

ACUTE TOXICITY OF THE HERBICIDE KARMEX (DIURON) IN *Macrobrachium acanthurus* AND *M. olfersii* PRAWNS

Toxicidad aguda del herbicida karmex (diuron) en langostinos *Macrobrachium acanthurus* y *M. olfersii*

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Key words: estuary, Decapoda, shrimp, diversity, pesticide.

ABSTRACT

Farmers have increased the production of food by using chemical substances such as diuron; however, these components affect the water quality and the species found in these ecosystems. *Macrobrachium acanthurus* and *M. olfersii* postlarvae present in the estuary of Río Jamapa, Veracruz were collected to measure the LC₅₀ and LD₅₀ using the herbicide Karmex (diuron: (3-(3,4-dichlorophenyl)-1,1-dimethylurea [DCMU]). The captures of the prawns were made with light traps. Acute toxicity bioassays were carried out at 96 h to determine the mean lethal concentration (LC₅₀). In the experimental design, a geometric series was used considering the control and five concentrations of the herbicide (1.75, 3.5, 6.99, 14, and 28 mg/L). The data analysis was carried out with the Probit method. A maximum LC₅₀ of 10.233 mg/L and LD₅₀ of 8.221 mg/L of Karmex were obtained for *M. acanthurus* and a maximum LC₅₀ of 9.242 mg/L and LD₅₀ of 7.504 mg/L of Karmex were obtained for *M. olfersii*. The average lethal concentration was consistent with those of other crustaceans, such as *Artemia salina* and *Daphnia*. Based on the response in the bioassays, *Macrobrachium acanthurus* and *M. olfersii* postlarvae can be used in toxicology studies.

Palabras clave: estuario, Decapoda, camarones, diversidad, plaguicida.

RESUMEN

Los agricultores han aumentado la producción de alimentos mediante el uso de sustancias químicas como el Diurón; sin embargo, estos componentes afectan la calidad del agua y las especies que se encuentran en estos ecosistemas. Se recolectaron postlarvas de *Macrobrachium acanthurus* y *M. olfersii* presentes en el estuario del Río Jamapa, Veracruz para medir la CL₅₀ y DL₅₀ utilizando el herbicida Karmex (diuron: DCMU (3-(3,4-diclorofenil)-1,1-dimetilurea). Se realizaron las capturas de los langostinos

con trampas de luz. Los bioensayos de toxicidad aguda se midieron a las 96 h para determinar la concentración letal media (CL_{50}). En el diseño experimental se utilizó una serie geométrica considerando el testigo y cinco concentraciones del herbicida (1.75, 3.5, 6.99, 14 y 28 mg/L). El análisis de datos se realizó con el método Probit. Se obtuvo una CL_{50} máxima de 10.233 mg/L y una DL_{50} de 8.221 mg/L de Karmex para *M. acanthurus* y una CL_{50} máxima de 9.242 mg/L y DL_{50} de 7.504 mg/L de Karmex para *M. olfersii*. La concentración letal promedio fue consistente con la de otros crustáceos, como *Artemia salina* y *Daphnia*. Con base en la respuesta en los bioensayos, se pueden utilizar postlarvas de *Macrobrachium acanthurus* y *M. olfersii* en estudios de toxicología.

INTRODUCTION

Farmers have implemented measures to increase the production of food due to its growing demand, including the use of chemical substances, such as herbicides (FAO 1997). In the Hydrological Region 28 of Papaloapan, Veracruz (SINA 2021), diuron is an agrochemical used in agriculture (Zuleta-Rodríguez and Vázquez-Torres 1992).

In the state of Veracruz, shrimp production comes from artisanal capture and aquaculture (CONA-PESCA 2018). However, the production of shrimp presents various problems that make it an activity vulnerable to external factors such as water quality in collection sites and cultivation activities (Gil-Díaz 2020).

