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Acute toxicity of pyrazosulfuron-ethyl and permethrin to juvenile *Litopenaeus vannamei*

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ABSTRACT. The objective of this study was to determine the LC_{50} (96h) of two pesticides: Sirius® 250 SC herbicide of the pyrazosulfuron-ethyl group, and Talcord® insecticide of the permethrin group, on juvenile *Litopenaeus vannamei*. Shrimp total hemocyte count (THC) was also determined as an indication of physiological alterations caused by the pesticides. Juvenile shrimp (5.0 ± 0.5 g) were exposed to the following concentrations: 0, 0.1, 1.0, 10, 100 and 1000 $\mu g\ L^{-1}$ Sirius® 250 SC; and 0, 0.001, 0.01, 0.1, 1.0 and 10 $\mu g\ L^{-1}$ Talcord®. The Talcord® LC_{50} (96h) was of 0.00933 $\mu g\ L^{-1}$ or 9.33 ng L^{-1}. There were no significant changes in the THC between control and test groups. No Sirius® 250 SC concentrations tested killed more than 50% of the shrimp; therefore, the herbicide was considered not toxic to the juveniles. However, the THC showed significant differences between the control and test groups, suggesting sublethal effects to *L. vannamei* juveniles. According to the results, the insecticide Talcord® is highly lethal for *L. vannamei* and the herbicide Sirius® 250 SC was not lethal in the concentrations tested but showed sublethal effects as lower THC. The results demonstrate the risks involved in farming *L. vannamei* shrimp near rice cultures where these pesticides are routinely used.

Key words: toxicology, pesticides, shrimp culture, *Litopenaeus vannamei*.

RESUMO. Toxicidade aguda de pirazossulfurom-etílico e permethrin em juvenis de camarão branco *Litopenaeus vannamei*. O objetivo deste trabalho foi determinar a CL_{50} (96h) dos agroquímicos Sirius® 250 SC, herbicida à base de pirazossulfurom-etílico, e Talcord®, inseticida à base de permethrin, em juvenis de *Litopenaeus vannamei*, bem como avaliar possíveis alterações fisiológicas por meio da contagem total de hemócitos (CTH) dos camarões. Juvenis de *L. vannamei* (5,0 ± 0,5 g) foram expostos às seguintes concentrações dos agroquímicos: Sirius® 250 SC, 0; 0,1; 1; 10; 100 e 1.000 $\mu g\ L^{-1}$, e Talcord®, 0; 0,001; 0,01; 0,1; 1 e 10 $\mu g\ L^{-1}$. A CL_{50} (96h) do inseticida Talcord® foi de 0,00933 $\mu g\ L^{-1}$ ou 9,33 ng L^{-1}. Não houve alterações significativas da CTH entre as médias dos grupos-controle e dos submetidos ao inseticida. Com base nas concentrações testadas do herbicida Sirius® 250 SC, não foi possível determinar a CL_{50} (96h), assim, este produto não foi considerado tóxico para os juvenis de *L. vannamei*. Porém, a CTH dos camarões expostos ao herbicida demonstrou diferenças significativas entre as médias do controle e dos tratamentos, o que evidenciou efeito subletal. Os resultados permitem concluir que o inseticida Talcord® é altamente letal para os juvenis de *L. vannamei* e o herbicida Sirius® 250 SC, apesar de não ter a mesma toxicidade, apresenta efeito subletal relacionado com a diminuição na CTH. Os resultados sugerem a existência de riscos em se cultivar *L. vannamei* nas proximidades de fazendas de arroz, em que defensivos agrícolas são usados rotineiramente.

Palavras-chave: toxicologia, pesticidas, carcinicultura, *Litopenaeus vannamei*.

Introduction

Marine shrimp farming has developed in areas traditionally dedicated to agriculture where pesticides are widely used (ROQUE et al., 2005). According to Galindo-Reyes et al. (2000), in coastal ecosystems of Sinaloa, in northwest Mexico, shrimp farms may be in risk as intensive agriculture of about 32 different cultures of fruits, vegetables and cereals is practiced with the use of large amounts of pesticides in crop protection. Studies in coastal ecosystems in Mexico have reported critical concentrations of restricted or forbidden chlorinated and organophosphate pesticides in the water, sediments and shrimp samples (GALINDO-REYES et al., 1999). In a study along an estuarine environment in Belgium, high concentrations of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were found in marine benthic organisms (VOORSPOELS et al., 2004). Wirth et al. (2001) reported that the deterioration of water quality and pesticide contamination within Florida Bay, USA, have affected crustacean recruitment.

