

Research on the changes of some physiological parameters in several fish species under the action of the thiametoxame insecticide

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Introduction

The thiamethoxame is an insecticide which belongs to a new class – the ‘neonicotine’ class, the third group of toxicity, and becomes active through contact and ingestion. The effect is that it inhibits the nicotine – acetylcholinic receivers of the nervous system in insects (it blocks movement and food ingestion).

The product investigated in our experiments – Actara (250 mg/l of thiamethoxame) has been initially introduced to control pests in the cotton fields; this insecticide controls pests efficiently, being used for the protection of crops and vegetables against bed bugs, the Colorado potato beetle, thrips, aphides, flea beetles, whiteflies, stink bugs, etc (5).

The thiamethoxame is considered a weak toxic product for fish, as in trout, for instance, its acute toxicity was observed only in concentrations higher than 100 mg/l (LC 50 after 96 hours).

Material and method

The concentrations of Actara used in all experiments and treatments were established by conducting preliminary survival tests. Since the toxic substance (the thiamethoxame) is highly soluble in water, irrespectively the species, the fish individuals were introduced into water after only 5 minutes of mixing and aeration. The water temperature was maintained at 18-20⁰C, and the ‘immersion’ solution was permanently aerated and changed every 48 hours. The fish were not fed during the experiments in order to avoid the additional influence of this factor.

Determinations were made between January and April 2005 on fish belonging to three species: prussian carp (*Carassius auratus gibelio* Bloch), bleak (*Alburnus alburnus*) and perch (*Perca fluviatilis*) caught in the surrounding lakes of Pitesti city. After 10 days of adaptation in the lab, when they were fed *ad libitum*, the fish were separated in lots, which were used separately for the following experiments:

The first experiment was carried out with prussian carps individuals separated in five lots, each lot being subdivided in two sublots:

- (1) individuals younger than one year, with an average weight of 8.52 g (C₀);
- (2) individuals with an average weight of 37.84 g (C₁).

The concentrations of thiamethoxame used in the first experiment were those of 16, 32, 160, and respectively 320 mg/l water. In order to determine the number of erythrocytes, two different lots of prussian carp, with an average weight of 34.58 g, were used.

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The second experiment was carried out with bleak individuals having an average weight of 1.2 g, which were subjected to thiamethoxame concentrations of 8, 16, and respectively 160 mg/l water.

The third experiment was carried out with perch individuals having an average weight of 12.56 g, which were subjected to thiamethoxame concentrations of 8 and respectively 16 mg/l water.

The following physiological parameters were investigated: (a) the lifespan (for all treatments); (b) the energetic metabolism; (c) the breathing frequency; the number of erythrocytes.

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) (Picoş and Năstăsescu, 1988). These determinations were made at intervals of 24, 48, 72, and respectively 120 hours. However, in some cases the determinations were made at intervals shorter than 24 hours from the immersion.

The breathing frequency was determined (only for the first treatment), at the same intervals as in the case of the energetic metabolism.

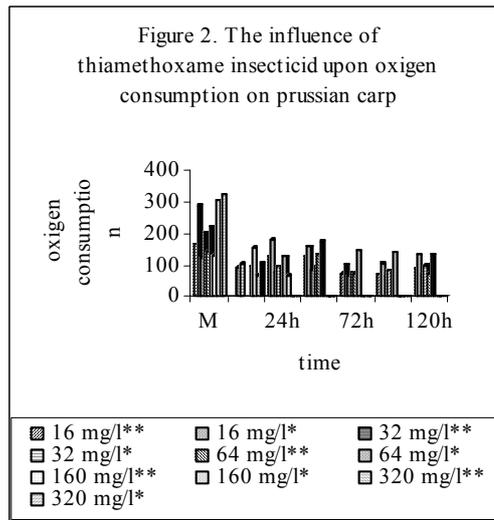
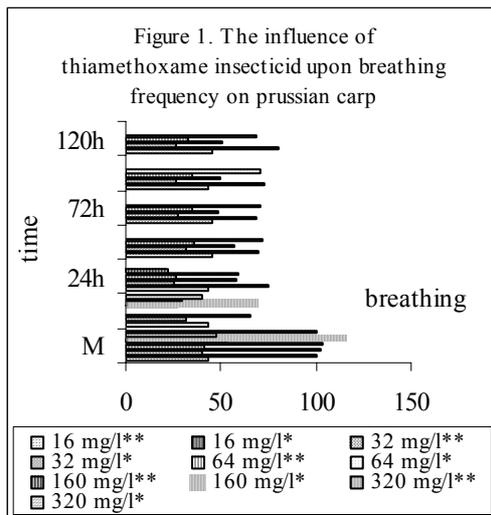
The number of erythrocytes was microscopically determined with a Thoma cells numbering chamber, by using a small amount of blood collected from the caudal artery (Picoş and Năstăsescu, 1988). The measurements were carried out only for the 7 days treatments with prussian carp individuals subjected to a concentration of 16 mg of thiamethoxame per liter of water.

