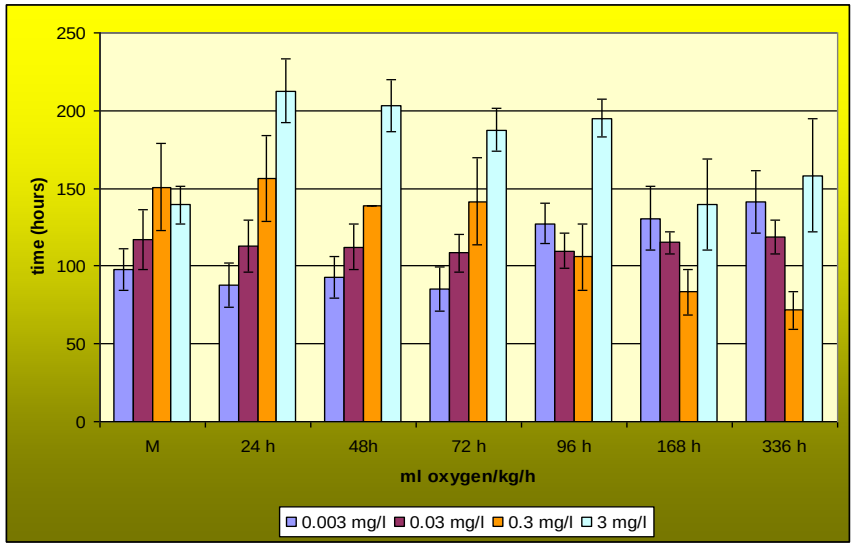

Fig.2. The influence of the Champion fungicide upon oxygen consumption on prussian carp

The oxygen consumption was found to be significantly influenced by the concentration of the used fungicide. Thus, as shown in figure 2, at a concentration of 0.003 and 3 mg fungicide/l water, this index increase (significantly differences for $p<0,05$) after four days of immersion (acute test); the respiratory metabolism diminished to 70.15% of the control value.

The values of the physiological index recorded after two weeks of exposure to Champion fungicide at a concentration of 0.003 and 3mg/l water are 144% and 113 % of the values recorded prior to fish exposure to toxicity. Decreased oxygen consumption under the action of some pesticides (Dithane M 45, Reldan, and Tilt) has also been noticed by Marinescu (Marinescu et al., 2004) and Ponepal (Ponepal et al., 2009).

Figure3 show the changes in the average values of erythrocytes after two weeks of exposure to Champion in a concentration of 0,003 mg/l water.

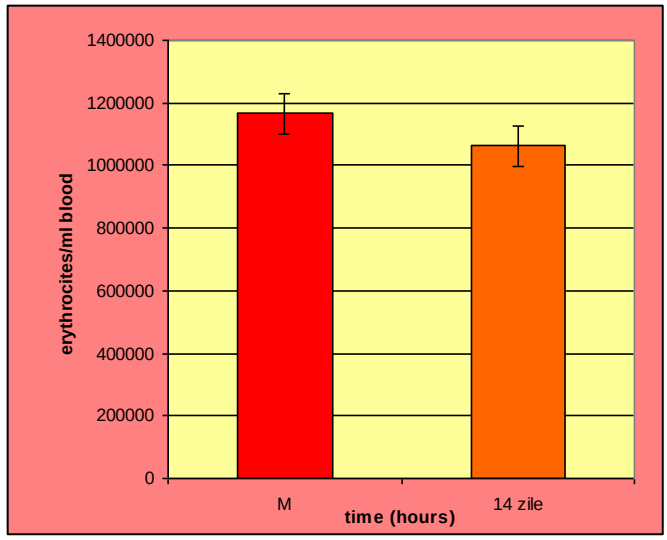

Fig. 3. The influence of Champion fungicide upon number of erythrocytes on prussian carp

The number of erythrocytes in the fish individuals subjected for 14 days to immersion into water with 0.003 mg/l of Champion was also affected. After 14 days of exposure to fungicide we found out a significant decrease in the number of erythrocytes (91.2 % of the control value). Similarly results were obtained in carp by Hughes et al (1995) after a brief exposure to Methadathion.

The decrease in RBC after 7 days exposure to some pesticides in fish was observed by Dhembare and Pondha (2000), Ponepal et al. (2009).

Table 1 shows the survival times on prussian carps during the 14 days of experiments.

Table 1

Lethal effect of the fungicide Champion on prussian carp

Experimental variants	Champion (mg/l water)	The number of living specimens					
		Immersion time (hours)					
		24	48	72	96	168	336
I	*0.003*	*10*	*10*	*10*	*9*	*9*	*9*
II	*0.03*	*10*	*10*	*10*	*9*	*8*	*8*
III	*0.3*	*10*	*10*	*10*	*9*	*8*	*7*
IV	*3*	*10*	*10*	*9*	*8*	*6*	*4*
V	*Control lot (0.00)*	*10*	*10*	*10*	*10*	*10*	*9*

The acute test (96 hours) records mortality in all fish variants, excepting control lot, but none of the variants record absolute mortality only in variant IV (3 mg Champion/l water).

CONCLUSIONS

With all concentrations (0.003, 0.03 and 0.3 mg/l water), excepting those of 3 mg Champion/l water, the fungicide caused a slowdown in Prussian carp breathing (shallow breaths) after two weeks of exposure. The fungicide Champion, under the concentrations of 0.003 and 3 mg/l water, had, after 96 hours of exposure, a stimulatory effect on oxygen consumption for the prussian carp. The fungicide had an stimulatory effect on the energy metabolism of prussian carps after two weeks of at concentration of 0.003 mg/l water. After 14 days of exposure to Champion (0,003 mg/l water at 16-18 ºC we found out a significant decrease in the number of erythrocytes of prussian carp.

In all variants we records fish mortality (at 10 to 60% after two weeks of exposure).

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