Shrimps can be affected by a number of products and substances used by man in aquaculture or other agricultural activities, as is the case of herbicides and insecticides. Insecticides are particularly toxic to shrimps as they are very close to insects in evolution (KRIEGER, 2001). In a study by the US Environmental Protection Agency Laboratory (EPA), penaeid shrimps were more sensitive than fishes or mollusks to the toxic effects of most pesticides and it was further suggested that pesticides in the water or in the soil compromises the shrimp immune system and triggers the outbreak of infectious diseases (ROQUE et al., 2005). Little information is available on the toxicity of pesticides to shrimp in farming ponds. Up to date, there is no data on the toxicity of most of the registered pesticides to marine organisms (ROBINSON, 1999). Studies on the subject have demonstrated that high concentrations of pesticides causes physiological and osmoregulatory alterations, which leads to reduced growth and consequent mortality of the farmed animals (GALINDO-REYES et al., 1996; HUANG et al., 2004; LUND et al., 2000).

In southern Brazil several pesticides are registered and indicated for use in flooded rice culture, but information on the toxicity of such products for non-target organisms is scarce, only toxicity tests of some pesticides to organisms that are not commonly found in flooded rice fields are available (RESGALLA et al., 2002). According to these authors, studies on the toxicity of insecticides and herbicides used in flooded rice culture to freshwater fish species are lacking.

Flooded rice culture is socially and economically important to the State of Santa Catarina because it involves more than 8,000 families and farmers in more than 130,000 hectares. However, most of the farmers use herbicides at least once in a cycle to control weeds, one of the main setbacks that have limited the growth of the rice production. Chemical control of weeds using herbicides has been widely used in rice cultures because it is a practical, efficient and fast method. In most of the rice culture farms in southern Brazil, flooding follows pesticide application or, as in many cases, e.g., in the pre-germinated system, pesticides are used directly in the flooding water (IRGA, 2001).

One of the risks of using pesticides is to directly or indirectly affect non-target organisms by contamination of their habitat or feeding source. Pesticide absorption in fish is passive until equilibrium is reached, but it depends on the concentration and the physical and chemical characteristics of the compound (RESGALLA et al., 2002). Acute toxicity of high concentrations in a short period of time is generally assessed by the Median Lethal Concentration (LC_{50}) after 96h of exposure, which is defined as the concentration that kills 50% of the organisms exposed for 96h to the test-compound (AMWEG et al., 2005).

Pyrazosulfuron-ethyl is the active compound of the herbicide Sirius® 250 SC efficiently used against a broad range of annual and temporary weeds, especially those with large and long leaves, and applied at very low concentrations at pre- or post-germination (NAKAGOME et al., 2006). Permethrin, the active compound of Talcord®, is efficient against a broad range of pest insects, particularly Lepidoptera and Choleoptera in cotton, fruits, tobacco, tomato, vegetables, and grapes by contact and action in the insect's stomach. It is also efficient against a wide variety of ectoparasites (lice) and flying insects (GARCIA et al., 2001).

Studies have described the biochemical and physiological alterations, e.g., reduced growth and survival, caused by pesticides to shrimp embryos, larvae and juveniles (GALINDO-REYES et al., 1996; 2000; 2002; HUANG et al., 2004; LUND et al., 2000). Usually, chronic stress can induce physiological compensations such as change in respiration rate and energy consumption, which can be related to growth, based on the concept that the energy exceeding the amount required for maintenance will be used for growth (GALINDO-REYES et al., 1996). Galindo-Reyes et al. (2000) reported *L. vannamei* reduced oxygen consumption in water contaminated with sublethal concentrations of the organochlorine pesticides Diazinon, Folidol and Gusathion.

In the present study, the acute toxicity of two pesticides to juvenile *L. vannamei* was assessed. The pesticides were used during the 2003/2004 rice crop in flooded rice farms located on the estuarine system in south Santa Catarina State, Brazil. Additionally, during the exposure period chronic effects such as alterations in behavior, physiology and immune system were assessed by the total hemocyte count (THC) of the surviving shrimp.

Material and methods

The toxicity of the herbicide Sirius® 250 SC (Pyrazosulfuron-ethyl) (IHARABRAS, Sorocaba, São Paulo State, Brazil) and the insecticide Talcord® (Permethrin) (BASF S.A., Brazil) widely used in the 2003/2004 crop of flooded rice were tested on juvenile shrimp. The use of Talcord® is not recommended for rice culture but it has been used by a farmer in the city of Jaguaruna (Santa Catarina State, Brazil).