Our dates was statistically interpreted using the Anova test.

Results and discussion

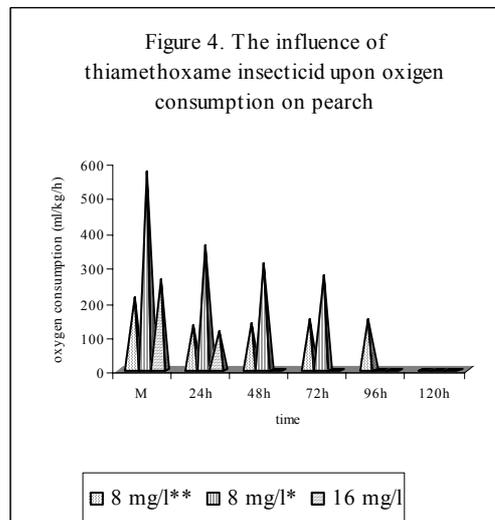
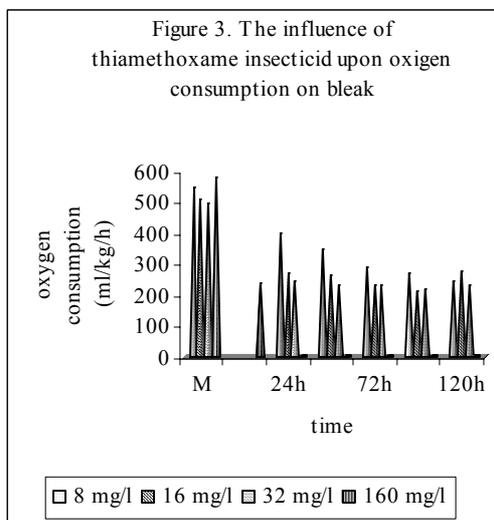
With all concentrations, excepting those of 16 mg/l water in prussian carp fish individuals younger than one year, the thiametoxame caused a slowdown in breathing (shallow breaths), as shown in Figure 1. The final values (after 120 hours of immersion), for a concentration of 32 mg thiamethoxame per liter of water, decreased at about 50% of the control value (established before the immersion) – significantly difference for $p < 0,05$. At this concentration, breathing slows down to almost 15% as compared to those recorded for the concentration of 16 mg/l water. As for the concentrations of 160 and 320 mg insecticide per liter of water, respectively, the decrease of respiratory rhythm occurred in the first six hours of immersion.

The oxygen consumption was found to be significantly influenced by the concentration of the used insecticide into the water. Thus, as shown in Figure 2, at a concentration of 16 mg/l water, this index decreases for both lots, since after 5 days of immersion the respiratory metabolism diminished to 46.05% of the control value for the C_0 generation, and respectively to 52.80% for the C_1 generation (significantly differences for $p < 0,05$). As for the concentration of 32 mg thiamethoxame per liter of water, the decrease of oxygen consumption occurs during the first 24 hours of immersion. These values were found to stabilize during the next days.



The decrease of oxygen consumption upon the action of some pesticides (Dithane M-45, 30 and 50 mg/l) was observed by Marinescu et al (2004).

The results of measurements on bleak individuals clearly revealed that higher the concentration, the more powerful was the toxic effect (Figure 3). Thus, lower values of oxygen consumption were found after 24 hours of immersion. The recorded values ranged from 72.7% (in a concentration of 8 mg/l water) to 41.65 % (in a concentration of 160 mg/l water) of the control value (significantly difference for $p < 0,05$). For the concentrations of 160 and 320 mg insecticide per liter of water, the decrease of respiratory rhythm occurred during the first six hours of treatment.

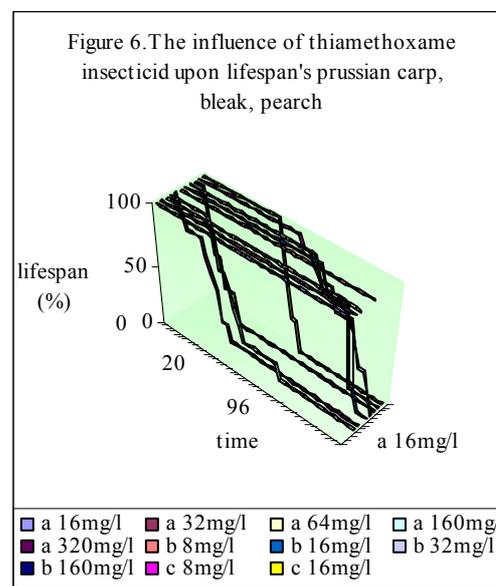
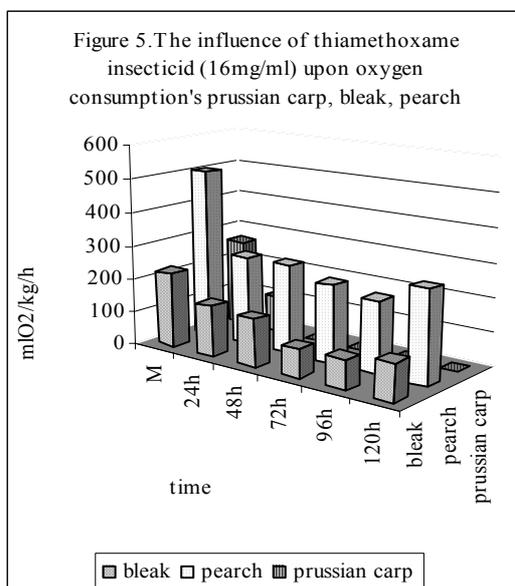


