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Research Article

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Acute Toxicity and Sublethal Effects of Methyl Parathion on Tambaqui (*Colossoma macropomum*)

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ABSTRACT

The aim of this study was to determine the acute toxicity and sublethal effects of methyl parathion on tambaqui (Colossoma macropomum). For this, juveniles of tambaqui (45.23 ± 0.43 g and 11.91 ± 0.08 cm) were transferred to tanks of 50 L and acclimated for 48 hours. For testing the acute toxicity the tambaquis were exposed to seven concentrations of methyl parathion (0.0; 1.0; 2.0; 3.0; 4.0; 5.0 and 6.0 mg L^{-1}), with three replicates, for 96 hours to determine the lethal concentration (LC_{50}). The water quality parameters were monitored during the assays and the feeding suspended in this period. After the determination of the LC_{50} fish were exposed to sublethal concentration and were evaluated the physiological responses by glucose and hematological analyses. The behavioral changes of the fish after the application of methyl parathion were: increase of opercular movements, erratic swimming, and exophthalmy. The LC_{50} 96 hours of methyl parathion for tambaqui was calculated to be 2.91 mg L^{-1}, indicating low tolerance of tambaqui to this organophosphorous. Although, the exposure of tambaqui to sublethal concentrations did not promote alterations in glucose and hematological indicators, that provided evidence of disturbances in the homeostasis of the species.

Keywords: *Colossoma macropomum*; Ecotoxicology; Fish culture; Organophosphorous

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INTRODUCTION

Tambaqui (*Colossoma macropomum*) is the main native species farmed in Brazil with a production of 136.99 thousand metric tons in 2016 [1]. The expansion in the tambaqui production is due to easy fry production, good acceptance of artificial and complete feed, high growth rate, desirable feed conversion ratio and resistance to low dissolved oxygen levels [2,3]. In intensive systems, tambaqui has been reaching weight of 2.62 kg and production of 18,530 kg ha^{-1} after 10 months of in ponds [3].

With the intensification of production systems, conditions such as high stocking densities, excessive feeding, inadequate fish management can contribute to fish chronic stress, with consequences to their immune system, which contributes to the development of various diseases, culminating in the use of chemotherapeutic agents and antimicrobials for treating parasitic and infectious diseases [4-9]. Among these, the pesticide are highlighted, whose indiscriminately use and without criteria puts at risk the entire food chain, the environment and human health [10-12].

Methyl parathion (O, O-dimethyl O-4-nitrophenyl phosphorothioate) is a non-systemic insecticide and acaricide organophosphate, is highly toxic to aquatic organisms and classified as extremely dangerous to the environment [13]. This organophosphorous has been used in aquaculture to avoid losses of fish by *Odonata nymphs* and

Chironomidae predation as well to control fish parasites as monogenean [14,15]. However, some studies show that exposure to organophosphorous in inadequate concentrations may result in inhibition of acetylcholinesterase activity in fish and impair fish reproduction [16-19], in addition to causing histological changes in gill lamellae [20] and cellular changes in the fish liver tissue [21].

Due to methyl parathion toxicological potencial, it is necessary to carry out ecotoxicological studies to evaluate the toxicity and adverse effects caused by these chemicals on aquatic organisms [21-23] especially to native fish whose production has been growing in recent years. Considering that, the tambaqui stands as an important species to be used as a test organism in acute toxicity studies in reason of being the most created species in northern Brazil [1], which registered the use of methyl parathion by fruit and vegetable farmers in Amazon floodplain areas [24] and also meet the standards of [25] recommending the use of fish belonging to the family Characidae. Therefore, the aim of this study was to determine the acute toxicity and sublethal effects of methyl parathion in tambaquis (*Colossoma macropomum*).

EXPERIMENTAL SECTION

Tambaqui juveniles (n=216; 45.23 ± 0.43 g and 11.91 ± 0.08 cm) were purchased at Fazenda Santo Antonio, Rio Preto da Eva, AM and transported to the experimental field of Embrapa Amazônia Ocidental, where they were acclimated in ponds of 200 m^2 for 30 days, receiving commercial feed for omnivorous fish with 32% crude protein (CP).

Preliminary tests were carried out to determine the approximate range of harmful effects of methyl parathion in tambaqui, to establish the lowest concentration that causes mortality to 100% of the organisms and the highest concentration at which was observed no mortality to use in the definitive test.

