RISK ASSESSMENT OF THE NEONICOTINOID INSECTICIDE ACETAMIPRID ON TWO NON-TARGET SPECIES, *DAPHNIA MAGNA* STRAUS, 1820 (CRUSTACEA, CLADOCERA) AND *PLEA MINUTISSIMA* LEACH, 1817 (INSECTA, HETEROPTERA)

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Abstract. Aquatic organisms are used to assess the health of aquatic systems. Extensive use of pesticides in agricultural and public health programs causes many environmental problems and toxic effects on aquatic animals especially against non-target organisms such as non-target organisms for mosquito larvae *Plea minutissima* Leach, 1817 and cladoceran biofilter and biomarker *Daphnia magna* Straus, 1820. These biological models were selected for their importance in the food chain and in the maintenance of homeostasis. Rustile is a multi-purpose toxic insecticide, manufactured by SARL DEKACHIM, consisting of 20% of the active ingredient Acetamipride. The lethal doses used for the insecticide in the treatment of these two non-target groups were LC_{50} = 1.45 ml/l and LC_{90} = 1.90 ml/l. The superiority of *Culex pipiens* larvae was recorded during three days of treatment. Both types showed a variable response to the tested product depending on doses and exposure times. Toxicity assays clearly showed a variable sensitivity of the two treated species recorded at high rates. The maximum values recorded were 86% and 97% for LC_{50} and LC_{90}, respectively. It was found that rusty pesticides affect the two non-target tested species. The most sensitive species to this pesticide was *Daphnia magna*.
Keywords: *ecotoxicity, Rustile, insect, crustasia, Heteropter*

Introduction

The extensive use of chemicals exposes the population to a high risk of contamination by these products (Svetlana, 2022). Numerous examples of disrupting the balance in the animal world confirm our statements. Pesticides in general and organochlorines in particular, because of their endocrine disrupting properties, represent potential promoters of various pathologies (Bor, 2020). A possible relationship is evoked between exposure to these substances and the appearance of a mammary tumor, early puberty, allergy or male fertility disorders. Chemicals have brought considerable benefits to society. However, some of them pose a threat to wildlife and humans. Indeed, chemical pollution can impact biodiversity (Brahic and Terreaux, 2009), potentially increasing the vulnerability of organisms to other threats such as climate change (Ravi, 2021). Insects and some crustaceans are excellent witnesses of the quality of the habitats. Thus, they are used as bioindicators of water quality (Yapo, 2012; Brahimi et al., 2021). Pest control is based mainly on the use of pesticides, and this is due to the presence of many substances that lead to the diversity of their composition, which increases their danger in the case of multiple uses (Hassaan, 2020). The world market for pesticides represents approximately 40 billion dollars; it has been stable since the year 2000. Algeria imports on average 8,827 tons of pesticides for an estimated cost of nearly 4.5 billion dinars per year. The

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intensive use of pesticides is often accompanied by fatal consequences, resulting in the destruction of crop pollinating insects with the effect of decreasing yields. Elimination of natural enemies of pests results in the loss of natural phytosanitary methods that maintain populations of natural crop enemies and vectors. The emergence of resistance to pesticides results in the intensification of their use, pollution of soil and water, and finally the degradation of biodiversity, especially of non-target organisms. This is due to the excessive and irrational use of pesticides (Goh, 2021). *Daphnia* are filter-feeding organisms phytophagous and bacteriophagous, and considered as primary consumers (Lovern et al., 2007). Heteroptera, formerly called bugs, are a suborder of hemimetabolic insects in the order of Hemiptera (Elder, 2012). They are also good bioindicators, especially for heavy metals (Brahimi et al., 2021). They are often formidable predators themselves and effectively contribute to the reduction of mosquito populations (Elder, 2016).

