EFFECT OF THE FUNGICIDE CHLOROTHALONIL (BRAVO) ON SOME PHYSIOLOGICAL PARAMETERS IN PRUSSIAN CARP

EFECTUL FUNGICIDULUI CLOROTALONIL (BRAVO) ASUPRA UNOR PARAMETRI FIZIOLOGICI LA CARAS

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Abstract. *In this paper we study the action of the Bravo 500 SC fungicide (the active substance is the chlorothalonil), under different concentration (0.78125 x 10^{-3}, 1.5625 x 10^{-3}, 3.125 x 10^{-3} , 6.25 x 10^{-3} and 12.5 x 10^{-3} ml /l water) on some physiological parameters on prussian carp (Carassius auratus gibelio Bloch). The fungicide Bravo had an inhibitory effect on oxygen consumption for the prussian carp. In all studied concentration the Bravo fungicide modified the values of breathing frequency (the fungicide effect is stimulating at first and then inhibiting of the breathing frequency).*

Key words: chlorothalonil, prussian carp, oxygen consumption, breathing frequency, number of erythrocytes.

Rezumat. *În această lucrare am studiat actiunea fungicidului Bravo 500 SC (substanţă activă clorotalonil) în diferite concentraţii (0.78125 x 10^{-3}, 1.5625 x 10^{-3}, 3.125 x 10^{-3} , 6.25 x 10^{-3} şi 12.5 x 10^{-3} ml /l apă) asupra unor parametri fiziologici la caras (Carassius auratus gibelio Bloch). Fungicidul Bravo a avut un efect inhibitor asupra consumului de oxigen al carasului. În toate concentraţiile studiate fungicidul Bravo a modificat valorile ritmului respirator (efectul fungicidului este stimulator la început, după care frecvenţa mişcărilor respiratorii scade).*

Cuvinte cheie: clorotalonil, caras, consum de oxigen, frecvenţa mişcărilor respiratorii, număr de eritrocite

INTRODUCTION

The commercial product Bravo 500 SC is a concentrated suspension of chlorothalonil (500g / l) produced by Syngenta Crop Protection AG, Basel, Switzerland. Chlorothalonil (2,4,5,6 tetrachlor isophthal-nitrile) is a contact fungicide with curative and preventive action (works by stopping germination and the development of spores) for combating a large number of pathogens (leaf spots, downy mildews, alternarioses, fruit rots, brown tor of fruit, scab) that threaten the main crops (12). Chlorothalonil has been marketed since 1969 under different trade names: Bravo, Anathalonil, Anadra, Anadac, Retador, Bravonil, Cheyene, Helmonil, Clorimex, Daconil etc. The fungicide is part of group IV of toxicity; it is not toxic to bees, warm-blooded animals and moderately toxic to insects (Kidd and James, 1991). Chlorothalonil and its metabolites are very toxic to fish, aquatic invertebrates and marine organisms: LC50 (96 h) is of 0.25

mg/l for rainbow trout (*Salmo gairdneri*), 0.3 mg/l for sun perch (*Lepomis macrochirus*), 0.43 mg/l for sea devil (*Ictalurus punctatus*), etc. (Kidd and James, 1991). Using an open breathing chamber and highly purified chlorothalonil, Davies and White (1985) report values of LC 50 (96 h) for aquatic organisms between 10.5 and 29.2 mg/l (Davies and White, 1985). Present in the animal organism, chlorothalonil reacts with glutathione in the intestine, giving mono- or diconjugates or triglutathione which can be excreted or further metabolized to thiol or mercapturic acid derivatives (Ernst et al., 1991). One also notices increases in levels of GSH in the liver from exposure to 10 mg / l after 96 hours, and significant decreases in the liver thiols (Davies and White, 1985). Chlorothalonil is not stored in the adipose tissue and is rapidly excreted outside the body, its rate of bioaccumulation being low.

This study was carried out to analyze the impact of lethal and sub-lethal concentrations of chlorothalonil on some physiological parameters of Prussian carp (*Carassius auratus gibelio Bloch*).