To determinate the mean lethal concentration (LC_{50}) of methyl parathion was used the commercial product Folisuper 600® (i.a. 600 g L^{-1} of methyl parathion). In the test, the tambaqui juveniles were transferred to polyethylene tanks with the volume fixed at 50 liters. After an acclimatization period of 48 hours, they were exposed to different concentrations of methyl parathion (0.0; 1.0; 2.0; 3.0; 4.0; 5.0 and 6.0 mg L^{-1}) for 96 hours.

The experimental tanks were equipped with static systems with constant aeration. The tests were performed in triplicate with groups of eight animals, fish feeding was suspended 24 hours before the start of testing and during the same. The dead animals during the LC_{50} 96 hours assays were counted and removed after 24, 48, 72 and 96 hours of the application of methyl parathion in water. During this period, observations were made in the behavior pattern of the fish, regarding loss of balance, increase of opercular movements and erratic swimming, and the occurrence of bleeding. The water quality parameters such as temperature (24.9 ± 0.70°C) and dissolved oxygen (7.3 ± 1.22 mgL^{-1}) were monitored using a YSI monitor 55, pH (5.31 ± 0.36) with a pH meter YSI - 100 pH, alkalinity (2.09 ± 0.24 mg $CaCO_3$ L^{-1}) determined by the titrimetric method EDTA, hardness (9.20 ± 2.59 mg $CaCO_3$ L^{-1}) by titrimetry with methyl orange and the total ammonia (0.07 ± 0.05 mg L^{-1}) by endofenol method (APHA, 1998), and these parameters daily assessed during the execution of experimental tests.

After determining the LC_{50} 96 hours of methyl parathion, an experiment was conducted to evaluate the possible effect of sublethal concentration of this organophosphorous in tambaqui. To this end, groups of eight fish were divided in six tanks of 50 liters with constant aeration and static system, corresponding to two treatments: control (0.0 mg L^{-1}) and sublethal concentration of methyl parathion (1.45 mg L^{-1}) with three repetitions in a completely randomized design, and the assay conducted for 96 hours. After the exposure, with the fish anesthetized (100 mg L^{-1} benzocaine), the blood harvested by caudal puncture with heparinized syringes for determination of blood glucose and hematological parameters.

Blood glucose was measured in digital reader (Advantage™), which method has been validated for use with tambaquis [25]. The hematological parameters determined were hematocrit (Ht) using microhematocrit metod, hemoglobin concentration (Hb), according to the method of cyanmethaemoglobin and red blood cells (RBC) count made in hemocytometer [26,27]. These data were used to calculate the Wintrobe hematimetric as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

The LC_{50} 96 hours of methyl parathion was determined by the Trimmed - Spearman Karber method [28], using the mortality rates of tambaquis. Values of glucose and hematological parameters of control group fish and those exposed to sublethal concentrations of methyl parathion were compared by the Student's T test at 5% probability.

RESULTS AND DISCUSSION

No mortalities were recorded in the control and in 1.0 mg L^{-1} of methyl parathion during the 96-hour exposure. On the other hand, no fish survived at concentrations of 5.0 and 6.0 mg L^{-1} in 96 hours of exposure to the organophosphorus (Figure 1). Mortality rate was directly proportional to the increase in concentration of methyl parathion added to the water in the experimental tanks, demonstrating a good dose-response relationship (Figure 1).

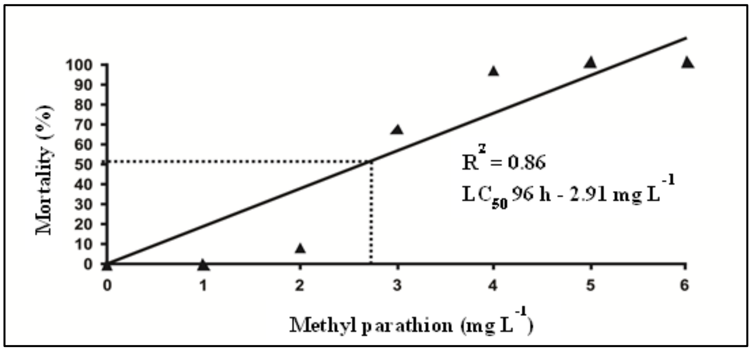

Figure 1: Mortality, after 96 hours, of juvenile tambaqui (*Colossoma macropomum*) exposed to different concentrations of methyl parathion

LC_{50} 96 hours of methyl paration for tambaqui was calculated at 2.91 mg L^{-1}, similar to the value obtained for tilapia (*Oreochromis niloticus*) which was 2.7 mg L^{-1} [19] while LC_{50} 96 hours values greater than these were seen in silver catfish fingerlings (*Rhamdia quelen*) and juvenile of matrinxã (*Brycon amazonicus*), mosquitofish (*Ganbusia affinis*) and pacu (*Piaractus mesopotamicus*), who had their LC_{50} 96 hours established at 4.8 ; 6.0; 9.86 and 8.4 mg L^{-1} of methyl parathion, respectively [16,29,30,31]. This result demonstrates that tambaqui has a low tolerance to this organophosphate.