The herbicide diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea [DCMU]) belongs to the group of nitrogenous cyclodiene pesticides. Its economic importance is due to its use in the control of broad-leaf weeds, grasses, cotton, banana, corn, sorghum, pineapple, citrus, and mainly sugarcane (UNA 2021). It is a potential contaminant of groundwater due to transport from farmland (INECC 2019, UNA 2021).

Studies in the field about the dissipation of diuron showed that the maximum amount in the sediment at the bottom was 0.76 mg/L immediately after application, which declined to 0.13 mg/L after four days and was detected at 0.066 mg/L in samples taken 256 days after application (Turner 2003). In analyses of water, Proia et al. (2011) reported pulses of up to 0.134 mg/L in the Llobregat river (NE Spain).

In Mexico, about 44 commercial brands contain the active ingredient in percentages of around 80 %. diuron is highly persistent (up to one year) in aquatic environments, and microorganisms are the main agents responsible for degrading diuron. It is classified as toxic to birds, amphibians, and crustaceans, as it is soluble in water, absorbed in sandy soils, and available for organisms associated with benthos

(INECC 2019), such as prawn shrimp of the genus *Macrobrachium*.

Some studies have been carried out on nitrogenous compounds on postlarvae of the species of the genus *Macrobrachium*. In this regard, an LC_{50} of 4.5 NO_2-N mg/L was determined for the acute toxicity of nitrite to larvae of the giant Malaysian prawn, *M. rosenbergii* De Man, 1879 (Armstrong et al. 1976). Alternatively, LC_{50} values of 21.14 and 21.65 mg/L were determined for the acute toxicity of ammonia to various life stages of the Amazon River prawn, *M. amazonicum*, Heller, 1862 (Dutra et al. 2016). Regarding the species used in this study, the acute toxicity of carbofuran in *M. olfersii* was assessed by Barbieri et al. (2016), who determined an LC_{50} of 0.42 mg/L. In addition, the acute toxicity of the herbicide Dasurquat (paraquat) in the shrimp was assessed, and a LC_{50} of 0.31 μ L/L (3.8 mg/L) was found (Gil-Díaz 2020). However, there are no studies about the use of diuron on the species *M. acanthurus* and *M. olfersii*.

An LC_{50} of 12 to 16 mg/L was obtained in other crustaceans with tests of acute toxicity for diuron, (Turner 2003, Koutsafitis and Aoyama 2008, Alyürük and Çavaş 2013, Shaalaa et al. 2015). Therefore, diuron produces mortality at high concentrations. However, lower concentrations can alter the neurotransmitters, hormones, immune response, reproduction, physiology, morphology, or behavior (Relyea and Hoverman 2006).

The prawn *M. acanthurus* (Wiegmann 1836) is distributed from North Carolina in the United States to Rio Grande do Sul in Brazil (García et al. 2013). The information available for this species is scattered, fragmented, or covers very specific aspects (Hernández-Hernández 2016). On the other hand, *M. olfersii* (Wiegmann 1836) is distributed from the Baluarte River, Sinaloa to El Naranjo, Chiapas, and along the Atlantic slope from St. Augustine Florida to Santa Catarina, Brazil (Hernández 2007). For larvae

and postlarvae of *M. acanthurus* and *M. olfersii* in the study area, only the studies of Salas-de la Rosa (2018) and Arias-Martínez (2020) are known. Given the above, the present study aimed to establish the mean lethal concentration (LC₅₀) in the postlarvae of the species *M. acanthurus* and *M. olfersii* to exposure to the agrochemical Karmex (diuron) used in crops in the Río Jamapa, Veracruz region.