MATERIAL AND METHOD

Determinations were carried out during February-April 2008 on samples of Prussian carp (*Carassius auratus gibelio Bloch*) caught in the lakes bordering Pitesti. Fish were acclimatized for two weeks before the completion of experiments in aquariums with a capacity of 100 l, under natural photoperiodic conditions in which they were fed once a day (*ad libitum),* at around 10. After acclimatization in the laboratory, fish were separated into groups and placed in the experiments.

Six experimental variants were completed, of two-three lots of 10 fish belonging to two generations (C_0 and C_1): variant I - fish subjected to the fungicide concentration of 0.78125×10^{-3} ml Bravo 500 SC/l (0.39 mg/l chlorothalonil) (I.1 - fish with average weight of 14.16 g, I.2 - fish with average weight of 35.42 g and I.3 - fish with average weight of 32.15 g), variant II - fish subjected to the fungicide concentration of 1.5625×10^{-3} ml Bravo 500 SC/l (0.78 mg/l chlorothalonil) (II.1 - fish with average weight of 13.24 g, II.2 – fish with average weight of 28.57 g and II.3 - fish with average weight of 34.85 g), variant III - fish subjected to the fungicide concentration of 3.125×10^{-3} ml /l Bravo 500 SC/l (1.5625 mg/l chlorothalonil) (III.1 - fish with average weight of 15.24 g and III.2 – fish with average weight of 27.45 g), variant IV - fish subjected to the fungicide concentration of 6.25×10^{-3} ml Bravo 500 SC/l (3,125 mg/l chlorothalonil) (IV.1 fish with average weight of 12.58 g and IV.2- fish with average weight of 33.12 g), variant V - fish subjected to the fungicide concentration of 12.5×10^{-3} ml Bravo 500 SC ml/l (6.25 mg/l chlorothalonil) and variant VI (control) - comprises three lots of 10 fish with no treatment, kept in dechlorinate tap water (VI.1 – fish with average weight of 15.24 g, VI.2 - fish with average weight of 36.84g and VI.3 - fish with average weight of 31.35 g).

In mentioned variants (lots I.1-VI.1, I.2-VI.2) determinations were made at intervals of 24, 48, 72, 96 hours and 7 days of oxygen consumption and the frequency of respiratory movements (fish mortality was observated during 14 days of treatment). Lots I.3, II.3 and VI.3 (the control lot) includes hematological determinations (number of erythrocytes) done on the lots of fish after 7 days of exposure to the toxic (after 7 days of immersion in the toxic solution, blood samples were collected to establish the number of erythrocytes and fish were killed).

Concentrations of Bravo 500 SC were established by preliminary tests of survival. Introducing fish into the solution was done after their good mixing and aeration for 5 minutes. Water temperature was of 16-18° C, the "immersion" solution was changed every 24 hours and water aeration was continuous; the fish were not fed during experiments to avoid interference of this additional factor. The testing method was semi-static with refreshing solution in 24 hours after the calculations of the day, in aquariums of 100 l for each experimental lot. Oxygen consumption was established by means of Winkler method and oximeter, glycemia was determined using o-toluidine method and the glucometer and erythrocytes were counted in a Thoma chamber, using a small amount of blood from the caudal artery on the optic microscope (Picos and Nastasescu, 1988). The statistical interpretation of the results was performed with the Anova test.

RESULTS AND DISCUSSIONS

For a good comparison between the toxic effects of chlorothalonil in the concentrations investigated, the average frequency of respiratory movements was represented graphically in Figure 1.

