THE CHANGES OF SOME PHYSIOLOGICAL PARAMETERS IN PRUSSIAN CARP UNDER THE ACTION OF THE FOLPAN 80 WDG AND THE PROTECTIVE ROLE OF THIOUREA

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Abstract
This study was carried out to analyze the effects of sublethal and lethal concentrations of Folpan 80 WDG ($30x10^{-5}$g Folpan 80 WDG /l water, $6x10^{-4}$g Folpan 80WDG /l water) and 1‰ thiourea on some physiological parameters (oxygen consumption, breathing frequency) on prussian carp (Carassius auratus gibelio Bloch 1782). The subacute and acute toxicity of Folpan 80 WDG fungicide and thiourea was evaluated in glass aquaria under semystatic conditions. Folpan 80 WDG produced, in all organized experimental variants a decrease in respiratory frequency and consumption of oxygen in the case of prussian carp, the more powerful the higher the concentration of the toxic was. Prussian carp anemia could be due to hypoxia that was induced by injuring the gills, as the red-pink colour of the gills became red-white, and at high concentrations the gills completely lost their red colour, while abundant secretions of mucus and even mucosal detachment with abundant bleeding could be observed. The antitoxic action of thiourea manifests itself by the fact that Folpan 80WDG are blocked by SH- groupings isothiourea, the mixture between Folpan 80WDG and thiourea produced no significant changes on the parameters physiological.

Keywords: prussian carp, oxygen consumption, respiratory rhythm, thiourea, Folpan 80 WDG.

1. INTRODUCTION

It has been made numerous researches in the biological effects of environmental pollution to detect functional changes of aquatic organisms under the action of chemical agents used in agriculture arriving in aquatic ecosystems.

Fungicides control spoilage of crops. Soft fruits, such as grapes, strawberries, and vegetables are susceptible to fungal attack that fungicides can control. In addition in the last 20 years fungicides have been used in boat paints to control colonization of hull surfaces by microalgae and the attachment and growth of seaweeds (Thomas, 1998). While fungicide contamination of foodstuffs and seawater is below restricted levels, they are a possible risk because of bioaccumulation.

Folpan 80WDG (active substance is folpet, (N-[(trichloromethyl)thio)phthalimide]) is a contact fungicide belonging to the dicarboximide family, a protective leaf-fungicide. Its mode of action inhibits normal cell division of a broad spectrum of microorganisms. It is used to control cherry leaf spot, rose mildew, rose black spot, and apple scab (Pesticide Information Project of Cooperative Extension Offices of Cornell University, *Folpet*,).

Folpan is a pyrethroid (Shan et al., 1997) that affects the central and peripheral nervous system and cause synaptic discharge, depolarization and ultimately death (Roberts et al., 1999). Pyrethroids, synthetic analogues of pyrethrins, belong to the chemical group of nonsystemic insecticides (Velisek et al., 2009). Like most pyrethroids, is also an ATP-ase inhibitor. Because they are highly lipophilic, pyrethroids are likely to be strongly absorbed by the gills, even from water containing low levels of pyrethroids (Smith et al., 1986).

Studies with typical end-use products indicate that folpet is highly toxic to both rainbow trout and bluegill sunfish. Rainbow trout were the most sensitive species and the folpet product tested was classified in the very highly toxic range of toxicity for his species (Pesticide Information Project of Cooperative Extension Offices of Cornell University, *Folpet*,). The 96-hours LC50 for bluegill

sunfish is 675 ppb and the 96-hours LC50 for rainbow trout is 185 ppb (Pesticide Information Project of Cooperative Extension Offices of Cornell University, *Folpet*,).

Thiourea is an organic compounds consisting of carbon, nitrogen, sulphur and hydrogen with the formula that is $SC(NH_2)_2$. Thiourea is a white and sparkling crystal in structure. The function of thiourea in aqueous solution is expected to be the protector from hydroxyl radicals (Mohamed et al., 2009). Thiourea derivatives and polyazomethines constitute important classes of chelating agents. Thiourea derivatives have been extensively studied as a potential anti-TB, anti- HIV, anti-tumor and plant growth regulators (Li et al., 2003; Liav et al., 2008; Kucukguzel et al, 2008). Polyazomethines have been widely studied for their high thermal stability, mechanical properties, electrical and magnetic properties, liquid–crystal properties, and non-linear optical properties (Cerrada et al., 1997; Kaya et al., 2006). Consideration of the high activity of a new thiourea derivative chelating macrocyclic ligand N, N--pyridine–2,6-diyl-bis [N--phenyl(thiourea)] (PDPT) has prompted us to undertake systematic studies on the complexation tendency (Nasser et al., 2010).

