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Research Article

Acute toxicity of diuron and glyphosate in megalopae of *Callinectes sapidus* from the Jamapa River Estuary, Veracruz

Sergio Cházaro-Olvera[1], Daniela Michel Solorzano-López[1], Jesús Montoya-Mendoza[2]
María del Refugio Castañeda-Chávez[2] & Fabiola Lango Reynoso[2]
[1]Laboratorio de Crustáceos, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla, Estado de México, México
[2]Laboratorio de Investigación Acuícola Aplicada, Tecnológico Nacional de México
Instituto Tecnológico de Boca del Río, Boca del Río, Veracruz, México
Corresponding author: Sergio Cházaro-Olvera (schazaro@gmail.com)

ABSTRACT. The objective of this work was to evaluate the acute toxicity of the commercial herbicides Karmex® (diuron: DCMU 3-(3,4-dichlorophenyl)-1,1-dimethylurea) and Herbipol® (glyphosate: N-(phosphonomethyl) glycine) on the megalopae of *Callinectes sapidus* and measure their relative abundance in the association of larval and postlarval species of crustaceans from the estuary of the Jamapa River, Veracruz. Collections were made overnight using white light traps. Likewise, dissolved oxygen, pH, temperature, total dissolved solids, and salinity were measured *in situ*. It was found that the association of larvae and postlarvae consisted of *Macrobrachium acanthurus*, *M. olfersii*, *Potimirim mexicana*, megalopae of *C. sapidus* and *Armases ricordi*, and zoeas of Brachyura. In total, 559 and 1057 *C. sapidus* megalopae were collected in November and March 2019, respectively. Dissolved oxygen was 6 and 7 mg L^{-1}, pH was 7 and 8, the temperature was 26 and 27°C, total dissolved solids were 700 and 1500 ppm, and salinity was 0.70 and 16 in November and March, respectively. Using Probit analysis, a 96 h LC_{50} of diuron (Karmex®) was 7.69 ± 1.07 mg L^{-1} in March and 6.64 ± 0.93 mg L^{-1} in November. The LC_{50} for glyphosate (Herbipol®) was 247.83 ± 34.54 mg L^{-1} in March and 288.18 ± 38.66 3 mg L^{-1} in November. The blue crab *C. sapidus* is tolerant to a wide range of physicochemical factors; however, it is sensitive in the postlarval stages, like other species of crustaceans, to the herbicides diuron and glyphosate, for which the megalopae can be used in ecotoxicological studies.

Keywords: *Callinectes sapidus*; Crustacea; Brachyura; Portunidae; postlarvae; blue crab; herbicides

INTRODUCTION

Some herbicides are considered by the U.S. Environmental Protection Agency (EPA) to be pollutants in rivers, coastal lagoons, estuaries, and irrigation canals due to their high toxicity, persistence, and mobility (Hirata 2002).

Among these herbicides is diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), a compound used in cotton, pineapple, and sugarcane crops for weed control. It is soluble in water and volatile; under certain conditions, it filters into groundwater. It is moderately toxic to most species of aquatic ecosystems (PPDB 2019a).

Another herbicide widely used in corn and sugarcane crops in Veracruz, Mexico, is glyphosate (Zuleta-Rodríguez & Vázquez-Torres 1992). In this region, many glyphosate-based herbicides are used, a compound that affects water quality and organisms by modifying their structure and functionality (Torres 2017). Glyphosate is highly soluble in water, and under certain conditions, it is moderately toxic to aquatic crus-

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taceans (PPDB 2019b). It is important to mention that in Mexico, a decree was published in the Official Gazette of the Federation on December 31, 2020, that establishes the actions necessary to gradually replace glyphosate and agrochemicals that contain it as an active ingredient with alternatives that do not harm human health and the environment (DOF 2020).

Surface runoff leads to the escape of agrochemicals and has caused serious effects on ecosystems such as estuaries and coastal lagoons (Vázquez-Botello et al. 1996). Some herbicides cause a decrease in the abundance of aquatic species of commercial importance, such as shrimps and prawns of the genera *Penaeus* Fabricius, 1798 and *Macrobrachium* Spence Bate, 1868, and crabs of the genus *Callinectes* Stimpson, 1860, either by reducing their reproduction rate or by physiological alterations that increase mortality (González 1998).