Juvenile shrimp *L. vannamei*, mean weight 5.0 ± 0.5 g, were taken from a pre-nursery, Marine Shrimp Laboratory (LCM), Department of Aquaculture, Federal University of Santa Catarina, and tested in the laboratory. Shrimp were acclimated for one week prior the tests in two 300-L tanks at 26°C with constant aeration and fed *ad libitum* with a commercial compound feed (35% crude protein). Fifty-percent of the water was changed every day. Dead shrimp or shrimp with stress signs (colorless abdomen) or disease (necrosis in carapace or appendices) were discarded. During acclimation, salinity was gradually adjusted to 20% and temperature to 24°C to simulate the shrimp farming conditions in the estuaries in south Santa Catarina State.

The culture system of the trials was semi-static, twenty 10-L buckets were individually aerated and each stocked with four shrimp. Each group of four buckets was placed in a 300-L tank water bath. The seawater used in the experiment was the same pumped from Moçambique beach, east Florianópolis shore (Santa Catarina Island) and supplied to LCM.

The pesticide concentrations tested in this study (Table 1) were based on previous studies. To obtain the test concentrations calculations were based on the concentration of the active ingredients of each pesticide. For each pesticide five concentrations were tested in triplicate plus a control. Four shrimp were randomly stocked in each replicate bucket. Each 300-L tank with four buckets represented one test-concentration with three replicates and a control replicate. Each pesticide was tested only once.

Table 1. Concentrations of the herbicide Sirius® 250 SC (Pyrazosulfuron-ethyl) and the insecticide Talcord® (Permethrin) used to test the toxicity to juvenile *Litopenaeus vannamei* shrimp (5.0 ± 0.5 g).

Sirius® 250 SC (μg L^{-1})	Talcord® (μg L^{-1})
0 (control)	0 (control)
0.1	0.001
1	0.01
10	0.1
100	1
1000	10

Experiment lasted 96h and mortality was registered every 12h. For standardization purposes, a shrimp was considered dead when the body was still and opaque. Shrimp were fed according to feed consumption at an approximate rate of 3% biomass per day. To avoid any disturbance, uneaten feed or feces were not siphoned out from the units, only aeration was adjusted every 6h. Daily water renovation was of 100% in the pyrazosulphuron-ethyl (Sirius® 250 SC) group because shrimp exposed to permethrin (Talcord®) died before the first 24h. Shrimp biomass was estimated at 0.35 g L^{-1} (dry weight). Total hemocyte count was the immune parameter analyzed at the end of the experiment in the LCM Microscopy Laboratory. Hemolymph samples were collected from shrimp with a plastic syringe (0.1 mL) and placed in a anti-clotting solution (1:4) (MAS: 27 mM sodium citrate, 336 mM sodium chloride, 115 mM glucose, 9 mM EDTA, pH 7.0) and hemocytes immediately counted with the aid of a Neubauer chamber.

The statistical analysis to find the LC_{50} was performed by the inverse accumulated distribution of the normal function or probit analysis (FINNEY, 1971). Total hemocytes count was analyzed by the Kruskal-Wallis non-parametric test.

Results and discussion

Figure 1 shows the probability of mortality of juvenile *Litopenaeus vannamei* shrimp after 96h exposure to the insecticide permethrin, including the lower and upper limits of the confidence interval.

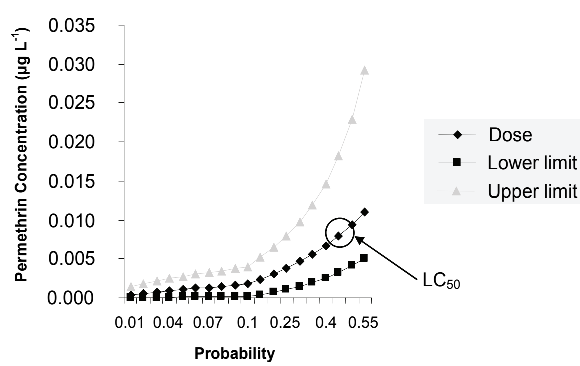

Figure 1. Relationship between the shrimp mortality probability and the permethrin concentration, including the lower and upper limits of the confidence interval after 96h exposure.