MATERIALS AND METHODS

Study area

The estuary of the Jamapa River is formed by the confluence of the Cotaxtla and Jamapa rivers. The Jamapa River arises at the limit of the states of Puebla and Veracruz with the name of Barranca de Coscomatepec. It continues its course towards the east, crosses farmlands, and forms meanders and alluvial terraces up to the Ixcualco stream. From there, it flows into the Cotaxtla River, which flows to the Moreno River and the Mandinga Grande Lagoon. Finally, it empties into the Gulf of Mexico in the town of Boca del Río, Veracruz. The Jamapa River basin is located between 18°45' and 19°14' north latitude, and between 95°56' and 97°17' west longitude. The area has a warm subhumid climate with a higher annual average temperature of 22 °C, and the temperature of the coldest month is 18 °C. The precipitation of the driest month is from 0 to 60 mm, and greater than 55.3 mm during the rainy season. In the season of cold fronts, the rain is from 5 to 10.2 % of the annual total. The main types of soil are Regosol and Vertisol (Fuentes-Mariles et al. 2014).

Specifically, the mouth of the Jamapa River is an estuarine system whose water discharges are rich in nutrients and reach the Sistema Arrecifal Veracruzano (SAV) (Leaño-Carrera et al. 2019). The level of the river in its estuarine part has a micro-tidal modulation (< 2 m) with a semi-diurnal (~ 12 h), diurnal (~ 24 h), and lunisolar component every two weeks (~ 15 days) (Salas-Monreal et al. 2019). The Jamapa River has a navigation channel in the southern part that generates important changes in the estuary dynamics (Salas-Monreal et al. 2019). The shipping channel produces stronger currents (> 0.5 m/s) and a continuous exchange of brackish water with the ocean (~ 12 h). The southern area is more dynamic (the exchange of water from the river and the ocean is continuous) than the northern area, where the water can remain static for periods longer than 24 h, due to its low speeds and the continuous supply of water from Arroyo Moreno, showing

greater pollution from the urban discharges that it receives directly (Salas-Monreal et al. 2020).

Fieldwork

The organisms were collected using light traps made with plastic boxes; their dimensions were 0.40 m long, 0.25 m wide, and 0.30 m high. The traps had four 0.025 m diameter perforations. In the lower part, a sample receiver was placed, which consisted of a mesh with a 330-μm aperture. Inside the box, a plastic grid with a mesh opening of 0.05 m and a white light lamp were placed (Cházaro-Olvera et al. 2018).

Night samplings were carried out considering the full moon phase. The traps were placed in three contiguous sites at 20:00 LT on the first day of sampling and were collected at 08:00 LT the next day. From the *in vivo* sample, prawns were selected for the bioassays. The environmental factors of temperature (°C), salinity (practical salinity units, PSU), dissolved oxygen (mg/L), and pH were measured *in situ* at 0.50 m of depth, using a Hanna HI9828 multiparameter meter equipped with HI769828DO and HI769828PH probes.

Laboratory work

The identification of the postlarvae of *Macrobrachium* was realized following the descriptions of Holthuis (1952): *Macrobrachium acanthurus* presents a straight rostrum that reaches slightly beyond the scaphocerite. The dorsal margin bears nine to 11 teeth, of which two are behind the orbit margin. The second tooth is sometimes placed partly over the posterior margin of the orbit. The lower margin bears four to seven (generally six) teeth, which proximally are placed closer together than distally. The carapace is smooth; it only bears short hairs, especially in the anterolateral region. The second legs are equal; they reach with the carpus or with a small part of the merus beyond the scaphocerite. The fingers are slender, close over their whole length and are only slightly shorter than the palm. The pleura of the fifth segment ends in an acute point. The sixth segment is 1.5 times as long as the fifth. The telson is 1.5 times as long as the 6th abdominal segment. *Macrobrachium olfersii* presents a straight rostrum, reaching the third joint of the antennular peduncle. The dorsal margin bears 14 to 15 teeth, of which two to three are behind the orbital margin. The ventral margin bears three to four teeth. The second pair of pereopods with chelas unequal in shape and size. The hepatic spine is smaller than the antennal. The second legs are very unequal; the larger reaches with the entire carpus and a small

part of the merus beyond the scaphocerite; outer surface with smooth central area surrounded by spines and ornamented with bristles and pubescence. The pleura of the fifth segment has a rectangular top or is slightly acute. The 6th segment is slightly longer than the fifth. The telson is 1.5 times as long as the 6th abdominal segment.