The test subjects in this study are from the blue crab *Callinectes sapidus* Rathbun, 1896, species with a wide distribution in tropical and semitropical environments. This species has a high tolerance to salinity changes, and its distribution patterns are usually related to protection, feeding, growth, maturation, recruitment, and reproductive events (Ortiz et al. 2007). The crabs have a complex life cycle, comprising planktonic, nektonic, and benthic stages in marine zones, estuarine systems, and even freshwater environments. *Callinectes* megalopae, juveniles, and adults are opportunistic carnivores, acting as predators of mollusks and other crustaceans (Ramírez et al. 2003). The blue crab *C. sapidus* is an important species in the fishing industry; it is distributed from Nova Scotia to southern Argentina, including the Gulf of Mexico, and can withstand wide variation in environmental conditions, as it has been found from freshwater systems to hypersaline lagoons (Williams 1984, Raz-Guzmán et al. 1992). In Mexico, *C. sapidus*, has been reported in estuaries and coastal lagoons of the Veracruz State (Rocha-Ramírez et al. 1992, Álvarez et al. 1999). In the estuary of Jamapa River, to the south of Veracruz, also captured the blue crab; even the migratory process of larvae and megalopae has been observed (Cházaro-Olvera et al. 2021). Is important to mention that the Jamapa River discharges its waters into the protected natural area Parque Nacional Sistema Arrecifal Veracruzano (PNSAV) (Liaño-Carrera et al. 2019).

Regarding the performance of glyphosate toxicity tests on *Callinectes*, only the study of Osterberg et al. (2012) is known, who found that the LC_{50} was between 6.279 and 316 mg L^{-1} for megalopae and juveniles of *C. sapidus*. Other research has reported LC_{50} values for other species of crustaceans, such as *Daphnia magna* Straus, 1820, *Gammarus pseudolimnaeus* Bousfield, 1958 (Folmar et al. 1979), *D. magna*, *Hyalella azteca* (Saussure, 1858) (Henry et al. 1994), and *Acartia tonsa* Dana, 1849 (Tsui & Chu 2003).

Regarding the herbicide diuron, no studies have been carried out on *C. sapidus*; however, the LC_{50} has been obtained in other species of crustaceans with a range from 6 to 47 mg L^{-1}, such as *Artemia salina* (Linnaeus, 1758) (Alyürük & Çavaş 2013, Shaalaa et al. 2015), *A. franciscana* Kellog, 1906 (Koutsaftis & Aoyama 2008), *Gammarus fasciatus* Say, 1818, *G. lacustris* G.O. Sars, 1863, *D. magna* (Crosby & Tucker 1966) and *Caecidotea brevicauda* (Forbes, 1876) (Mayer et al. 1986).

As noted, there is little information on the effects of herbicides on megalopae of *C. sapidus*, so the present study aims to assess the acute toxicity of the herbicides Karmex® (diuron: DCMU 3-(3,4-dichlorophenyl)-1,1-dimethylurea with 80% active ingredient) and Herbipol® (glyphosate: N-(phosphonomethyl) glycine with 36% active ingredient) on the megalopae of *C. sapidus* collected in the estuary of the Jamapa River, Veracruz, SW Gulf of Mexico.

MATERIALS AND METHODS

Study area

The study site was in the Jamapa River originates at the border of the states of Puebla and Veracruz and begins with the Ixcualco stream, which flows into the Cotaxtla River. This river joins the Jamapa River, which continues its route until it empties into the Gulf of Mexico in the municipality of Boca del Río, Veracruz. The estuary of the Jamapa River has other tributaries, such as the Arroyo Moreno stream and El Estero (Fig. 1). The Jamapa River basin is located between 18°45'-19°14'N and 95°56'-97°17'W. It has a warm sub-humid climate, with an average annual temperature greater than 22°C. The rainfall in the driest month ranges from 0 to 60 mm, with a summer rainy season over 55.3 mm in winter from 5.0 to 10.2% of the annual total (Fuentes-Mariles et al. 2014). The level of the Jamapa River estuary has a micro-tidal modulation of 2 m, with a semi-diurnal, diurnal, and lunisolar synodic component (Salas-Monreal et al. 2019). Therefore, this area shows greater pollution from the urban discharges it receives directly (Salas-Monreal et al. 2020).

Fieldwork

Callinectes sapidus postlarvae were collected in two samples using white light traps made from plastic boxes (Cházaro-Olvera et al. 2018).

