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# Effect of malathion on AChE activity, humoral and cellular response in juvenile shrimp *Litopenaeus vannamei* (Bonne, 1931)

# Efecto del malatión sobre actividad de AChE, respuesta humoral y celular en juveniles de camarón *Litopenaeus vannamei* (Bonne, 1931)

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**Abstract**

Malathion is an organophosphate pesticide used in agricultural fields in the northwestern part of the state of Nayarit. Shrimp production systems are located near the fields, and it could be thought that chemical residues could be present in the shrimp fattening ponds. Due to the environmental risk of pesticides and the economic risk they represent in the shrimp farming area, the present work consisted of knowing the effect of three sublethal concentrations of malathion, by means of a 96-hour toxicity bioassay, with the purpose of evaluating the humoral, enzymatic, total protein and cellular response of shrimp (*Litopenaeus vannamei*). Initially, a batch of unexposed shrimp (time 0) was analyzed, after the application of sublethal doses of malathion and at 96 hours, another batch of shrimp was analyzed. Coagulation time was not altered by exposure to malathion, acetylcholinesterase (AChE) activity was inhibited 75.5 % at the highest malathion concentration, and a decrease in protein ($p<0.05$) was recorded in the shrimp from the different treatments and at the two times analyzed (0 hours and 96 hours). The total number of hemocytes was higher when the concentration of malathion was increased, which demonstrates with the results obtained that the effect of malathion on the variables analyzed was evident in spite of having used sublethal concentrations.

**Shrimp, Enzyme, Hemocytes, Pesticide, Toxicity, Shrimp, Toxicity**

**Resumen**

El malatión es un plaguicida organofosforado que se usa en campos agrícolas del Noroeste del Estado de Nayarit, los sistemas de producción de camarón se encuentran cerca de los campos de cultivo, y se podría pensar que los residuos de sustancias químicas pudieran estar presentes en los estanques de engorde de camarón. Debido al riesgo ambiental de los plaguicidas y al riesgo económico que representa en la zona camaronícola, el presente trabajo consistió en conocer el efecto de tres concentraciones subletales de malatión, mediante un bioensayo de toxicidad de 96 horas, con la finalidad de evaluar la respuesta humoral, enzimática, proteína total y la respuesta celular del camarón (*Litopenaeus vannamei*). Inicialmente un lote de camarones no expuestos (tiempo 0) fueron analizados, posterior a la aplicación de dosis subletales de malatión y al tiempo de 96 horas, otro lote de camarones fue analizados. El tiempo de coagulación no fue alterado por la exposición a malatión, la actividad de acetilcolinesterasa (AChE) fue inhibida 75.5 % en la mayor concentración de malatión, además fue registrado una disminución de proteína ($p<0.05$) en los camarones de los diferentes tratamientos y en los dos tiempos analizados (0 horas y 96 horas). El número total de hemocitos fueron mayores cuando se incrementó la concentración del malatión, lo que se demuestra con los resultados obtenidos, que el efecto del malatión sobre las variables analizadas fue evidente a pesar de haber utilizado concentraciones subletales.

