

STUDY ON SOME TOXIC EFFECTS OF THE INSECTICIDE FENDONA 15 SC ON THE PRUSSIAN CARP (*CARASSIUS GIBELIO*, BLOCH, 1782)

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Abstract

*Fendona 15 SC (15g/L alpha-cypermethrin formulation) is an insecticide with fast and reliable action against a broad range of insect pests. The toxicological effects of Fendona 15 SC (0,0002 ml/L water and 0,0004 ml/L water) upon prussian carp (*Carassius gibelio*, Bloch, 1782) recorded respiratory, haematological, biochemical and behavioral changes. The experiments were of acute and subchronic type and the semistatic test method was used. Data obtained from the toxicity tests were evaluated using the one-way analysis of variance (Anova) method.*

Keywords: Fendona 15 SC, prussian carp, erythrocytes, blood, behavior.

1. INTRODUCTION

Among aquatic organisms, fish have suffered especially, following the use of pesticides (Shankar et al., 2013; Jabeen et al., 2015). Pesticide residues can persist in the aquatic environment long after their application, due to their low biodegradability (Bálint et al., 1997).

Pyrethroids are synthetic derivatives of pyrethrins and have been employed as substitutes for organochlorines, organophosphates and carbamate insecticides because of their low persistence in the ambient and comparatively lower mammalian toxicity (Aldridge, 1990; Parvez and Raisuddin, 2006). Because they do not contaminate groundwater, pyrethroid insecticides are a good alternative to conventional insecticides (Bradberry et al., 2005).

Pyrethroids are from commonly used insecticides worldwide (Kuivila et al., 2012; Alonso et al., 2012) due to their short biodegradation period and weak tendency to accumulate in organisms (Laskowski, 2002). Pyrethroids have been widely used in agriculture and to control ectoparasitic infections in humans and animals (Barbini et al., 2007; Anadón et al., 2009).

Except that this class of pesticides shows a low toxic action for birds and mammals (Bradbury and Coats, 1989), pyrethroids are pesticides with high risk for aquatic organisms with high toxicity for fish (Dobsikova et al., 2006; Velisek et al., 2006, 2007; Bille et al., 2017).

The toxic effect of pyrethroids in fish increases with the increase of their fat solubility (Muir et al., 1985); due to their strong lipophilic character, pyrethroids are well absorbed by the gills, even from waters with a low content of these pesticides (Smith and Stratton, 1986; Moore and Waring, 2001). Because fish are not able to produce the enzymes that hydrolysis the insecticides, the metabolism

and elimination of these compounds being very slow, the elimination rate of some pyrethroids in trout is greater than 48 hours, while the elimination rate in mammals is 6-12 hours) (Bradbury and Coats, 1989; Haya, 1989).

Pyrethroid insecticides can bioaccumulate in fish, with the bioaccumulation factor being 1200 X for cypermethrin and 400 for esfenvalerate in *Oncorhynchus mykiss* (Walker and Keith, 1992).

Although a large number of specialized works report the possible negative impact of the cypermethrin on aquatic ecosystems (Selamoglu, 2018; Khafaga et al., 2020), the insecticide is one of most broadly used pesticide (Corcellas et al., 2015; Farag et al., 2021) in a broader range of crops (Carriquiriborde et al., 2007; Ullah and Zorriehzahra, 2015)

Jaensson et al. (2007) reported high levels of cypermethrin in the surface water. Laabs et al. (2002) revealed cypermethrin in rainwater at 0.376 g/L concentration.

Cypermethrin is highly toxic to fish, which is why it is classified as a restricted use pesticide by the EPA (Saha and Kaviraj, 2009). Important toxic effects of this insecticide, including lethal effects, have been reported, leading to important economic losses (Farag et al., 2021).

In laboratory tests, the 96-hour LC₅₀ has a rate of 0.4–2.8 µg/l (Stephanson, 1982; Bradbury and Coats, 1989; Davis et al., 1993; Polat et al., 2002; Sarkar et al., 2005; Corcellas et al., 2015). In some works, however, the 96 h LC₅₀ value of cypermethrin was higher: 27.07 g/l on the guppy fish (Salako et al., 2020), 38.38 g/L on *Poecilia reticulata* males (Dangi et al., 2012).