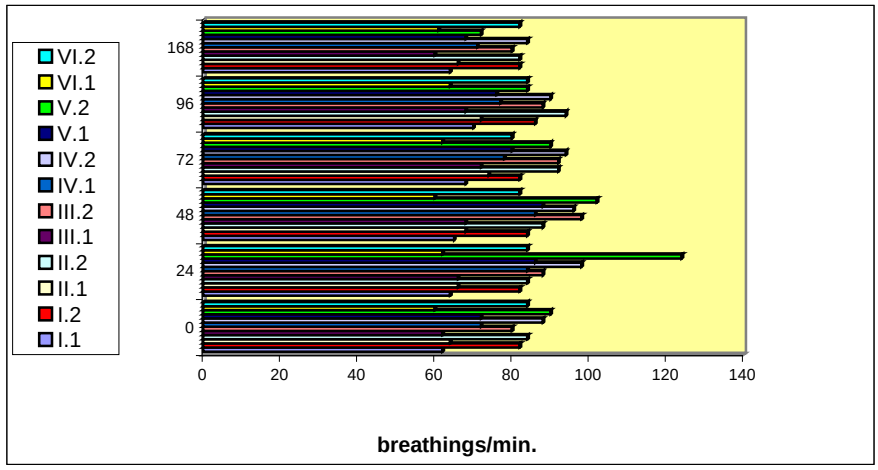

Fig. 1. The influence of Bravo fungicide upon breathing frequency on prussian carp

Chlorothalonil has changed the respiratory rhythm of prussian carps in all investigated concentrations. For all concentrations tested the effect of the fungicide is initially stimulating and inhibitory as regards the frequency of respiratory movements. Similar effects on prussian carps were also noticed at another fungicide - propiconazole (Ponepal et al., 2008). Variations of the respiratory rhythm in both generations of fish exposed to chlorothalonil concentration of 0.39 mg / l are insensitive to the threshold of significance $p < 0.05$. The stimulating effect of respiration is maintained for 72 hours after exposure to three concentrations, the strongest stimulation being recorded after 24 hours of exposure to the concentration of 12.5×10^{-3} ml / l Bravo 500 SC (37.77%

more than the value recorded at the beginning of the experiment, a significant increase for $p < 0.05$).

Analysis of Figure 1 shows that chlorothalonil effect is stronger on smaller fish. This physiological parameter values recorded after 7 days of exposure to toxic action, return to normal for the four concentrations tested, both for fish of larger sizes, and for those of smaller size. Figure 2 shows the average oxygen consumption at prussian carps exposed to the fungicide Bravo in different concentration for 7 days.

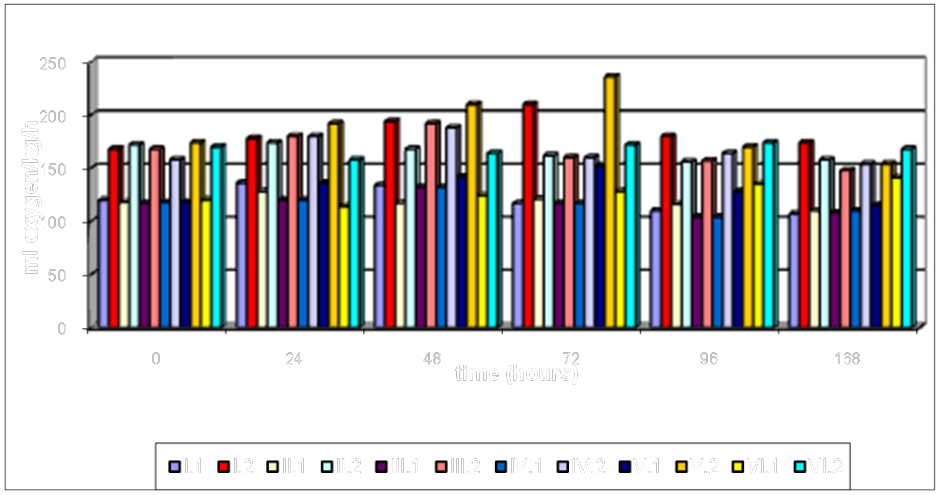

Fig. 2. The influence of Bravo fungicide upon oxygen consumption on prussian carp

After 7 days of exposure, for all lots of fish tested, oxygen consumption values fall below the value recorded before the introduction of fish in experiments, significant reductions of between 88.09 and 97.45 of the control lots. Similar changes (for specific concentrations) were reported by Marinescu, under the action of another fungicide - Dithane M-45 (Marinescu et al., 2004).