Based on the statistical analysis, the LC_{50} (96h) for Talcord® is equivalent to 0.00933 μg L^{-1} or 9.33 ng L^{-1}. It is important to notice that this concentration refers to the commercial product and not to the active principle permethrin, which, in this case, would be of 0.002333 μg permethrin L^{-1} or 2.33 ng permethrin L^{-1}. Although a high concentration of the herbicide Sirius® 250 SC was tested, i.e., 1 mg L^{-1} (1000 μg L^{-1}), no LC_{50} (96h) was determined. According to the probit analysis, the LC_{50} (96h) for the compound would be 2427 $\mu g\ L^{-1}$ or 2.43 mg L^{-1}, with a confidence interval from 0.53 to 2600 mg L^{-1}, demonstrating that the value is not reliable. For the pesticides studied, the safety indexes are shown in Table 2.

Table 2. Values of LC_{50} (96h) determined in this study for the pesticides Sirius® 250 SC and Talcord® for juvenile *L. vannamei*, and recommended concentrations, safety index, and toxicology class of the pesticides according to the manufacturers.

Parameters	Sirius® 250 SC	Talcord®
Chemical group	Pyrazosulfuron-ethyl	Permethrin
LC_{50} (96h)	> 1000 (mg L^{-1})	0.00000933 (mg L^{-1})
Recommended concentration	0.08 (mg L^{-1})	0.000008 (mg L^{-1})
Safety index	12500	1.17
Toxicology class	IV	III

The mean THCs of shrimp exposed to Talcord® are presented in Table 3. Hemocytes were counted in surviving shrimp, i.e., in the control group and at two lower concentrations. Twenty-five percent of the shrimp stocked in each concentration were sampled (five from a total of 20 shrimp and three of 12 shrimp per treatment group).

Table 3. Values are means (± standard deviation) of Total Hemocyte Count (THC) of juvenile shrimp *Litopenaeus vannamei* that survived the exposure to Talcord®.

Talcord® Concentration (mg L^{-1})	Mean THC (cell mL^{-1})*
0	269,058.35 ± 169,242.20 [a]
0.001	138,737.00 ± 109,370.30 [a]
0.01	123,967.25 ± 46,824.96 [a]

*Mean values followed by the same superscript letter are not significantly different by the Kruskal-Wallis test.

Mean THC of shrimp exposed to the herbicide Sirius® 250 SC are presented in Table 4. Twenty-five percent of the shrimp were sampled.

Table 4. Values are means (± standard deviation) of total hemocyte count (THC) of shrimp *Litopenaeus vannamei* exposed to the herbicide Sirius® 250 SC and survived.

Sirius® 250 SC Concentrations (mg L^{-1})	Mean THC (cells mL^{-1})*
0	428,262.40 ± 133,429.35 [a]
0.1	294,155.92 ± 236,290.71 [a]
1	275,212.50 ± 87,319.30 [a]
10	123,579.58 ± 72,871.784 [b]
100	119,415.42 ± 43,869.10 [b]
1000	97,463.75 ± 131,065.85 [b]

*Mean values followed by the same superscript letter are not significantly different by the Kruskal-Wallis test.

In the shrimp group exposed to Talcord®, LC_{50} (96h) was of 0.00933 $\mu g\ L^{-1}$ but it can also be considered LC_{50} (12h) as shrimp mortality was observed a few minutes after the insecticide was added into the water. In the groups exposed to higher concentrations, shrimp were highly agitated and constantly jumping out the water immediately after exposure and a few seconds later they were lying on the bottom of the tank shivering and died quickly. This behavior can be a sign of the neuromuscular disturbance caused by the insecticide on acetylcholinesterase (AChE), an enzyme involved in the deactivation of acetylcholine at nerve endings, preventing continuous nerve firing, which is vital for normal functioning of sensory neuromuscular systems (COMOGLIO et al., 2005). Studies have demonstrated that many organophosphorus and carbamate insecticides are effective AChE inhibitors (GALGANI et al., 1992; KUMAR; CHAPMAN, 1998; KEY; FULTON, 2006). The inhibition of AChE in estuarine organisms has been established as an indicator of the contamination by insecticides (FULTON; KEY, 2001). Similarly, in the grass shrimp *Palaemonetes pugio* the inhibition of AchE is a relevant biomarker of the exposure to insecticides (KEY; FULTON, 2002). In addition to organophosphorus and carbamate insecticides, other classes of environmental contaminants, heavy metals and agrochemicals have shown to be potential inhibitors of AChE in organisms exposed to such chemicals (HABIG et al., 1988).