**Camarón, Enzima, Hemocitos, Plaguicida, Toxicidad**

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## Introduction

Malathion ($C_{10}H_{19}O_6PS_2$) is an organophosphate pesticide used to control pests affecting agricultural crops, the constant use of the pesticide in crop fields can lead to high-risk residues that may be moved by rainfall or agricultural drains and incorporated into water bodies. Residues in water can cause toxicity and mortality of sensitive organisms. Malathion is derived from phosphoric acid and has phosphorus and sulphur atoms (US EPA, 2013), the main effect is the inhibition of the enzyme acetylcholinesterase, which, by not carrying out the hydrolysis of the neurotransmitter (acetylcholine), accumulates in the post-synaptic membranes and affects the nervous system (García-de la Parra et al., 2006). Pacific shrimp (*Litopenaeus vannamei*) is a sensitive species when exposed to organophosphate pesticides, requiring only 0.078 mg $L^{-1}$ of malathion to cause mortality in 50% of exposed organisms (LC50-96 hours) (Bautista, 1996). Organophosphate pesticides; malathion, chlorpyrifos, diazinon and parathion among others, are considered immunotoxic because they alter humoral and cellular responses even when present in sub-lethal concentrations (Yeh et al., 2005; Jose et al., 2011). They also have an effect on acetylcholinesterase (AChE) activity which causes loss of balance and erratic swimming in crustaceans (Mwila et al., 2013). Among the adverse effects of malathion, genotoxicity of haemocytes in Penaeus monodon (Jose et al., 2011) and effects on growth, decreased food consumption and altered respiration process in the crustacean *Macrobrachium nipponense* (Yuan et al., 2004) have been recognised. In the present study, the objective was to evaluate the effect of three sublethal concentrations of malathion on haemolymph coagulation, acetylcholinesterase (AChE) activity, total protein and haemocyte number in shrimp (*L. vannamei*), before and 96 hours after the application of the pesticide, in order to identify the dose at which an effect on the defence response, enzymatic alteration and effect on total protein of the experimental organisms could be observed.

Theoretical framework

The continuous use of chemical substances in the world is something that is carried out in a normal way, with the intention of improving and protecting investments in agricultural fields in order to have sustainable productions ensuring food for the population, in such a way that this has led to the excessive use of chemical substances. In Mexico, the use of pesticides is currently unregulated and is a source of pollution that has a negative impact on the environment and causes a decline in sensitive species. In the last 20 years, agrochemicals applied in the environment of northwestern Mexico (Sonora, Sinaloa and Nayarit) have been studied and it has been concluded that residues are present in sediments and aquatic organisms (Hernández et al., 2018). White shrimp is one of the main products for consumption and export; production is in second place in Mexico as a result of its high demand (CONAPESCA, 2015). However, gas chromatography has determined residues (0.00035 mg g-1) of malathion and chlorpyrifos (0.000013 mg g-1) in shrimp from farms in the state of Sonora, Mexico (Burgos-Hernández et al., 2006). Due to the presence of residues, experiments with white shrimp L. vannamei exposed to chlorpyrifos (0.00036 mg L-1 and 0.00018 mg L-1) and after 21 days of exposure, slow growth and gill cell damage were observed (Pawar et al., 2019). Other effects caused by organophosphorus pesticides are the decrease in glycogen, triglycerides and total protein, in addition to causing mortality of experimental organisms even at sublethal concentrations (Osuna-Flores et al., 2019).

## Method and tools

The juvenile shrimp used were donated by the farm "Los Sauces" located in the municipality of San Blas Nayarit, Mexico. They were transported to the Laboratory of Biological Indicators of Environmental Stress (LIBEA) of the National School of Fisheries Engineering of the Autonomous University of Nayarit. The organisms were acclimatised to 25 practical salt units (PSUs) for 72 hours. The shrimp (40 in total) were placed in 60-litre glass tanks (60 cm x 40 cm x 35 cm). The conditions of the experiment were; ambient temperature of 25 °C and 24 °C in the water of the tanks, pH 7.5 and oxygen concentration was 7.45 mg L-1.

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The organisms were fed with the commercial feed Api-shrimp at 8 % of the biomass. Dilutions of malathion were prepared based on the LC50-96 hours obtained by Bautista (1996), which were; 0 %, 1 %, 10 % and 50 %. Ten organisms taken at random were placed in each aquarium, including a control group (0 %). Before the application of the different malathion concentrations, three shrimp were analysed (clotting time, acetylcholinesterase activity, total protein and total haemocyte count). After the corresponding percentage concentration was applied (0.0, 0.00078 mg L-1, 0.0078 mg L-1 and 0.039 mg L-1), at the end of 96 hours of exposure the same variables were evaluated in seven organisms for each treatment.