One of the interesting characteristics of thiourea, an organic compound is to act as free radical scavenger (Mohamed et al., 2009).The SH-groupings of thiourea manifests an antitoxic action blocked herbicides (Păunescu et al., 2005).

The aim of the present study was to compare the effect of Folpan 80 WDG and thiourea used in single and mixed solutions on the oxygen consumption and breathing frecvency of prussian carp (*Carassius auratus gibelio* Bloch 1782).

2. MATERIAL AND METHOD

Determinations were made between September and November 2011 on prussian carp (*Carassius auratus gibelio* Bloch 1782), caught in the surrounding lakes and rivers of Piteşti city. We choose this species of fish because these are the most frequent in Arges River. After 10 days of adaptation in the lab, when they were fed *ad libitum* once a day, the fish were separated in lots, which were used separately for the following experiments:

The first experiment was carried out with prussian carps individuals having an average weight of 34.16g, which were subjected to thiourea 1‰.

The second experiment was carried out with ten prussian carp individuals having an average weight of 37.18 g, which were subjected to Folpan 80 WDG concentrations of 30x10-□g Folpan 80 WDG /l water.

The third experiment was carried out with ten prussian carp individuals having an average weight of 30.24g, which were subjected to Folpan 80 WDG /l water concentrations of $6x10^{-4}$ g Folpan 80 WDG /l water.

The fourth experiment was carried out with ten prussian carp individuals having an average weight of 33.64g, which were subjected to Folpan 80 WDG /l water concentrations of $30x10^{-5}$ g Folpan 80 WDG /l water and thiourea 1‰.

The fifth experiment was carried out with ten prussian carp individuals having an average weight of 36.48g, which were subjected to Folpan 80 WDG /l water concentrations of $6x10^{-4}$ g Folpan 80 WDG /l water and thiourea 1‰.

The sixth experiment was carried out with ten prussian carp individuals having an average weight of 35.76g, which were control lot.

The Folpan 80 WDG and thiourea concentrations that have been used have been established by preliminary survival test. The immersion of fish in these solutions has been made after they have been well stirred and aired for five minutes. The water temperature has been between 18º Celsius and 19º Celsius and the immersion solution has been changed every 24 hours and the water has been continuously aired; the fish have not been fed during the experiments, in order to avoid the intervention of this factor (Picos and Năstăsescu, 1988). The energetic metabolism, expressed by the oxygen consumption, was determined by using the closed respiratory chamber method (the oxygen dose in the water was established by using the Winkler chemical method) (Picos and

Năstăsescu, 1988). These determinations were made at intervals of 6, 24, 48, 72, 96, 168 and respectively 336 hours. However, in some cases the determinations were made at intervals shorter than 24 hours from the immersion. The breathing frequency was determined at the same intervals as in the case of the energetic metabolism. There have been made determinations of oxygen consumption and frequency of respiratory movements at intervals of 24, 48, 72, 96 and 336 hours on all samples of these lots.

3. RESULTS AND DISCUSSIONS

In studied concentrations, the Folpan 80WDG mg/l water ($30x10^{-5}$ g Folpan 80 WDG /l water, $6x10^{-4}$ g Folpan 80 WDG/l water) and thiourea 1‰ modified the values of breathing frequency as shown in figure 1.