Bioassays

Live specimens for bioassays were placed in transparent 500-mL containers with 100 mL of water from the site previously filtered and constant aeration using Elite 802 air pumps. To carry out the bioassays, 10 postlarvae were used for each 500-mL plastic container following the criteria of Mohapatra and Rengarajan (1995). The agrochemical used was obtained from the commercial brand Karmex (diuron: 3-(3,4-dichlorophenyl)-1,1-dimethylurea [DCMU] with 80 % active ingredient). Preliminary 96 h static bioassays were carried out to determine the concentration ranges of the definitive bioassays. The concentrations used were 0, 1.75, 3.5, 6.96, 14, and 28 mg/L (Castro-Castro et al. 2005). In each bioassay, three replicates were used. Mortality readings were taken after 1, 2, 4, 8, 18, 24, 36, 48, and 96 h of exposure (Uc-Peraza and Delgado-Blas 2012). For the acute toxicity bioassays, the guidelines of the US Environmental Protection Agency (USEPA 2016a) were followed for ecological effects tests (USEPA 2016b). On the other hand, to complement the information about toxicity of Karmex (diuron) on *Macrobrachium*, the LD₅₀ with Probit-Log(dose) lineal regression models were obtained. Subsequently, ANOVA tests to assess significance and goodness of fit to the regression models were applied ($p < 0.05$) (Bustos-Obregón and Vargas 2010). To test if LD₅₀ were significantly lower than LC₅₀ a paired t test was applied (Ross and Willson 2017).

Statistical analysis

To determine the statistical significance of the concentration variability of Karmex (diuron) for the two species of *Macrobrachium*, a one-way analysis of variance (ANOVA) and the Tukey comparisons test were used (Zar 1999). The mean lethal concentration (LC₅₀) was determined with the statistical program Minitab v. 18.1 (Minitab, LLC, State College PA, USA), and the 95 % confidence intervals were obtained. The LD₅₀ linear regression models and the respective graphics were obtained with the statistical program Microsoft Excel 365. The paired t test was applied with PAST software v. 3.26 (Harmer et al. 2001).

RESULTS AND DISCUSSION

The dissolved oxygen in the Río Jamapa estuary was 6.7 mg/L, pH was 8.7, temperature was 27 °C, and salinity was 0.72 ± 0.18 and 13.89 ± 5.48 PSU in March and October, respectively. For the bioassays, the values of the environmental parameters were maintained following the collection values (**Table I**). The values of the parameters are consistent with those recorded in the study area by Houbbron (2010), Jasso-Montoya (2012), Aké-Castillo et al. (2016), Castañeda-Chávez et al. (2017), González-Vázquez et al. (2019) and Salas-Monreal et al. (2020).

TABLE I. PHYSICAL AND CHEMICAL FACTORS: ENVIRONMENTAL CONDITIONS FOR THE COLLECTION OF *Macrobrachium acanthurus* AND *M. olfersii* IN THE BOCA DEL RÍO, VERACRUZ, ESTUARY, AND ENVIRONMENTAL CONDITIONS IN THE LABORATORY DURING THE BIOASSAYS.

Environmental conditions in the Boca del Río estuary		
Environmental factors	March	October
Dissolved oxygen (mg/L)	6.63 ± 0.22	6.69 ± 0.12
pH	8.6 ± 0.21	8.61 ± 0.11
Temperature (°C)	25.11 ± 0.12	25.08 ± 1.02
Salinity (PSU)	0.70 ± 0.01	14.09 ± 0.58
Environmental conditions in the laboratory during the bioassays		
Environmental factors	March	October
Dissolved oxygen (mg/L)	6.72 ± 0.12	6.73 ± 0.15
pH	8.70 ± 0.32	8.71 ± 0.32
Temperature (°C)	27.41 ± 0.45	27.38 ± 1.09
Salinity (PSU)	0.72 ± 0.18	13.99 ± 5.48

PSU: practical salinity units.