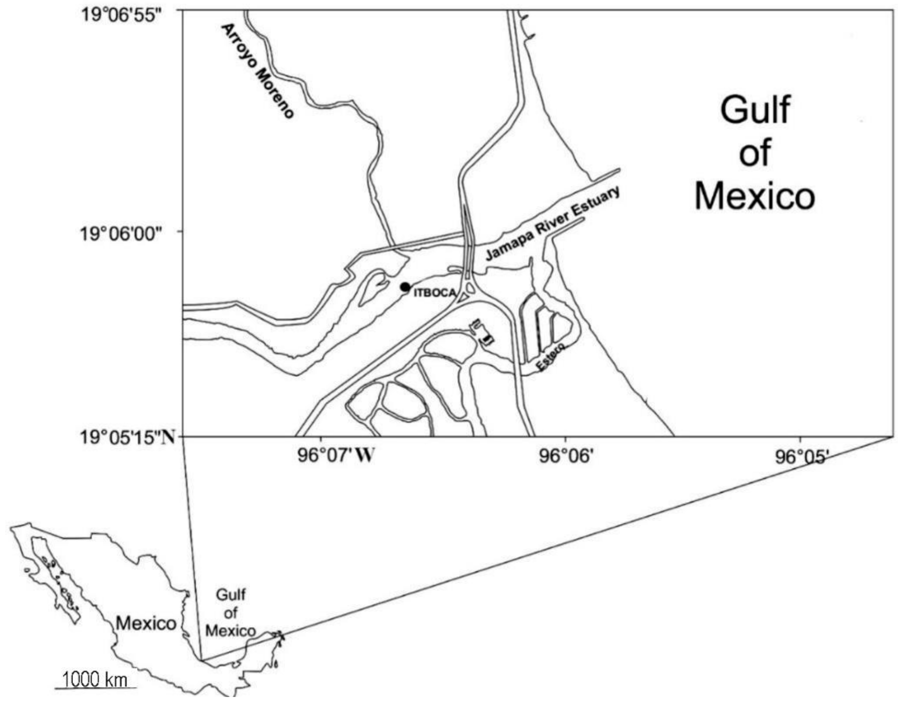

Figure 1. Location of the sampling site in the Río Jamapa estuary, Boca del Río, Veracruz. Black circle: sampling site.

The traps were placed overnight on the jetty of the Technological Institute of Boca de Río Veracruz at the site located at 19°05'50.23"N and 96°06'36.97"W (Fig. 1). The boxes for the collection were placed at 20:00 h on the first day and removed at 09:00 h the next day. The physicochemical parameters were measured at each site with a previously calibrated Hanna® HI 9828 multiparametric. For the in vivo sample, *C. sapidus* megalopae were selected under the microscope in 60 mm diameter Petri dishes to perform the bioassays. The rest of the sample was fixed with 70% alcohol and labeled with the date and place of sampling.

Laboratory work

The samples fixed were transported to the Crustacean Laboratory of the Faculty of Higher Studies Iztacala of the National Autonomous University of Mexico for separation and counting. To identify the megalopae of *C. sapidus*, we used a Motic model SMZ-168 optical microscope following the original description of Costlow & Bookhout (1959).

Bioassays

Water collected from the estuary was acclimatized after being filtered with 50 µ mesh 7 h prior and aerated using Elite 802® pumps to perform bioassays. Ten postlarvae were used for each 500 mL of water with different concentrations of dissolved pesticide in a plastic container of 1000 mL to carry out the bioassays, following Mohapatra & Rengarajan (1995). The water in the containers was maintained with constant aeration using Elite 802® pumps. A 5 mm perforation was made in the lid of each container to insert a hose that was also 5 mm in diameter and injected air. At the end of the hose, a diffuser was placed to control the air entrance to avoid damaging the megalopae. The agrochemicals used were the commercial brands Karmex® (diuron: DCMU 3-(3,4-dichlorophenyl)-1,1-dimethylurea with 80% active ingredient) and Herbipol® (glyphosate 36% active ingredient). Preliminary 96 h static bioassays were carried out to determine the concentration ranges of the definitive bioassays; in this regard, the concentrations of the active ingredients and the concentrations used in bioassays for other species of macrocrustaceans were considered (Koutsaftis & Aoyama 2008, Alyürük & Çavaş 2013, and Shaala et al. 2015 for diuron, and Henry et al. 1994 for glyphosate).

The concentrations of diuron used were 1.75, 3.5, 6.96, and 14 mg L^{-1}, and glyphosate was 101.25, 202.5, 405, 810, and 1620 mg L^{-1}. A control without any agrochemical was also performed for all treatments. Mortality readings were taken after 1, 2, 4, 8, 18, 24, 36, 48, and 96 h of exposure (UC-Peraza & Delgado-Blas 2012). The U.S. Environmental Protection Agency guidelines (US-EPA 2021) for toxicity bioassays were considered, following the guidance for ecological effects testing (US-EPA 2016).