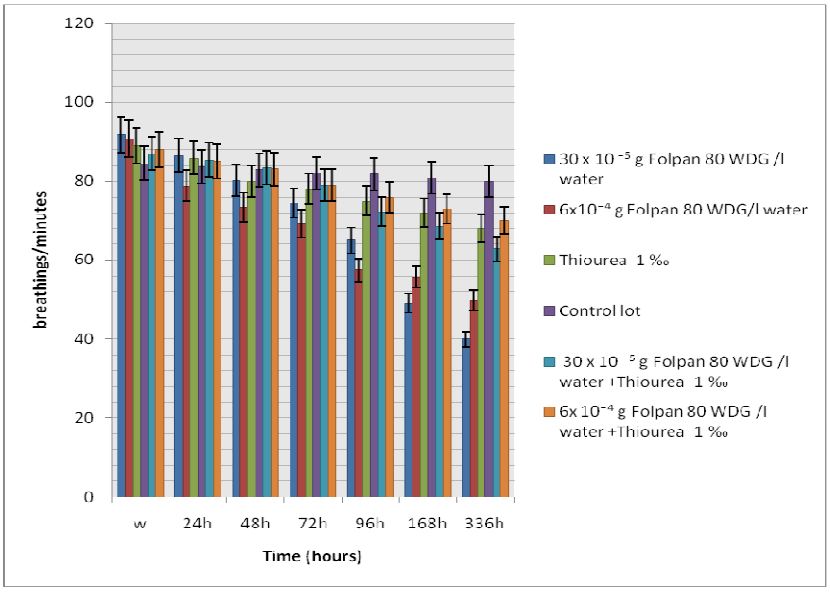

Figure 1. The influence of Folpan 80WDG and thiourea upon breathing frequency on prussian carp

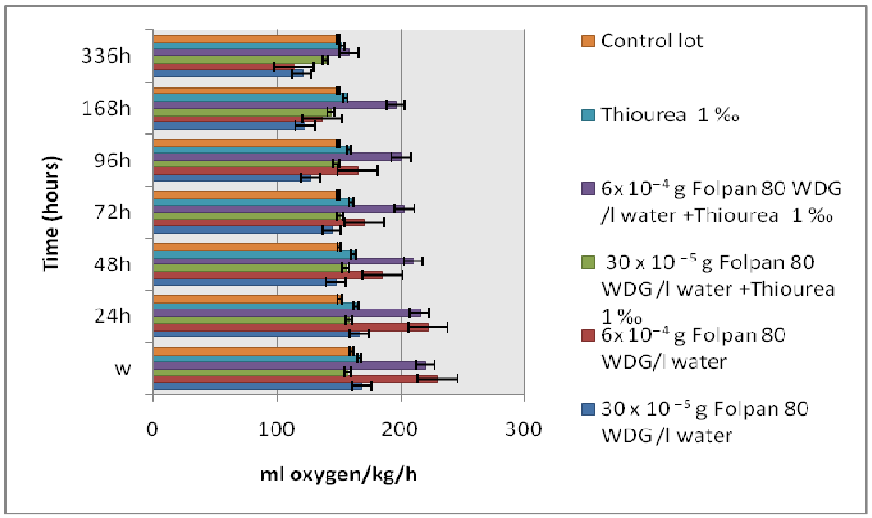

Figure 2. The influence of Folpan 80WDG and thiourea upon oxygen consumption on prussian carp

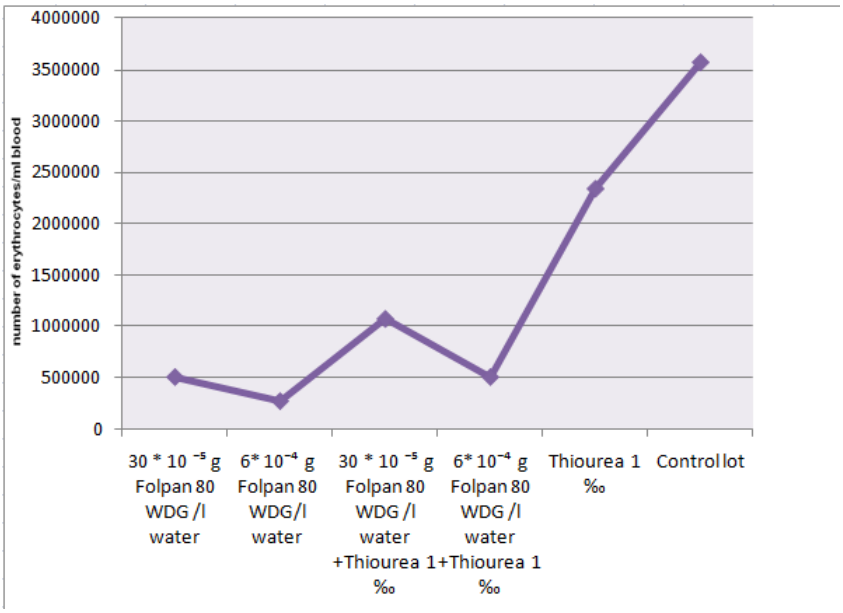

Figure 3. The influence of Folpan 80WDG and thiourea upon number of erythrocytes on prussian carp