The differences in mortality among the concentrations of Karmex (diuron) for the two species of *Macrobrachium* determined by ANOVA are greater than would be expected by chance; there is a statistically significant difference ($p < 0.05$), which estimated with Tukey's test was found between all concentrations ($p < 0.05$), except for 1.75 and 3.5 mg/L ($p = 0.056$).

The maximum concentration of Karmex (diuron) of 28 mg/L caused mortality rates of 100 % in both *Macrobrachium* species (**Table II**). With the Probit analysis, an LC₅₀ of 8.77 mg/L of Karmex (diuron) for *M. acanthurus* at 96 h for March was found, with

a 95 % confidence interval of 7.67 to 9.92 mg/L (**Fig. 1a**). For October, the LC_{50} was 9.04 mg/L, with a 95 % confidence interval of 7.92 to 10.23 mg/L (**Fig. 1b**).

TABLE II. CUMULATIVE MORTALITY (%) AT 96 HOURS IN THE BIOASSAYS WITH *Macrobrachium acanthurus* AND *M. olfersii* CARRIED OUT ON TWO SAMPLING DATES (MARCH AND OCTOBER).

<i>Macrobrachium acanthurus</i>							
Initial date	Final date	Karmex (diuron) concentration (mg/L)					
		0	1.75	3.5	6.96	14	28
		Cumulative mortality (%)					
12/03/2019	17/03/2019	0	10	20	40	50	100
12/03/2019	17/03/2019	0	10	30	50	60	98
02/10/2019	06/10/2019	0	20	20	40	60	100
02/10/2019	06/10/2019	0	10	20	40	50	98
<i>Macrobrachium olfersii</i>							
Initial date	Final date	Karmex (diuron) concentration (mg/L)					
		0	10	20	50	60	100
		Cumulative mortality (%)					
12/03/2019	17/03/2019	0	10	30	50	60	98
12/03/2019	17/03/2019	0	10	30	50	60	98
02/10/2019	06/10/2019	0	20	20	60	80	100
02/10/2019	06/10/2019	0	10	20	40	60	98

With the Probit analysis, we found an LC_{50} of 8.14 mg/L for Karmex (diuron) in *M. olfersii* at 96 h for March, with a 95 % confidence interval of 7.14 to 9.24 mg/L (**Fig. 2a**). For October, the LC_{50} was

7.57 mg/L, with a 95 % confidence interval of 6.62 to 8.55 mg/L (**Fig. 2b**).

With the Probit-Log(dose) linear regression model, LD_{50} values of 7.974 and 8.221 mg/L for March and October, respectively, were obtained for *M. acanthurus* (**Fig. 3a, b**). For *M. olfersii*, LD_{50} values of 7.504 and 7.008 mg/L were obtained for March and October, respectively (**Fig. 3c, d**). The four correlation coefficients were statistically significant ($p < 0.05$). The values calculated for LC_{50} were higher than for LD_{50} . Differences statistically significant between the LD_{50} and LC_{50} for *M. acanthurus* ($t = 70.217$, $p = 0.009$) and *M. olfersii* ($t = 16.189$, $p = 0.0393$) were obtained. Kamau et al. (2012) found an LD_{50} of 153.2 mg/L for *Artemia salina* with plant extracts as an alternative to synthetic insecticide. In this regard, Reed (2017) mentions that the smaller the LD_{50} values of a substance under test, the greater its toxicity.