In an effort to understand the environmental impact of this contamination, we have examined the degradation of diflubenzuron, a chitin synthesis inhibitor commonly used in crop protection, in saltwater (Soltani and Morsli, 2003) and freshwater (Zaidi et al., 2013). In addition, ecotoxicological risks of some insecticides were evaluated on non-target species: diflubenzuron and novaluron chitin synthesis inhibitor insecticides, on structure and biochemical composition of cuticles and stress biomarkers in crustacean species *Penaeuskerathurus* and *Palaemonadspersus* (Morsli and Soltani, 2003; Soltani et al., 2009; Morsliet al., 2015; Berghiche et al., 2016; Benradia et al., 2016; Lechekhab and Soltani, 2018) and on a larvivorous fish, *Gambusiaaffinis*, testing diflubenzuron and flucycloxuronon on growth, enzymatic activities and pattern of recovery (Zaidi and Soltani, 2010, 2011, 2013) and thiametoxam a neonicotinoid insecticide on growth and biomarker responses (Cheghib et al., 2020). More recently, Benchaabane et al. (2022) have reported the effect of a sublethal concentration of thiamethoxam on selected biomarkers in the African honeybee Apismelliferaintermissa. The lipophilic nature of neonicotinoids such as acetamiprid facilitates their passage through all biological barriers. The digestive distribution is rapid and complete, the plasma peak is obtained at the second hour, while the blood-brain barrier of mammals is very little permeable to acetamiprid (David et al., 2007; Terayama et al., 2016).

These products used to protect crops pose significant and potential hazards to non-target aquatic organisms due to their seepage and deposition into surface waters and their inability to break down, so they travel to rivers and lakes to pollute them (Morsli et al., 2015).Therefore, the present study aimed to examine the impact of Rustile, trade formulation of a neonicotinoid insecticide acetamiprid intensively used in agriculture, on two non-target species *Daphnia magna*and *Plea minutissima* collected from Garaat Djamel (Northeast Algeria). The two non-target species were treated with two lethal doses of Rustile LC_{50} and LC_{90} were obtained after testing. It is an insecticide from the chemical family of Acetamiprid, works on insect pests, and is considered an environmental pollutant (xenobiotic).

Materials and methods

Presentation of the study area Garaate Djamel

Garaate Djamel is located in the commune of El Chat, daira of Ben Mhidi on the left side of the wilaya road number 109 connect the wilaya of Annaba with El- Kala, and it's

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located at 36° .88 N and 07° .90 E. This body of water does not discharge into the sea water which minimizes their variation under the effect of currents (*Figs. 1* and *2*).

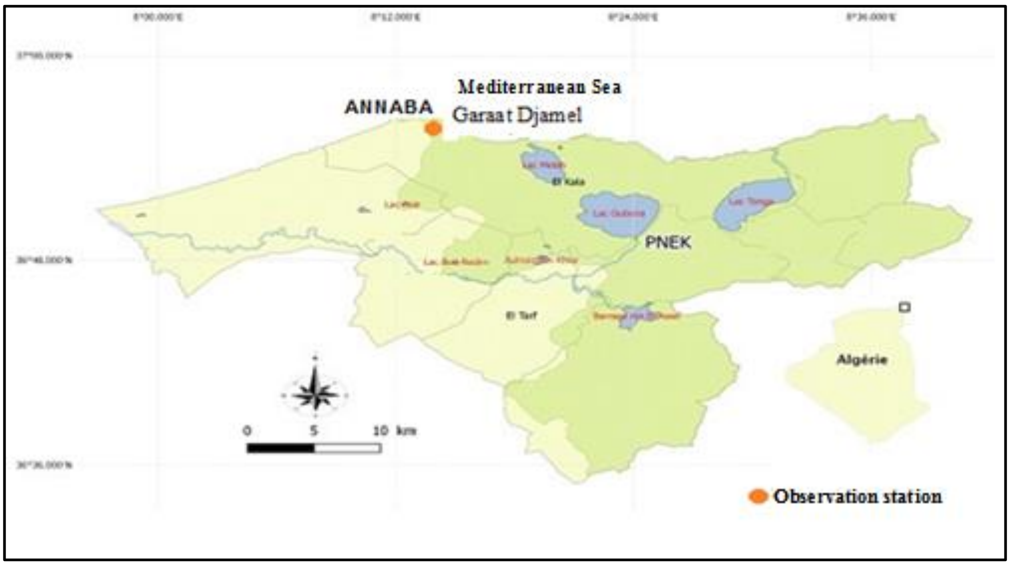

Figure 1. *Location of observation station (Garaat Djamel)*

Figure 2. *Presentation of the Garaat Djamel study site (Personal photos)*

Presentation of the two tested species

Daphnia magna Straus, 1820 is a micro crustacean widely distributed in non-flowing fresh waters of temperate climatic zones. Its size varies between 3 and 5 mm as an adult. The head includes the eyes, the mouth and 2 antennas which are used for locomotion. The body is protected by a transparent carapace which is changed during the moults. This carapace is finished by an apical spine. The ventral part is equipped with appendices which filter the phytoplankton (Vesseron, 2000; Santiago et al., 2002). The use of *D. magna* for Eco toxicological tests has many advantages such as relatively easy rearing,