The mean lethal concentration (LC_{50}) was determined using the statistical program Minitab Version 18.1 (Minitab©, LLC, State College PA, USA), and a 95% confidence interval was obtained.

RESULTS

The values of the physicochemical parameters registered were as follows: the dissolved oxygen concentration was between 6 and 7 mg L^{-1}, the pH was between 7 and 8, the temperature was between 26 and 27°C, the total dissolved solids were between 700 and 1500 ppm, and the salinity was between 0.70 and 16, in November and March 2019, respectively (Table 1a). The values of the physicochemical parameters were maintained during the bioassays (Table 1b).

The sampling method was efficient for postlarvae collection of *Macrobrachium acanthurus* (Wiegmann, 1836), *M. olfersii* (Wiegmann, 1836), *Potimirim mexicana* (de Saussure, 1857), megalopae of *Callinectes sapidus* and *Armases ricordi* (H. Milne Edwards, 1853), and zoeas of the infraorder Brachyura Latreille, 1802. A total of 1616 megalopae of *C. sapidus* were used, of which 559 were obtained in November and 1057 in March, to perform bioassays.

When analyzing the response to herbicides, a 100% mortality of *C. sapidus* was obtained with the maximum concentration of 28 mg L^{-1} of diuron (Karmex®) in November and March. Based on Probit analysis, the CL_{50} of diuron (Karmex®) for *C. sapidus* at 96 h was 7.69 mg L^{-1} in March (6.62-8.76 mg L^{-1}) (Fig. 2). A CL_{50} of 6.64 mg L^{-1} was obtained in November (5.71-7.57 mg L^{-1}) (Fig. 2).

With the maximum concentration of 1620 mg L^{-1} of glyphosate (Herbipol®), 100% mortality of *C. sapidus* was recorded at both time points. The estimated CL_{50} of glyphosate (Herbipol®) was 247.83 mg L^{-1} in *C. sapidus* at 96 h in March (213.29-281.37 mg L^{-1}) (Fig. 3). For November, the CL_{50} was 288.18 mg L^{-1} (249.52-326.84 mg L^{-1}) (Fig. 3).

DISCUSSION

The dissolved oxygen value is consistent with that of Torres et al. (2007), who reported a dissolved oxygen value of 5.9 mg L^{-1} during the winter (November-March). The pH values of the water are also similar to those obtained by Torres et al. (2007), who recorded 7.5 to 7.9. The temperature remained between 27 and 28°C. However, another study found temperatures of up to 33.6°C in the river (Torres et al. 2007). The total dissolved solids presented values higher than those SEMARNAT (2002) mentioned, reaching 179 ppm. The interval of salinity includes the value recorded by Torres et al. (2007), whit 11.6. Miranda et al. (2016) mention that high levels of dissolved solids are associated with spills derived from the region's economic activities, such as agriculture.

The larvae and postlarvae found in this study are common elements of other estuarine systems throughout Veracruz (Álvarez et al. 1999, 2011, Rodríguez et al. 2019). The highest abundance de megalopae registered in March could be explained because the main spawning season of *C. sapidus* in some estuarine systems Gulf of Mexico occurs from March to July and the second from August to October (Tagatz 1968, Loran et al. 1993).

The LC_{50} value of diuron (Karmex®) obtained in this study is consistent with the concentrations recorded for *Artemia salina* and *A. franciscana*, for which an LC_{50} of 6.0 mg L^{-1} has been found (Shaala et al. 2015) and is close to the LC_{50} registered for *Daphnia magna* at 8.4 mg L^{-1} (48 h). (Crosby & Tucker 1966). However, this value is higher than that found for the amphipods *Gammarus lacustris* and *G. faciatus*, with

Table 1. Physicochemical factors. Environmental conditions when *Callinectes sapidus* megalopae were collected in the Boca del Río Veracruz estuary, and in the laboratory during the bioassays.