Figure 3 represents graphically the average values of erythrocytes at prussian carps exposed to the toxic chlorothalonil concentrations of 0.39 and 0.78mg / l, compared to the average control. The values obtained for the control variant are higher than those presented in the specialized literature (Schliecher, 1927, Mc Cay, 1931, cited from Motelica et al., 1965), probably due to the period in which experiments were carried out. For both experiments carried out – I.3 and II.3, the average values of erythrocytes after 7 days of immersion in the toxic solution have decreased by 4.81% and 12.83% compared to control, significant decreases for the threshold of significance $p < 0.05$, which can be correlated with decreased consumption of oxygen after 7 days of exposure to toxic.

Decreases in the number of erythrocytes after 7 days of exposure to the action of a pesticide have also been reported by other authors (Dhembare and Pondha, 2000, Hughes et al., 1995, Ponepal et al., 2006). Table 1 shows the data on Prussian carps mortality during the experiments.

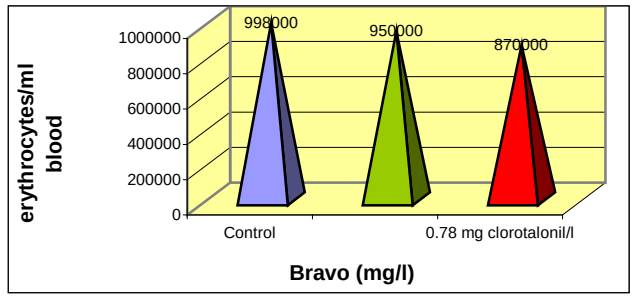

Fig. 3. Changes in the number of erythrocytes in prussian carp after 7 days of exposure to the fungicide Bravo

Table 1

Lethal effect of the fungicide Bravo on Prussian carp

Experimental variants	The number of living specimens					
	Immersion time (hours)					
	24	48	72	96	168	336
VI.1	10	10	10	10	10	10
VI.2	10	10	10	10	10	10
I.1	10	10	10	10	10	10
I.2	10	10	10	10	10	10
II.1	10	10	10	10	9	9
II.2	10	10	10	10	9	8
III.1	10	10	9	9	8	7
III.2	10	10	9	9	8	8
IV.1	10	10	9	9	8	6
IV.2	10	9	7	7	6	4
V.1	10	10	9	8	7	2
V.2	9	8	7	6	4	0

The acute test (96 hours) records mortality from the concentration level of 3,125 x 10^{-3} ml / Bravo 500 SC, but none of the variants record absolute mortality. Chlorothalonil toxicity is lower than that indicated in the literature (Kidd and James, 1991, Davies and White, 1985), which is due both to the testing method (semi-static) and the fact that no pure chemical product has been used.

CONCLUSIONS

Chlorothalonil (commercial product from Bravo 500 SC) in concentrations of 0.78125 x 10^{-3}, 1.5625 x 10^{-3}, 3.125 x 10^{-3}, 6.25 x 10^{-3} and 12,5 x 10^{-3} ml / l Bravo, had an overall stimulating effect on oxygen consumption of Prussian carps in the first phase (with variable duration: 24-96 hours after exposure) followed by restoration of energy metabolism after 7 days of exposure to toxic.

In all concentrations studied, chlorothalonil (0.39, 0.78, 1.5625, 3125, and 6.25 mg/l water) has significant changed (for $p<0.05$) the values of the respiratory rhythm at Prussian carps during the acute test (96 hours), the toxic effects being initially stimulating, followed by reducing the physiological parameter. The

values recorded after 7 days of exposure to the fungicide were in all cases very close to the control.

The fungicide Bravo 500 SC, in a concentration of 0.78125 x 10^{-3}, 1.5625 x 10^{-3} ml/l water, has produced a significant decrease in the number of erythrocytes (by 4.81% and 12.83% as compared to the control, a significant decrease of the threshold of significance $p<0.05$).

During the acute test (96 hours) the fungicides Bravo 500 SC has caused mortality only in concentrations of 0. 3125 x 10^{-3}, 6,25 x 10^{-3} and 12.5 x 10^{-3} ml / l the smaller species being more sensitive to the toxic than the larger ones.

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