Generally, for all concentrations tested fish showed signs of asphyxia, repeatedly rising to the surface for oxygen uptake, showing themselves as hyperactive. An increase in the opercular movements and an intense release of mucus after 24 hours exposure were observed, followed by erratic swimming, exophthalmos and nosebleeds in tambaquis exposed to concentrations of 5.0 and 6.0 mg L^{-1} of methyl parathion. In several species of fish, it has been reported that the pattern of erratic swimming is due to the action of the pesticide in the inhibition of acetylcholinesterase [16,19], and the inhibition of this enzyme is also responsible for altering the breathing pattern as observed in Nile tilapia (*Oreochromis niloticus*), which showed a decrease in respiratory rate indicating the occurrence of lesions in the gills [19]. Tambaqui exposure to sublethal concentration of methyl parathion (1.45 mg L^{-1}) for 96 hours did not promoted changes in hematocrit, hemoglobin, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, as well as in blood glucose (Table 2). These results are contrary to those observed in Nile tilapia, where low concentrations (0.5 and 1.0 mg L^{-1} methyl parathion) for 6 hours causes changes in hematological parameters, whose response is attributed to possible injury in the gills, resulting in internal hypoxia and stimulation of erythropoiesis [19]. In this study, tambaquis exposed to sublethal concentration did not exhibit physiological responses that showed disorders in maintaining their homeostasis, since the levels of glucose and hematologic parameters evaluated are within the amplitude of variation for the species [32].

Table 2: Blood glucose and hematological parameters of tambaqui, *Colossoma macropomum*, after exposure to sublethal concentration of methyl parathion for 96 h

Parameters	**Methyl parathion (mg L^{-1})**	
	0	**1.45**
Ht (%)	34.19 ± 3.78 a	34.39 ± 2.90 a
Hb (g dL^{-1})	9.20 ± 1.28 a	9.72 ± 1.28 a
RBC (10^6 mm^{-3})	2.44 ± 0.32 a	2.50 ± 0.26 a
MCV (μm^3)	141.93 ± 19.86 a	139.44 ± 21.06 a
MCH (pg)	37.93 ± 4.13 a	39.21 ± 5.99 a
MCHC (%)	27.01 ± 3.39 a	28.40 ± 4.17 a
Glucose (mg dL^{-1})	41.61 ± 8.54 a	41.94 ± 10.26 a

Means followed by the same letter do not differ by Student's t test at 5% probability.
Ht - hematocrit, Hb - hemoglobin, RBC - red blood cell count, MCV - mean corpuscular volume, MCH - mean corpuscular hemoglobin and MCHC - mean corpuscular hemoglobin concentration.

The determination of LC_{50} 96 hours of methyl parathion for tambaqui is important to establish the limits of tolerance to the use of this organophosphorous, since methyl parathion is used indiscriminately in aquaculture to control aquatic insect larvae that prey on larvae fish, as well as controlling parasites [12,15]. Regarding the control of monogenoidea *Anacanthorus penilabiatus* in pacu, methyl parathion concentration used was 7 mg L^{-1} in 24 hours of exposure, which provided an 97% efficacy [15]. However, this concentration of methyl parathion that showed effectiveness in long-term bathing is far above the tolerated for tambaqui in 96 hours of exposure, therefore if used for this species it can cause mortality of animals during the administration period, highlighting the importance of conducting ecotoxicological studies in aquaculture due to the use of chemical products with high toxicological potential. Although, others studies are necessary to evaluate the toxic effects of methyl parathion in the inhibition of acetylcholinesterase (AChE) in different tissues and organs of tambaqui as well as others alterations that compromises heart function and others vital organs.

CONCLUSION

The LC_{50} 96 hours of methyl parathion for tambaqui was calculated at 2.91 mg L^{-1}, showing the high toxicological potential of this organophosphate to the species.
Tambaqui exposure to sublethal concentration of methyl parathion (1.45 mg L^{-1}) for 96 hours does not promote alterations in blood glucose and hematological parameters.

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