The 24-h LC₅₀ value of alpha cypermethrin for 20.0 µg/l for silver barb and 4.50 µg/l for common mirror (Grayson et al., 1990). Whalon et al. (1990) find 24-hour LC₅₀ of 4.50 µg/l and 20 µg/l for *Cyprinus carpio* L. and *Ctenopharyngodon idela*, respectively. Polat et al. (2002) found the 48-h LC₅₀ value of beta cypermethrin in male guppies as 21.4 µg/l.

Cypermethrin can induce genotoxicity and oxidative stress in *Danio rerio* (Paravani et al., 2018, 2019), malformations in *Labeo rohita* during the early developmental stages (Dawar et al., 2016), DNA damage, apoptosis, and histopathological alterations in *Cyprinus carpio* (Khafaga et al., 2020), hepatotoxicity in *Catla catla* (Sharma and Jindal, 2020), neurotoxicity and apoptotic changes in the brain of *C. catla* (Jindal and Sharma, 2019).

Low levels of cypermethrin having a significant long-term effect on Atlantic salmon populations through disruption of reproductive functions (Moore and Waring, 2001).

This work aimed to assess the toxicological effects induced by Fendona 15 SC insecticide (active substance alpha cypermethrin 15g/L) on a non-target species - prussian carp (*Carassius gibelio*, Bloch, 1782) - by evaluating oxygen consumption, respiratory rate, blood figure elements, glucose and behavioral change.

2. MATERIALS AND METHODS

The biological material used is prussian carp samples (*Carassius gibelio*, Bloch, 1782), with an average weight of 16.28 ± 3.48 g, caught from the Argeş River.

The concentrations that have been used have been established by study of the literature on cypermethrin levels reached in surface waters and preliminary survival test. The immersion of fish in these solutions has been made after they have been well stirred and aired for five minutes.

Fish were kept in 100 L glass aquaria with gently aerated tap water, at dissolved oxygen 7.45 ± 0.58 mg/L, pH 7.85 ± 0.5, temperature 18-20°C, total hardness 100 mg/L CaCO₃ with a natural light: dark photoperiod (according to the protocol previously described by Păunescu et al., 2022). The immersion solution has been changed every 24 hours by transferring the fish to another aquarium

and the water has been continuously aired (semi-static method). The fish have not been fed during the experiments, in order to avoid the intervention of this factor (Picoş and Năstăsescu, 1988).

After 10 days of adaptation in the lab, when they were fed *ad libitum* once a day, the fish were separated into three groups of 10 specimens each, as follows: the control group, without the addition of insecticides, and two experimental groups exposed to Fendona 15 SC insecticides at concentrations of 0.0002 and 0.0004 ml/l (3 and 6 µg L⁻¹ alpha cypermethrin).

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), by chemical dosing of oxygen dissolved in water with thiosulfate (Picoş and Năstăsescu 1988). These determinations were made at intervals of 24, 48, 72, 96, 168 and respectively 336 hours. The breathing frequency was determined at the same intervals as in the case of the energetic metabolism.

Blood samples were taken from the caudal artery (according to the method described by Picoş and Năstăsescu, 1988) after 14 days of setting up the experiments and the average number of erythrocytes and leukocytes was determined (with Thoma chamber to Olympus microscope according to the method described by Picoş and Năstăsescu, 1988). Glucose determination was performed from blood drops with an Accutrend Plus GCT device.

Values are given as arithmetic means ± standard error of the mean (SEM). The data were statistically analysed using one-way analysis of variance (ANOVA) was performed to compare among experimental variants. Significance was accepted at p<0.05.

3. RESULTS AND DISCUSSIONS

By determining the oxygen consumption of fish kept in toxic solutions and by recording the respiratory movements, the concentrations at which changes in respiration begin to appear can be determined. As a result of fish exposure to pesticides, Gray and Söderlund (1985) reports cardio-respiratory changes and blood chemical parameter changes (increased glucose, oxygen consumption).

The gills of fish are the main site of ionic exchanges with the environment and also the route through which toxic substances enter. Being in constant contact with water, any change in water composition can damage gill permeability and their functions.

The respiratory rate of prussian carp intoxicated with Fendona 15 SC is constantly reduced during the experiment for both investigated concentrations, the values determined after 14 days of exposure being 27.87 and 41.38% lower, respectively, compared to the values determined before immersion in the toxic (Table 1).