Environmental factors	Boca del Río Veracruz		Laboratory bioassays	
	March	November	March	November
Dissolved oxygen (mg L^{-1})	6.93 ± 0.12	5.79 ± 0.12	6.82 ± 0.12	6.73 ± 0.15
pH	7.54 ± 0.38	7.81 ± 0.21	7.61 ± 0.12	7.71 ± 0.32
Temperature (°C)	26.68 ± 0.12	26.08 ± 0.92	27.01 ± 0.15	27.38 ± 1.09
Total dissolved solids (ppm)	732.5 ± 34.44	1443.33 ± 65.91	722.8 ± 175.33	1252.66 ± 106.69
Salinity	15.35 ± 0.65	0.89 ± 0.19	13.99 ± 5.48	0.72 ± 0.18

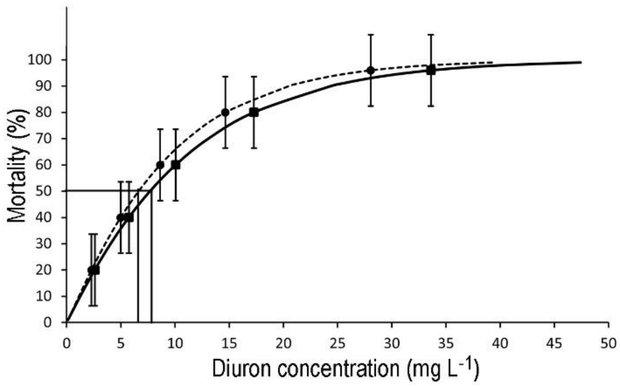

Figure 2. Mean lethal concentration (LC_{50}) of Karmex® (Diuron) in *Callinectes sapidus* megalopae at 96 h with upper and lower confidence intervals of 95%. Black circle: March, black squared: November.

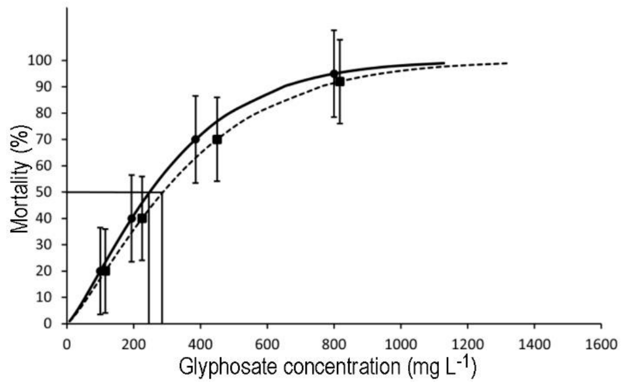

Figure 3. Mean lethal concentration (LC_{50}) of Herbipol® (Glyphosate) in *Callinectes sapidus* megalopae at 96 h with upper and lower confidence intervals of 95%. Black circle: March, black squared: November.

with a CL_{50} of 0.16-0.7 mg L^{-1} (Sanders 1969, 1970). In this regard, Cornejo et al. (2021) found that some species of the bottom fauna of freshwater amphipods are less tolerant to pesticide pollution.

The CL_{50} found in the present study for glyphosate (247.83-288.18 mg L^{-1}, Herbipol® Glyphosate 36% active ingredient) (is between those obtained for *D. magna* and *Hyalella azteca*, 218 and 720 mg L^{-1}, respectively (Trademark used Rodeo® Glyphosate, active ingredient 53.8%) (Henry et al. 1994), and close to that recorded for the copepod *Acartia tonsa* (177 mg L^{-1}) (Trademark used Roundup® Glyphosate, active ingredient 41%) (Tsui & Chu 2003). In a study on megalopae of *C. sapidus* conducted by Osterberg et al. (2012), a CL_{50} of 6.28 mg L^{-1} was recorded (Trademark used Roundup-Pro® Glyphosate, active ingredient 50.2%). In other crustacean species, such as *D. magna* and *Gammarus pseudolimnaeus*, the values found were between 3 and 62 mg L^{-1} (Trademark used Roundup® (MON02139) Glyphosate, active ingredient 48%) (Folmar et al. 1979). The difference in the crustacean species of LC_{50} may be related to glyphosate composition and the surfactants or co-formulants. Formulations vary between brands and countries (Parlapiano et al. 2021). There are glyphosate compounds as the active ingredient (36-50.2%), water, salts, and co-formulants such as polyoxyethylene-amine (POEA) (Parlapiano et al. 2021). Compared to other formulations, the POEA incorporation into glyphosate compounds is more toxic (Mesnage et al. 2013, Mesnage & Antoniou 2018). For example, formulations with POEA were more toxic to *A. salina* than formulations without POEA (Brito-Rodrigues et al. 2017).

The blue crab *C. sapidus* has a high tolerance to changes in environmental factors (Williams 1984); however, in postlarval stages, it is sensitive to contaminants such as the herbicides diuron and glyphosate, so that it can be used at this stage in ecotoxicological studies.

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