The oxygen consumption was found to be significantly influenced by the concentration of the used Folpan 80WDG into the water. Thus, as shown in Figure 2, in all concentrations of Folpan 80WDG, this index decrease after 24 hours of immersion, oxygen consumption decreases significantly.

Marinescu et al., (2004) and Ponepal et al., (2009a-c) has also been noticed decreased oxygen consumption under the action of some pesticides and changes in respiratory rate. For all the concentrations investigated, Folpan 80WDG produced, since the first hours of exposure, a significant reduction in the oxygen consumption of the carp, as an index of their energy metabolism, reducing toxic directly proportional to the concentration and duration of exposure to its action. When Folpan 80WDG solution was replaced every time, *Carassius auratus gibelio* Bloch *(*1782) was vivacious at the beginning and then relaxed. Folpan's effects on prussian carp are positively related to the concentrations of the chemical, suggesting an obvious dose-response relationship. As for the mortalities of $25x10^{-5}$ g Folpan 80 WDG, only one piece of prussian carp died on the 10th day. In $6x10^{-3}$g Folpan 80 WDG concentration the mortality of prussian carp was 2 on the 12 th day and increased to 4 on the 14^{th} day. The mortality of $6x10^{-3}$g Folpan 80 WDG and thiourea 1‰ was 3 on the 14th day It is suggested that water organisms can be poisoned by Folpan 80 WDG such as prussian carp, and the toxicity relies on the chemical concentrations and the exposure duration.The Folpan's toxic effect was proven to be more powerful in the first 24 hours from the fish's immersion.

Respiratory irregularities are thought to be caused by mucus precipitation on the gill epithelium in response to a toxicant (Schaumburg et al., 1967). The use of respiratory stress to monitor sublethal effects of intoxication was previously applied to a variety of toxicants and subjects (Schaumburg et al., 1967; Walden et al., 1970). This may result in a decrease in the dissolved oxygen at the gill surface, initiating the cough reflex which is an attempt to clean the respiratory surface.

The Folpan 80WDG, in all concentrations, has changed the fish respiratory rhythm and oxygen consumption, but, the mixture between Folpan 80WDG and thiourea determined a smaller decrease. The thiourea 1‰ decresed oxygen consumption and repiratory rhythm significantly after 24 hours. The mixture because the SH – groupings of tissual tioenzymes are protected against action of toxic substances, and the enzymes under discussion remain totally or partially unaltered accordind to the

quantity of isothiourea (Picoş et al., 1983; Păunescu et al., 2005), maintain he physiological indices steady. Haematological studies in fishes have assumed greater significance because these parameters were to be used as an effective and sensitive index to monitor physiological and pathological changes induced by natural or anthropometric factors (Dhembare et al., 2000). Prussian carp anemia could be due to hypoxia that was induced by injuring the gills, as the red-pink colour of the gills became red-white, and at high concentrations the gills completely lost their red colour, while abundant secretions of mucus and even mucosal detachment with abundant bleeding could be observed (Figure 3).

4. CONCLUSIONS

Folpan 80 WDG produced, in all organized experimental variants a decrease in respiratory frequency and consumption of oxygen in the case of prussian carp, the more powerful the higher the concentration of the toxic was.

After two weeks of exposure to the $6X10^{-4}$ g Folpan 80 WDG number of erythrocytes in prussian carp decrease significantly compared to the control groups, can say that these concentration produce hemolysis, is impaired erythropoiesis.

The Folpan 80WDG has changed the respiratory rhythm and oxygen consumption of prussian carp in all concentrations.

The antitoxic action of thiourea manifests itself by the fact that Folpan 80WDG are blocked by SH-groupings isothiourea, the mixture between Folpan 80WDG and thiourea produced no significant changes on the parameters physiological.

5. ACKNOWLEDGMENTS

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