Haemolymph extraction was performed with the aid of a 1 mL insulin syringe, which was inserted at the base of the first abdominal segment between the pereiopods. The clotting time of the haemolymph was counted with a standard digital stopwatch (H56-70), a 1.55 mm capillary haematocrit microtube (brandSD) was used (Jussila et al., 2001). To quantify acetylcholinesterase activity, the methodology described by Ellman et al. (1961), adapted to microplate (Herbert et al., 1995), which is based on a colorimetric method, was used. Protein concentration was determined by the method of Bradford (1976), adapted to microplate (Herbert et al., 1995). Bio-Rad reagent was used as reaction solution. Total haemocyte count (THC) was performed with an optical microscope; 17 µL of haemolymph mixed with formalin according to Costa et al. (2009) was used. Haemocyte counts were performed in a Neubauer chamber at 400X, placing the total number of cells in the four corner quadrants and one additional quadrant randomly selected for each case (Arredondo & Voltolina, 2007).

Statistical analysis of the data was by normality, homoscedasticity, one-way ANOVA and two-way ANOVA tests, and when differences were identified, mean comparison tests (Holm-Sidak) were applied. All statistical analyses were performed with a significance level α= 0.05.

## Results

The highest haemolymph coagulation time occurred before applying the malathion concentration (at the concentration 0.00078 mg L-1, corresponding to 1 % of the LC50) while the lowest value occurred 96 hours after applying the pesticide concentration (Table 1). The two-way ANOVA analysis showed significant differences (p=0.05) as a function of time, concentration and the interaction between the two sources of variation. On the other hand, the coagulation time of the control group shrimp showed no significant difference between their mean values.

| | | *Malathion concentrations (mg $L^{-1}$)* | | | |
|---|---|---|---|---|---|
| Variable | Time | 0 | 0.00078 | 0.0078 | 0.039 |
| Coagulation (seconds) | 0 hours | 73.33±10.21a | 167.33±13.31d | 101.66±25.42bc | 118.33±30.61c |
| | 96 hours | 70.85±17.08a | 85.57±15.76a | 67.20±11.09a | 113.00±19.13b |

**Table 1** Mean values (seconds ± standard deviation) of coagulation of L. vannamei haemolymph exposed to three sub-lethal concentrations of La LC50-96 hours of malathion

*Two-way Anova and Holm-Sidak method*

The acetylcholinesterase (AChE) activity present in shrimp at the time (0 hours) before pesticide application did not show significant differences in their mean values. However, once the pesticide was applied and at the time of 96 hours, AChE decreased in the presence of the highest concentration of malathion, which corresponded to half the value of the LC50-96 hours, reflecting the effect caused by the pesticide used in the experiment.

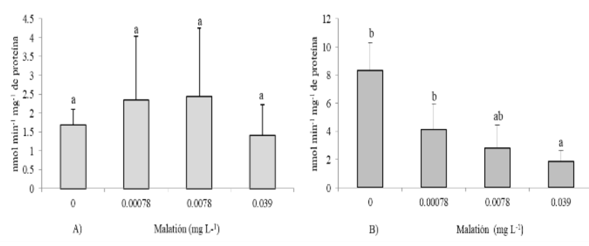


**Figure 1** Mean values of AChE activity (± standard deviation), A) time 0 hours, before malathion application, B) 96 hours of exposure to the pesticide.

The total protein concentration was variable at the beginning of the experiment, the highest average value was presented in treatment 0 (control), and before the application of the pesticide (A), significant differences were determined between the treatments with respect to shrimp from the control group. Once the pesticide was applied and after 96 hours of exposure, the trend of total protein *in L. vannamei* muscle was to decrease in shrimp exposed to the highest concentration (Figure 2), with significant differences (p=0.05) with respect to the average values of total protein of shrimp in the control group. However, the mean values of total protein in shrimp exposed to the 0.039 concentration did not represent significant differences (p>0.05) with respect to the mean values of total protein of shrimp from the two pesticide treatments (0.00078 mg $L^{-1}$ and 0.0078 mg $L^{-1}$).