When the perch individuals having an average weight of 1.3 g were subjected to a concentration of 8 mg insecticide per liter of water, after a rapid decrease of oxygen consumption in the first 24 hours of immersion (62.73% of the control value), the values of this index decreased slowly in the next few days, so that, after the fourth day of treatment, the average value was only 43.29% of the control value for the fish which survived (Figure 4). A similar evolution of this index was found for the fish individuals with an average weight of 12.63 g, which resulted in 100% mortality during the 5 days of the experiment. In a concentration of 16 mg insecticide per liter of water, the oxygen consumption decreased by 68.22% of the control value during the first 24 hours, and 75% of the fish individuals died in the first 48 hours of immersion. Similar results were obtained for the concentration of 64 mg

insecticide per liter of water. However, some differences occurred for the recorded values in the second day of immersion, when we noticed a metabolic ‘recurrence’ for the C₀ generation.

With the concentrations of 160 and 320 mg insecticide per liter of water, the toxic effect of the thiametoxame was proven to be much more powerful, as all the fish individuals died even during the acute test.

Analyzing comparatively the evolution of oxygen consumption in the three species investigated, when subjected to a concentration of 16 mg insecticide per liter of water, we found that the decrease (in percentage) of this metabolic index, in bleak, was similar to those recorded for the prussian carp; unlike the prussian carp, in the bleak, a 16% mortality was recorded after the four days interval of the acute test. On the other hand, in the case of perch, the effects were different: after the decrease of respiratory metabolism recorded after the first day of immersion (the oxygen consumption does not even reached 30% of the control value), the toxic substance had a lethal effect during the acute test (96h).



As shown in Figure 6, the ‘survival curves’ revealed that sensitivity of the investigated fish species to thiametoxame was different, with the perch being the most sensitive and the prussian carp being the less sensitive. The thiamethoxame had the greatest toxicity in a concentration of 320 mg/l water on small prussian carp (in which the average lifespan was found to be only 3 hours).

The number of erythrocytes in the fish individuals subjected for seven days to immersion into water with 16 mg/l of thiamethoxame, was also significantly affected. The difference between the number of erythrocytes which was determined for the control (1616250 per ml) and the ‘treated’ lot (1511437 per ml), an average decrease of 6.49% was found in the treated fish individuals, which seemed to be related to the acute decrease in the oxygen consumption.

The decrease in RBC after 7 days exposure to LC₅₀ of some insecticides in fish was observed by Dhembare and Pondha (2000). Similarly results was obtained in carp by Hughes et al (1995) after a brief exposure to Methadathion.

Conclusions

The thiamethoxame have had in all experimented concentrations (8, 16, 32, 64, 160, and respectively 320 mg/l water) an inhibiting effect on the energetic metabolism and the

breathing frequency (excepting the prussian carp in the C₁ generation, in a concentration of 16 mg insecticide per liter of water) in the fish species investigated (prussian carp, bleak, perch).

A concentration of 16 mg/l of thiamethoxame caused a decrease by 6.49% in the number of erythrocytes in the prussian carp, after seven days of immersion.

The thiamethoxame's toxic effect was proven to be more powerful in the first 24 hours from the fish's immersion. However, in some cases, after this period of time, a certain degree of 'adaptation' was observed.

From the three investigated fish species, the perch proved to be the most sensitive to the action of the toxic substance, followed by the bleak and the prussian carp.

Summary

In recent years, sustained research has been conducted on obtaining an efficient insecticide to fight against pests. Such an insecticide is supposed not to cause the resistance effect or to harm the environment. We investigated the action of such a product – Actara (in which the thiamethoxame is the active substance), in different concentrations, on some physiological indices – lifespan, oxygen consumption, breathing frequency and the number of erythrocytes – in the case of three species of fish: prussian carp (*Carassius auratus gibelio* Bloch), bleak (*Alburnus alburnus*) and perch (*Perca fluviatilis*).

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