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parthenogenetic reproduction ensuring a genetically stable population (Sison-Mangus et al., 2015). Moreover, its global distribution and its role in the aquatic ecosystem make this model an interesting indicator of the potential impact of a pollutant on the environment (Thushari, 2020).

Plea minutissima are heteroptera, formerly called Bedbugs, they are a suborder of hemimetabolous insects of the order Hemiptera (Akhoundi, 2020). Which is characterized by often incomplete metamorphoses, with a size at a length of 2.5 - 2.7 mm. The color can vary from white to brown to black. The body is convex, is composed of 3 distinct parts although the head, and thorax are closely related. They hold an important place in the ecology of freshwater (Moulton, 2018). They constitute a food resource for various organisms such as amphibians, fish, birds. They are often formidable predators themselves and effectively contribute to the reduction of mosquito populations (Elder, 2016).

Insecticide and treatment

Rustile insecticide

Rustile is the trade name of the product, it belongs to the Neonicotinoid family and targets the nervous system, its action at the level of nicotinic acetylcholine receptors, the active molecule is Acetamiprid (Whitehorn et al., 2012). It is a polyvalent insecticide in powder form, its active ingredient is acetamiprid (20% WP) (WHO, 2019). Acetamiprid is an active ingredient with systemic action (penetrates into the sap of the plant then diffuses in all parts). Due to its systemic action, Acetamiprid has a broad spectrum of efficacy, particularly on biting-sucking insects and leaf miners and certain beetles. It also attacks virus vectors, and acetamiprid metabolic pathways pass through brain cytochromes P450 (Dively, 2015).

Bioassays

Sampling was conducted during the month of February 2021. Using a fine mesh dip net. The two species tested *Daphnia magna* and *Plea minutissima* were collected at the lake shore. The individuals collected were kept in containers filled with water, hermetically sealed and well labeled (Date, station and species name) and were transported to the laboratory, where they will be sorted and identified systematically. Under laboratory conditions, the toxicity tests of the product were carried out on the fourth larval stage of a species of Culicidae, which presents an abundance-dominance in the study area *Culex pipiens* L.1758 (Arroussi, 2021). These letters were conceded it as target species. The LC_{50} and the LC_{90} were tested on two non-target species, living in association with the Culicidae. The two lethal concentrations obtained from the toxicological analysis of the treated species *Culex pipiens*, the LD_{50} and the LD_{90}, were tested on two non-target species, living in association with Culicidae. The tests carried out on the two species treated by the two doses, include for each dose and each species, a control and 5 repetitions. For this purpose, 150 ml of water from the bed was filtered and introduced into cups in the presence of 20 individuals of *D. magna* and *Plea minutissima*. Therefore, both lethal doses were administered: the first LD_{50} (1.45 ml/l) and the second LD_{90} (1.90 ml/l). The tests were performed for 3 days and the mortality was recorded every day (24 h; 48 h; 72 h).

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Statistical analysis

Results are presented by mean ± standard error (SD). Significance between the different series was tested using the ANOVA test: (AV1; AV2). To verify the existence or absence of possible variations between the treatments of the factors studied with the Tukey test. Statistical analyses were performed using the statistical software Minitab 16.

Results

Toxic effect of two Daphnia magna and Plea minutissima

The lipophilic nature of neonicotinoids such as acetamiprid facilitates their passage through all biological barriers. The digestive distribution is rapid and complete, the plasma peak is obtained at the second hour, while the blood-brain barrier of mammals is very little permeable to acetamiprid (David et al., 2007; Terayama et al., 2016).

The lethal doses tested (LC_{50} and LC_{90}) are recommended by the product manufacturer. The results obtained after three days of exposure of the specimens, showed mortality for the two doses and that during the three days of the test. The percentages of mortality concerning *D. magna* varied from 78% to 86% for the LC_{50}, and from 84% to 97% for the LC_{90}. The second species was more resistant to the insecticide during the first day compared to *D. magna* with a percentage varying from 68% to 80% on the third day for the LC_{50} and from 83% to 91% for the LC_{90}" (*Table 1*).