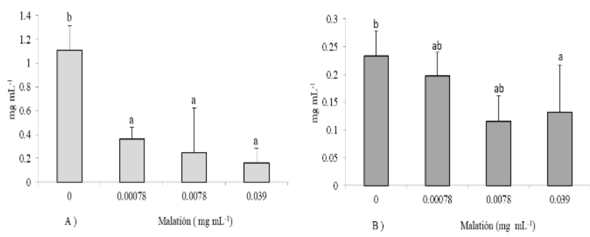


**Figure 2** Mean values of total protein (± standard deviation) in *L. vannamei* muscle. A) not exposed, B) exposed 96 hours to malathion

The mean values of total haemocytes of shrimp in the three treatments tended to decrease with respect to shrimp in the control group. The decrease occurred even though the shrimp were not exposed to the pesticide (time 0), the trend determined was significantly different (p=0.05) and the lowest mean value was 1.060±0.440 X 106 cells mL-1 (Figure 3). Once the pesticide concentrations were applied, the total haemocytes of the exposed shrimp showed a tendency to increase in number (B), which were higher in the 0.0078 mg L-1 malathion treatment (10 % of the LC50), than the average values of haemocytes of shrimp in the control group, and there were only significant differences (p<0.05) between the averages of the malathion treatment with respect to the average values of the control group.

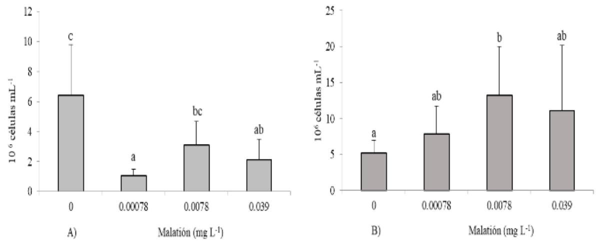


**Figure 3** Mean haemocyte values (± standard deviation) of *L. vannamei*. A) not exposed, B) exposed 96 hours to malathion.

## Discussion and conclusions

Malathion is a widely used and broad-spectrum pesticide in pest control, and it is possible that residues may reach shrimp production systems. Some of its effects are reported in several studies focused on evaluating the effect on the gill system, as well as on immunological and neurotoxic processes. Clotting time is an important response and is considered a defence response to different viruses (Song et al., 2003). In addition, increased clotting time may be indicative of stress conditions (Jussila et al., 2001). In this work the clotting time showed increase in time and according to the different sublethal concentrations of malathion, the increase happened at the sublethal concentration of malathion (0.039 mg L-1). This result coincides with that reported for Fenneropenaeus indicus species exposed to 0.00005 mg L-1 malathion, the coagulation time was altered (Sabu & Gopal, 2016) reflecting that for Litopenaeus vannamei a higher concentration of malathion is required to cause an effect on haemolymph coagulation time. AChE activity was inhibited by sublethal concentrations of malathion, the inhibition value was approximately 4.4 times lower than the value obtained in control shrimp, while Tassanee Eamkamon et al. (2012). in the study of exposure to sublethal concentrations of chlorpyrifos, determined an effect on AChE that was 1.9 times less than the activity of control shrimp. Studies show that the pesticides malathion and paraquat affect the feeding rate in Macrobrachium nipponense and cause a decrease in body weight and consequently the loss of total protein (Yuan et al., 2004), which also with Litopenaeus vannamei, malathion caused a decrease in total protein. On the other hand, haemocytes are important in the innate defence mechanism, both humoral and cellular responses represent the immune defence to the presence of pathogenic microorganisms and exogenous particles (Jose et al., 2011).

A decrease in the number of haemocytes has been shown to occur in the presence of metals, viruses and bacteria (Lorenzon et al., 2001; Abad-Rosales et al., 2019). In this experiment and in the presence of malathion, haemocytes tended to increase in the treatment (0.0078 mg L-1), perhaps due to the low concentration of malathion used, which is described as induction by the stress caused by the pesticide used. In addition, the present work also corroborates that malathion alters AChE activity and total protein concentration in the presence of sublethal concentrations of malathion.

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