Regarding the toxicity bioassays with 80 % diuron, an LC_{50} of 84 to 300 mg/L for *Lepomis macrochirus* Rafinesque, 1819, 16 to 23.8 mg/L for *Oncorhynchus mykiss* (Walbaum 1792), and 0.5 to 6.0 mg/L for *Morone saxatilis* (Walbaum, 1792) were found. The Pesticide Properties Database (PPDB 2021) determined that the LC_{50} for aquatic crustaceans is 1.1 mg/L; however, Shaalaa et al. (2015) found that for *Artemia salina* (Linnaeus, 1758) nauplii, the increase in diuron concentration increases mortality since they determined diuron LC_{50} values of 23.27 mg/L, 12.19 mg/L, and 6.00 mg/L after 24, 48, and 72 h of exposure, respectively. Likewise, Alyürük and Çavaş (2013) found that in *A. salina* larvae, the EC_{50} value for diuron was 12.01 mg/L. Meanwhile, Koutsafitis and Aoyama (2008) reported

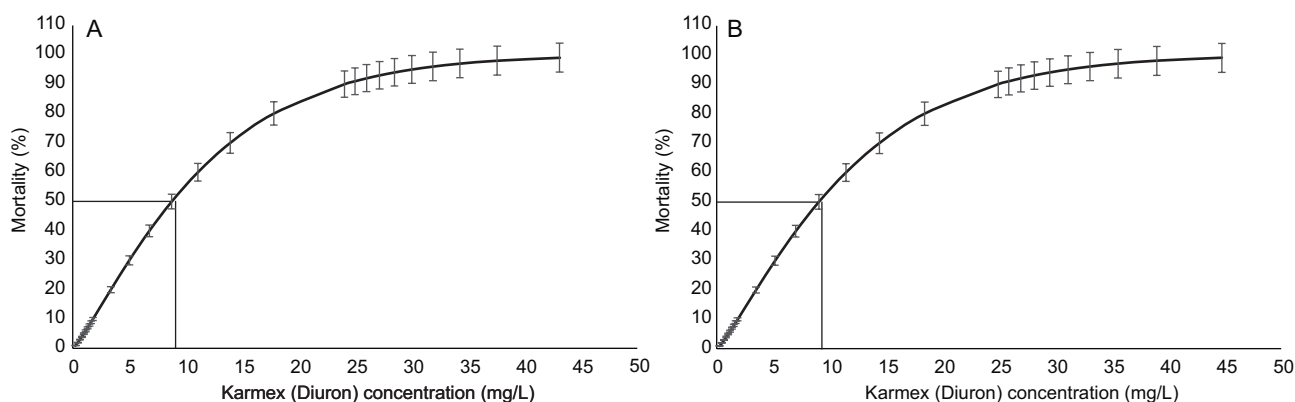


Fig. 1. Mean lethal concentration (LC_{50}) of Karmex (diuron) for *Macrobrachium acanthurus* at 96 h with upper and lower 95 % confidence intervals: (a) March, (b) October.