Table 1. Variations in the average respiratory rate (breaths/minute) and the standard deviation of the *Cassius gibelio* exposed to Fendona 15 SC insecticide at two concentrations

Lots	0 h	24 h	48 h	72 h	96 h	168 h	336 h
Control	65.26±4.41	64.52±4.36	62.14±4.44	61.48±3.64	62.62±6.52	61.98±3.26	61.56±2.44
Lot I Fendona 15 SC 0.0002 ml/l	61.24±2.14	56.74±4.36*	53.55±4.24*	51.74±3.66*	46.25±2.58*	42.32±3.83*	44.23±5.38*
Lot II Fendona 15 SC 0.0004 ml/l	58.42±2.14	52.46±6.62*	48.25±3.58*	44.42±6.12*	39.54±4.26*	36.54±2.28*	34.56±2.51*

* the mean difference is significant at the 0.05 level

Fish consuming a higher amount of energy to alleviate toxic stress (Ferrando and Moliner, 1992), which results in the improvement of oxygen use on hypoxia and even anoxia conditions.

Oxygen consumption in prussian carp exposed to Fendona 15 SC insecticide at two concentrations (0.0002 and 0,0004 ml/l) are shown in Table 2.

Table 1. Variations in the average oxygen consumption (ml oxygen/kilogram/hour) and the standard deviation of the *Cassius gibelio* exposed to Fendona 15 SC insecticide at two concentrations

Lots	0 h	24 h	48 h	72 h	96 h	168 h	336 h
Control	165±18.58	168.84±28.36	162.36±20.44	160.58±18.54	158±21.58	162.67±11.74	161.66±12.14
Lot I Fendona 15 SC 0.0002 ml/l	162.5±26.3	175.8±14.2*	182.5±19.6*	164.4±20.6*	142.2±10.3*	138.3±11.3*	136.8±8.6*
Lot II Fendona 15 SC 0.0004 ml/l	164.4±15.85	176.4±11.6*	154.5±8.8*	136.2±11.1*	114.6±9.3*	95.7±12.4*	112.8±17.2*

* the mean difference is significant at the 0.05 level

In the case of exposure of the prussian carp to the insecticide Fendona in a concentration of 0.0002 ml L⁻¹, the oxygen consumption, as an indicator of the energy metabolism of the fish, changed significantly even after the first 24 hours after the immersion of the fish in the solution, registering an increase of 8.02% compared with the values determined before immersion in the toxic. After 48 hours of exposure to insecticides, the values of this parameter show a constant decrease until the end of the experiment, when the average value determined is 16% lower compared to the control values. At the concentration of 0.0004 L⁻¹, the values determined after 14 days show reductions of 32% compared to the control; the evolution of oxygen consumption is similar, with the difference that the reduction in metabolism occurs after 24 hours of exposure.

A similar situation was also described by Philip et al. (2002) to *Labeo rohita* subjected to sub-lethal concentration of cypermethrin under in vitro conditions.

In fish, pesticides first pass through the gills, so that any disorder at this level will have a great influence on the adaptive changes. Decreased oxygen consumption may also be due to insecticide damage to the gills. Many scientific papers mention this type of effect of cypermethrin: haemorrhages of secondary gill lamellae and other histopathological changes (Korkmaz et al., 2009; Andem et al., 2016; de Moraes et al., 2018) and changes in the activity gill ATP-ases (Suvetha et al., 2010).

Several reports demonstrated that the blood profile of fish species may be impacted by pesticide exposure (Banaee et al., 2011; Ullah and Zorriehzakra, 2015). Blood glucose, protein, and lipid concentrations could be utilized as necessary biomarkers for monitoring fish health under toxic stress (Javed et al., 2016).

Table 3 shows the mean values of erythrocytes, leukocytes and blood glucose for the three experimental groups.

After 14 days of exposure of the Prussian carp to Fendona 15 SC in concentrations of 0.0002 and 0.0004 ml/l we recorded significant decreases, both in the average number of erythrocytes (by 15.5 and 24.95%, respectively, compared to the control group) and in the average number of leukocytes (by 16.57 and 39.19%, respectively, compared to the control group).