Table 1. *Percent mortality of Daphnia magna and Plea minutissima treated with LC_{50} and LC_{90} of the pesticide Rustile for 3 days. N= 60 individuals (20 individuals/repeat)*

Temps (H)	**D. magna**				**P.minutissima**			
	Witness		**Treated**		**Witness**		**Treated**	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
24h	0%	0%	78±2.8%	84±2.3%	0%	0%	68±2.3%	83±1.3%
48h	0%	0%	84±2.1%	93±2.0%	0%	0%	74±1.6%	88±0.8%
72h	1±0 %	2±0 %	86±1.3%	97±1.4%	0%	01±0%	80±2 %	91±1.4%

Table 2 summarizes the means and standard deviations of the mortalities obtained after 24 h. The two doses presented almost equitable mortalities, which are respectively (14.33±4.03); (15.33±4.17) and the third day (15.83±3.54); (17.83±4.02) concerning *D. maghna*. However, the results obtained from the treatment of *P. minutissima* on the first day and that concerning the two doses were 3.60±2.30; 16.60±1.34, respectively. Then, after 72 h, the means were (16.00±2.00); (18.20±1.48)"(*Table 2*).

Table 2. *Summary of the results of two species Daphnia magna and Plea minutissima mortality treated with 2 doses of the insecticide "Rustile". Time: 3 days; (Mean ± SD); N= 60 individuals (20 individuals/repeat)*

	Time / Dose	**24 h**	**48 h**	**72 h**
Daphnia magna	LC_{50}	14.33±4.03	15.50± 3.72	15.83±3.54
	LC_{90}	15.33±4.17	17.00±4.33	17.83±4.02
Plea minutissima	LC_{50}	3.60±2.30	14.8±1.64	16.00±2.00
	LC_{90}	16.60±1.34	17.6±0.89	18.20±1.48

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The analysis of variance with a fixed model classification criterion, showed that there was a very highly significant difference between the doses and time for *D. magna* (P=0.000) as well as for the species *P. minutissima* (P=0.000) (*Table 3*). The Tukey test allowed us to study the mean values and time groups which were homogeneous for *D. magna* with two groups; on the other hand, for *P. minutissima*, the means and time groups are homogeneous and present three groups (*Table 4*).

Table 3. *Comparison, using analysis of variance with a fixed model classification criterion, between the 3 exposure times to Rustile for each of the 2 doses tested (LC_{50}, LC_{90}) for 2 treated species*

Species	Dose	SV	ddl	SCE	CM	Fobs	P
Daphnia magna	LC_{50}	Time	5	205.778	41.156	32.21	0.000***
	LC_{90}	Time	5	253.611	50.722	21.74	0.000***
Plea minutissima	LC_{50}	Time	5	299.111	59.822	23.41	0.000***
	LC_{90}	Time	5	381.778	76.356	62.47	0.000***

SV: sources of variation; ddl: degrees of freedom; SCE: sum of squares of variances; CM: mean square; Fobs: observed value of Fisher's F variable; P: probability of finding; Significant differences; P>α = 0.05: (ns) non-significant differences; P≤α = 0.05: (*) just significant differences; P≤α= 0.01: (**) highly significant differences; P≤α = 0.001: (***) very highly significant differences

Table 4. *Search for homogeneous time groups by dose and species (TUKEY test); T: Time*

Species	Dose	Mean and homogeneous time groups			Number of groups
Daphnia magna	LC_{50}	T24 13.7	T48 13.0	T72 4.7	A B
	LC_{90}	T24 17.3	T48 15.7	T72 5.3	A B
Plea minutissima	LC_{50}	T24 16.7	T48 13.0	T72 8.7	A B C
	LC_{90}	T24 18.0	T48 15.3	T72 9.0	A B C

Two-criteria analysis of variance (ANOVA)

The results of the analysis are summarized in *Table 5* and reveal a non-significant difference for the species and a significant difference for both doses after 24 h. After 48 h the differences were highly significant for the species, and a non-significant difference for the doses. Then, after 72 h of treatment, the difference was highly significant for the species and not significant for the doses (*Table 5*).