The THC of shrimp exposed to Talcord® was not significantly different between the control group and the two concentrations in which shrimp survived. First, possibly because of the low concentrations tested (0.001 and 0.01 $\mu g\ L^{-1}$) that might not have altered the number of hemocytes per milliliter of hemolymph in a short period of exposure. Second, permethrin causes alterations in the shrimp nervous and muscular systems and does not interfere in the production of defense cells. And third, the high variability in the data as the number of shrimp sampled for THC was low. The THC does not seem to be one of the best hemato-immune parameters to be used as a tool to assess alterations in the immune system caused by a contaminant. Although the high individual variability in the number of cells it is mostly used to determine the health status of crustaceans (LE MOULLAC et al., 1998).

The high sensitivity of juvenile *L. vannamei* to the insecticide Talcord® can be explained by the phylogeny between shrimp and insects, with many similarities, e.g., in the nervous and muscular systems. In general, penaeid shrimp are highly sensitive to permethrin, differently from mollusks that are much more resistant (FAO, 1999; IMGRUND, 2003). Cripe (1994) suggested that crustaceans are more sensitive to agrochemicals during molts, especially the larval forms, as molts occur more frequently in the early life stages.The LC_{50} (96h) of 0.00933 $\mu g\ L^{-1}$ determined for Talcord® is an extremely low concentration and it is an environmental concern. Considering the total volume of the southern estuarine complex in the State of Santa Catarina, i.e., 20 trillion L of water (20,000 ha) it means that 187 L of the insecticide could kill all indigenous crustaceans in the lagoons of the estuary (this estimation did not take into account the dilution by the daily tide water renewal).

Although the herbicide Sirius® 250 SC did not show mortalities above 50% at a concentration of 1,000 μg L^{-1} it is important to observe that the time of exposure was of only 96h and a longer exposure could result in sublethal or lethal effects affecting shrimp feeding and growth. Studies have demonstrated low toxicity of pyrazosulfuron-ethyl to aquatic animals. Fleck (2000) studied the same compound in rainbow trout *Oncorhynchus mykiss* and reported LC_{50} (96h) > 180 mg L^{-1}. On the contrary, Resgalla et al. (2002) reported a LC_{50} (96h) of 0.32 pyrazosulfuron-ethyl mg L^{-1} for carp *Cyprinus carpio*.

Nevertheless, reduction in the THC of shrimp exposed to Sirius® 250 SC (57.5%) as compared to the control group demonstrate that it has a sublethal effect and such reduction in the number of defense cells may leave shrimp more susceptible to infectious diseases. The mortality of only six shrimp of a total of 80 shrimp exposed to the herbicide Sirius® 250 SC could be related to acute effects, or to sublethal effects that interfere in biochemical and physiological processes in the shrimp, despite the low toxicity.

No substrate was used in the toxicity tests of this study. Toxicity of agrochemicals can be enhanced by the presence of sediment (HOLMES et al., 2008). Hartman and Martin (1984) tested the toxicity of glyphosate to *Daphnia pulex* and the LC_{50} (48h) was of 3.2 mg L^{-1} with sediment and of 7.9 mg L^{-1} without it.

Imgrund (2003) reported that products with safety indexes > 20 present lower risks of environmental impact. For the herbicide Sirius® 250 SC, the safety index was very high, much above the concentrations recommended for use in agriculture, and three times higher than the safety index determined by Resgalla et al. (2002) for juvenile carp. On the other hand, the insecticide Talcord® presented a low safety index, indicating a higher risk to the environment. According to the calculated safety index, the Talcord® concentration used in rice culture (8 ng L^{-1}) is close to the LC_{50} (96h) of 9.33 ng L^{-1} determined in this study for juvenile *L. vannamei*, demonstrating the risk of farming shrimp *L. vannamei* in areas close to rice farms. The safety level of a compound recommended by Sprague (1971) for aquatic organism is equivalent to 10% of the LC_{50}, therefore, farmed shrimp should not be exposed to concentrations above 0.93 ng permethrin L^{-1}.

Conclusion

The permethrin-based insecticide tested in this study was highly toxic to juvenile *Litopenaeus vannamei*.

The herbicide pyrazosulfuron-ethyl did not present acute toxicity but reduced significantly the total hemocyte count in shrimp.

The results indicated the potential risk of contamination in farming *Litopenaeus vannamei* near rice farms that use agrochemicals routinely.

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