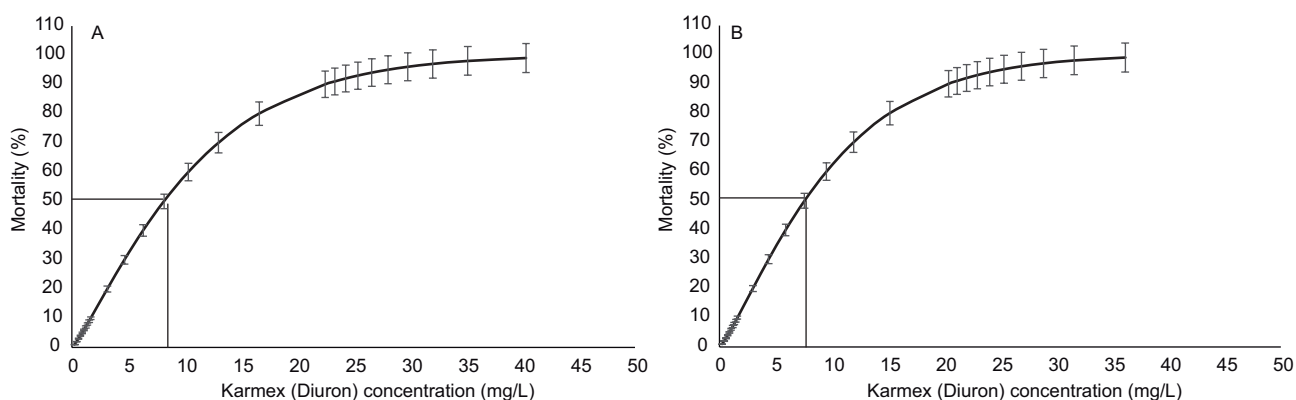


Fig. 2. Mean lethal concentration (LC_{50}) of Karmex (diuron) for *Macrobrachium olfersii* at 96 h with upper and lower confidence intervals of 95 %: A, March, and B, October.

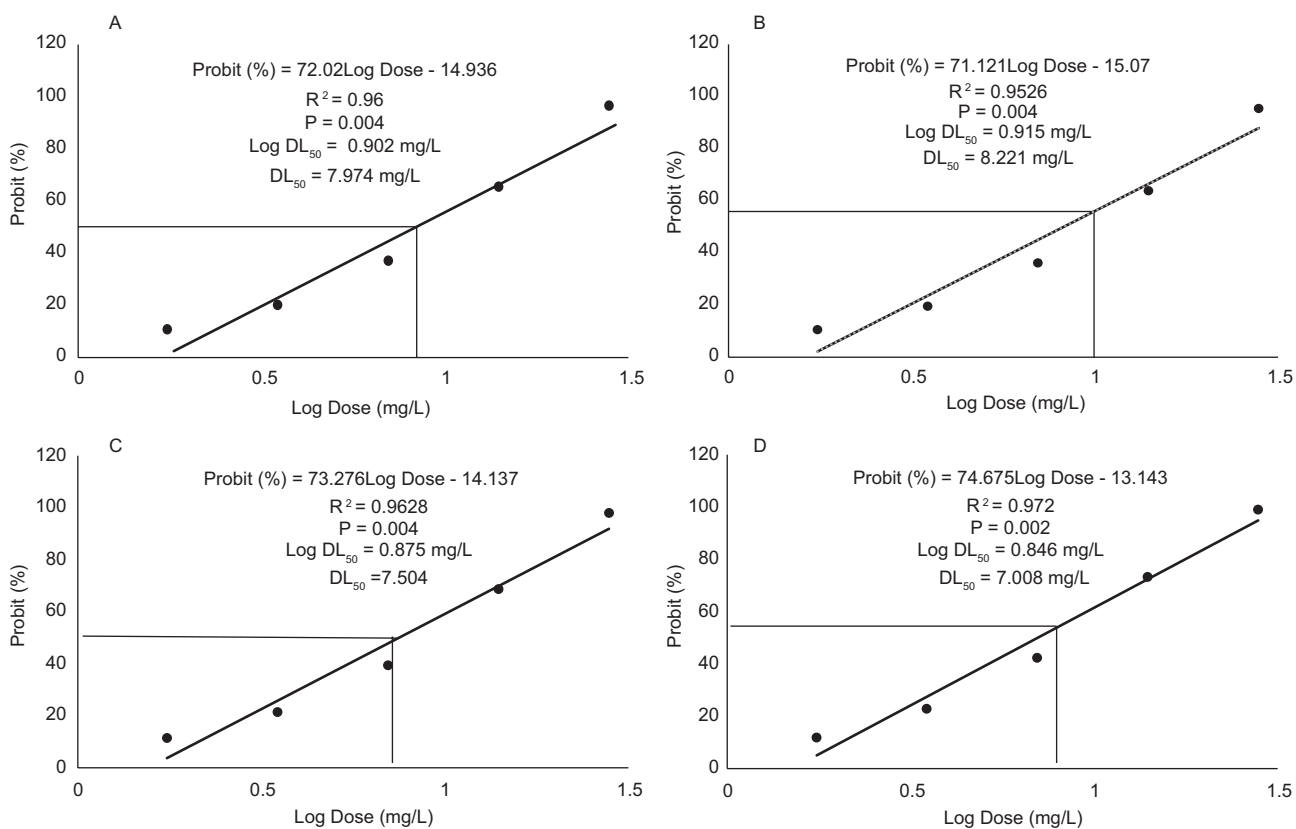


Fig. 3. Lethal dose (LD_{50}) of Karmex (diuron) with the Probit-Log(dose) lineal regression model for *Macrobrachium acanthurus*: (a) March, (b) October; and *M. olfersii*: (c) March, (d) October.