Look for homogeneous groups between doses and species and time

The TUKEY test was used to determine the groups of means that are identical or in other words, the groups of mortality that are as homogeneous as possible, that is, the toxicity has the same effect on both species. The results of the TUKEY test, give only 1 homogeneous group for the 2 doses after 24, 48 and 72 h. On the other hand, there are 2 homogeneous groups for the species after 48 h and 72 h and only one group after 24 h (*Table 6*).

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Table 5. *Analysis of variance at two classification criteria (species & doses) for the 3 treatment times of the 2 species at LC_{50} and LC_{90} of Rustile. 2 h of treatment the difference was highly significant for the species, and not significant for the doses*

Species	SV	Ddl	SCE	CM	Fobs	P
24 H	Species	1	6.05	6.050	1.15	0.3 NS
	Dose	1	22.05	22.050	4.18	0.05*
48 H	Species	1	26.45	26.45	8.46	0.01**
	Dose	1	11.25	11.25	3.60	0.07 NS
72 H	Species	1	24.20	24.20	9.98	0.006**
	Dose	1	7.20	7.20	2.97	1.000 NS

Table 6. *Search for homogeneous time groups between doses and species after 24 h, 48 h and 72 h: results of the TUKEY test*

Time	Dose and Species	Mean and homogeneous time groups		Number of groups
24 H	**Dose**	LC_{50} 14.6	LC_{90} 16.7	A
	Species	Esp1 15.1	Esp2 16.2	A
48 H	**Dose**	LC_{50} 16.2	LC_{90} 17.7	A
	Species	Esp1 15.8	Esp2 18.1	B A
72 H	**Dose**	LC_{50} 17.1	LC_{90} 18.3	A
	Species	Esp1 16.6	Esp2 18.8	B A

Esp1: *Daphnia magna*, Esp2: *Plea minutissima*

Discussion

Cladoceran species are of great ecological interest for freshwater environments judging by their wide distribution and their role in the food chain. Indeed, the genus Daphnia is widely distributed in stagnant fresh waters from the Arctic to the tropics. The presence of this genus is reported in different trophic states of streams and in different sizes and morphologies of aquatic habitats ranging from ponds or shallow lakes to the pelagic zone of large lakes and reservoirs (Jeppesen et al., 2014). In the majority of these systems, Daphnia dominates the zooplankton community in terms of abundance and biomass during certain time intervals. Because Daphnia play an important role in the food chain of lakes, scientists have designated them as keystone species in pelagic systems. Compared to other zooplankton, Daphnia can achieve high filtration rates and particle consumption potential in the range of 0.5 to 50 µm. In temperate lakes, filtration by daphnids participates in the clarification of the water surface, and thus Daphnids play an important role in the spatial and temporal structuring of the phytoplankton community (Wickramarathna, 2014). Under favorable conditions, Daphnia can achieve total filtration of the medium per day (Serra, 2019). The relatively large size of *D. magna* compared to other species in the same genus has also contributed to its wide use. Daphnia are filter

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feeders, considered to be primary consumers. They are phytophagous, and bacteriophagous (Wirtz, 2013). The species *Daphnia magna* was the main biological model for this study. This cladoceran was chosen because it combined several advantages, the most important being its role in the food chain, its sensitivity and its ease of use for long-term laboratory testing. Heteroptera include all field, wood, and house bugs and of course aquatic species (Suhett, 2015). They are separated into seven infra-orders, two of which are primarily aquatic (Gerromorphaand Nepomorpha), one semi-aquatic (Leptopodomorpha), and the other four terrestrial (Enicocephalomorpha, Discocoromorpha, Cimicomorphaand Pentatomomorpha). *Daphnia magna* is a freshwater zooplanktonic microcrustacean in the family Daphnideae (Berchi, 2015). Rustil is a very effective insecticide against crawling insects, especially cockroaches; it has been widely used for public health treatment. This is what 2007 pointed out by showing that the impact of insecticides on non-target arthropods varies greatly depending on the nature of the product, the dose used, the date of treatment, the taxon considered, and the environments concerned. Unfortunately, there are no studies related to the effect of pesticides on aquatic heteropterans. However, other studies have been conducted on the effects of these products on non-target insects such as bumblebees. The effects at the colony level have been clearly demonstrated; exposed colonies grow slower, and produce significantly fewer queens (Jayaraj et al., 2016). Other work has studied abactamine toxicity in different biological models; abamectin does not persist or accumulate in the environment. Its instability, as well as its low water solubility and high affinity for soils, limits its bioavailability in non-target organisms, which also prevents its leaching into groundwater and entry into the aquatic environment. Abamectin may have adverse effects on pollinators and biological control organisms (Seshadri, 2015). Other studies done by EPA (2002) show that acetamiprid toxicity is selective to insects, but some uses may pose a risk to some non-target aquatic invertebrates. However, conventional biological indices do not diagnose the types of pollution, and do not take into account the natural variations, temporal or spatial, of the communities (Abreu-Villaça and Levin, 2017).