Table 3. Mean number of figured elements and glucose with standard deviation in *Cassius gibelio* exposed to Fendona 15 SC insecticide at two concentrations

Lots	Red blood cells/ml blood	White blood cells/ml blood	Glucose (mg/100 ml blood)
Control	899 780 ± 5540	50 700 ± 5480	68.25 ± 8.40
Lot I Fendona 15 SC 0.0002 ml/l	760 400 ± 9 550*	42 300 ± 6420*	80.30 ± 9.12*
Lot I 15 SC 0.0004 ml/l	675 300 ± 4200*	30 780 ± 8400*	88.70 ± 7.80*

* the mean difference is significant at the 0.05 level

Similar results have been reported by other authors. Thus, a decrease in red blood cells was observed in long-term exposure of *Labeo rohita* to sublethal concentrations of cypermethrin (Das and Mukherjee, 2003; Adhikari et al., 2004). Anaemias resulting from exposure to cypermethrin for 14 and 21 days, respectively (0.015, 0.030 and 0.045 µg/l) were also reported by Babu Velmurungan et al. (2016) in *Anabas testudinaeus*. Reduction in the number of erythrocytes after exposure for 2.5 and 8 days to cypermethrin at concentrations of 0.3 and 0.6 µg/l was reported by Parma et al (2007) in *Prochilodus lineatus*.

Intoxication with Fendona 15 SC also resulted in a notable increase in blood glucose, for both investigated concentrations (by 17.65% more than in the control group at the concentration of 0.0002 ml L⁻¹ and by 29.96% at the concentration of 0.0004 ml L⁻¹).

Decreases in liver glycogen and increases in plasma glucose were also reported by Kaviraj and Gupta (2014) in acute tests performed on *Oreochromis niloticus* exposed to the action of the insecticide cypermethrin in concentrations of 1.25 and 2.5 µg/l and by Saha and Viraj (2009) in *Heteropneustes fossilis* after 4 hours of exposure to cypermethrin in concentrations of 0.3 and 0.5 µg/l. Also, by Moraes et al. (2018) found increases in blood glucose levels in *Brycon amazonicus* after 96 hours of exposure to cypermethrin at a concentration of 20% of the LC50.

Exposure to cypermethrin decreased total protein in muscle, gill, brain, and liver and increased blood glucose in *Tor putitora* (Ullah et al., 2014). Similar results were reported by Das and Mukherjee, 2003 and Nayak et al., 2004.

Also, Reddy et al. (1991) reported a decrease in glycogen and pyruvate levels of *Tilapia mossambica* exposed to sub-lethal concentration of cypermethrin.

No mortality was observed in the control group during the experiment. The changes in behavioural response started 1-2 hours after dosing, depending on the concentration of toxicant.

During the acute tissue (96 hours) mortality recorded was 20% for the concentration of 0.0002 ml L⁻¹ Fendona 15 SC water and 30% for the concentration of 0.0004 ml L⁻¹. In both experimental variants with insecticide, mortality was recorded during the experiment (14 days) - 40 and 50%, respectively.

After 24-48 hours from the exposure of the fish to the insecticide, in both tested concentrations, the swimming movements became weaker and weaker, the fish showed loss of balance, increased secretion of gill mucus.

Similar results were also reported by Yılmaz et al. (2004) reported that behavioral changes of male guppies manifested themselves starting at alpha cypermethrin concentration of 15 µg/L. Aendem et al. (2016) also reported erratic movement in specimens of *Clarias gariepinus* exposed to cypermethrin in different concentrations, and Sarikaya (2009) found behavioral changes in Nile tilapia intoxicated with the same insecticide. Edwards et al. (1986) report the presence of symptoms

of acute intoxication in trout exposed to cypermethrin: hyperactivity, loss of balance and the impossibility of maintaining the normal position.

Exposure to synthetic pyrethroids has resulted in various behavioral changes in fish: slow movement, disturbed swimming, inability to maintain position, reduced feeding, hypo- or hyperexcitability, increased respiratory movements, loss of balance, frequent surfacing, vertical position, sinking to the bottom, hypo- or hyperactivity, jumping, loss of balance, immobilization (Bradbury and Coats, 1989; Velisek et al., 2006, 2007; Ullah et al., 2014).

4. CONCLUSIONS

This paper is a contribution to knowledge of toxicity and effects of Fendona 15 SC insecticide on prussian carp (*Carassius gibelio*, Bloch, 1782).

Fendona 15 SC insecticide reduced the energy metabolism and the respiratory rate, decreases red blood cells and white blood cells, increase glucose level in blood, and induce behavioral changes in prussian carp.

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