that the LC_{50} value for diuron in *A. franciscana* (Kellog, 1906), was 12.5 mg/L. With an active ingredient concentration of 80-95 %, LC_{50} values of 0.16 to 0.7 mg/L were found for *Gammarus fasciatus*, 0.16 mg/L for *Gammarus lacustris*, 47 (26 h) to 8.4 (48 h) mg/L for *Daphnia magna*, and 15.5 mg/L for

Asellus brevicauda (Turner 2003). Jinlin et al. (2020) report that the LC_{50} of diuron (48 h) to *Daphnia magna* was 17.1 mg/L and Sipcam (2020) mentions that for the same species it is 6.3-13 mg/L.

The LC_{50} of diuron found in this study for both *M. acanthurus* at 96 h (10.23 mg/L) and *M. olfersii*

(9.24 mg/L) were slightly lower than that found for *Artemia* species and is consistent with species of the Cladocera suborder and peracarids.

No change in the appearance of the organisms of the two *Macrobrachium* species was observed. Regarding the behavior, a decrease in movement was recorded, since organisms remained for a longer time at the bottom of the container with concentrations of 6.96 to 28 mg/L; at lower concentrations, active movement of the organisms was observed throughout the container.

It is important to mention that since 1980, diuron has been used in the state of Veracruz, Mexico at a concentration of 2.0 kg/ha (Ramírez-Mora et al. 2018). This compound, like other pesticides, has the potential to enter water bodies through rain that flows down streams and rivers into estuaries and oceans (NPIC 2021). Giacomazzi and Cochet (2004) reported that diuron is a biologically active pollutant present and persistent in water and sediments. In a three-year study, Xu et al. (2013) found that the annual average amount of diuron in seawater and sediments presented a slow-growing trend, indicating that diuron poses a potential risk to the aquatic ecosystems, like in our case, the estuary of the Jamapa River.

Thus, according to the previous values, there are differences in the resistance of exposure to diuron among species, which highlights the importance of knowing the effect of concentration of contaminants (such as herbicides) in species used in aquaculture.

According to the above and considering the studies of toxicity realized by Barbieri et al. (2016) with carbofuran and Gil-Díaz (2020) with paraquat in *M. olfersii*, as well as Marques et al. (2021) with chlorpyrifos in *M. acanthurus*, it is possible to consider that the postlarvae of *Macrobrachium* can be used as a suitable test organisms to evaluate the toxicity of diuron and other pesticides. Given the relevant role of prawn shrimp in aquatic environments and its use for human consumption, its inclusion in ecotoxicological studies is recommended.

CONCLUSIONS

The lethal concentrations of Karmex (diuron) for two species of *Macrobrachium* were greater than 3.5 mg/L. The maximum concentration of diuron (28 mg/L) caused a 100 % mortality rate in both *Macrobrachium* species. The LC_{50} and LD_{50} of diuron found in this study for both *M. acanthurus* ($LC_{50} = 10.23$ mg/L, $LD_{50} = 8.221$ mg/L) and *M.*

olfersii ($LC_{50} = 9.24$ mg/L, $LD_{50} = 7.504$ mg/L) at 96 h were lower than that found for *Artemia* species and are consistent with the values for species of the Cladocera suborder. Considering the above, *Macrobrachium* postlarvae can be used as test organisms to evaluate the toxicity of Karmex (diuron).

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