The use of neonicotinoids around the world varies depending on the molecule used, and acetamiprid is one of them, was released around 1995 and was used to specifically kill insects, in grapevine and in cotton and ornamental plant farming (Marieb et al., 2014). Acetamiprid is an odorless-organochlorine pesticide widely used in Algeria. It is intended to control sucking insects that affect plants (Sahraoui et al., 2017). In the body, a fraction of the order of 10% of this pesticide is eliminated in unchanged form. Acetamiprid is converted into methylamine, which is more active than the parent molecule. There is no accumulation of acetamiprid in the body. More than 90% of an oral dose is eliminated in less than 72 hours, all in 96 hours. The urinary and fecal tracts are the main routes of elimination (Seifert, 2005; Sheets, 2010; Chen et al., 2014; Nakayama et al., 2019). This pesticide promotes the accumulation of Ach in synaptic clefts during nerve stimulation leading to hyperstimulation of cholinergic receptors (Kimura et al., 2012; Nawaza et al., 2015; EFSA, 2016). Their mechanism of action is mainly based on the covalent binding to the Acetylcholinesterase of the central nervous system by opposing the physiological hydrolysis of Ach which then becomes irreversibly non-functional (Seifert et al., 2005).

This work was devoted to the evaluation of the toxicological risk, following the intensive use in the agricultural environment of Rustile and that it could alter the degradation of the biodiversity of the aquatic environment, at the time of pluviometric leaching in Algeria. Considering the biological interest of the two species (*D. magna* crustacean biofilter and *P. minutissima* bioindicator), tested by the LC_{50} and LC_{90} of this

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product, the results show the effect of the insecticide for the two doses and that the daphnia are the most sensitive. Both species proved their sensitivity to the doses used and the exposure time. We can conclude that Rustile has an impact on the non-target population. This confirms the results of Balança and Visscher (1997) who indicate that the impact of insecticides on non-target arthropods varies according to the nature of the product; the dose used; the time of treatment; the taxon considered and the environments concerned. Many insecticides have been tested on *Daphnia magna,* such as chlordane, Deltamethrin and Malathion, except for Rustile. Other studies done by the EPA (2002) show that the toxicity of Acetamiprid is selective for insects, but some uses may present a risk to non-target aquatic invertebrates. Also, the second tested species *Plea minutissima*, was sensitive to Rustile with a mortality almost 100% and after three days of treatment. The results obtained lead us to say that the two tested species are sensitive to Rustile and can be threatened with disappearance, if the use of Rustile in our orchards, continues to be prolonged, at least in Cladocera and aquatic Heteroptera.

Conclusion

The general objective of our study is to compare the toxic effect of an insecticide (Rustile) widely used in agriculture in Algeria, on two non-target groups of Heteroptera (*Plea minutissima*) and Cladosiran (*Daphnia magna*) collected in the village of Djamel in the Tarf Region (extreme north-east Algeria). After treatment of these species with two lethal doses of Rustile (LC_{50} & LC_{90}), the results obtained showed the sensitivity of the treated species, in particular *Daphnia magna*, translated by high mortality rates, depending on the concentrations. In view of the results obtained and the importance of the organisms tested (aquatic heteropterans, good predators, usable in biological control of carcinogens and *Daphnia magna*, known for their important role as biomarkers of water pollution), the use of pesticides must be carried out in strict compliance with the rules of biodiversity conservation. Some species also play an important role in the ecological balance and in improving the living conditions of the population. Despite the toxic effects recorded for many organisms, these insecticides are widely used in agriculture.

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