RISK ASSESSMENT OF TWO INSECTICIDES ON WATER MITES, A NON-TARGET SPECIES WERE COLLECTED IN FAR NORTH-EASTERN ALGERIA

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Abstract. Aquatic organisms are used as bioindicators to assess the health of aquatic ecosystems. The use of pesticides can harm non-target species. Our study aims to compare the toxic effect of two different types of insecticides used in agriculture, Rustile and Thiam, at two different doses, on the water mite biomarker. This biological model was chosen because of its importance in the food chain. The samples were collected from Lake Tonga, located in the far north-east of Algeria. The lethal doses used for the two insecticides in treating this group are CL_{50} = 1.45 mL/L and CL_{90} = 1.90 mL/L for Rustile, CL_{50} = 1.90 mL/L and CL_{90} = 4.22 mL/L for Thiam. The first test included 10 water mite individuals in three replicates with 200 ml of lake water and CL_{50} of the two insecticides. The second test included 10 individuals of the same insect in three replicates with 200 ml of lake water at a lethal concentration of 90 for both pesticides. Mortality was assessed after 24, 48, and 72 hours. The results showed that sensitivity was reflected in high mortality rates. This indicates that the two insecticides contain toxic elements.
Keywords: Hydracariens, lake, biomarkers, *Piona uncata*, Tonga

Introduction

Biodiversity can include the study of differences; however for the preservation of biodiversity, which is a global issue, the knowledge of the distribution of animals and plants is essential. Arthropods play an essential role in forest ecosystems (Couturier et al., 1985). Arthropods constitute an important branch of animals and are among the most diverse and widespread organisms on Earth, which contributes to greatly increasing animal diversity. Most arthropod species consist of insects (Morin, 2002). Spiders represent the first terrestrial arthropods and constitute the most important class of Chélicérates, as they form a polyspecific group. All diets exist within the group: predators, parasitoids, and symbionts have an amazing quality and remarkable ability to adapt. Aquatic Acariens, also known as Hydrachnellae, d'Hydrachnidia, are a monophyletic group, found in the aquatic environment. The regulation of life cycles and genetic development allows group dispersal and significant diversification of aquatic environments (Smith and Cook, 1991). Insecticides are active substances or phytosanitary preparations that have the property of killing insects, their larvae and eggs. They are themselves included in the family of biocides, and both are regulated in Europe through specific directives. The general term "insecticides" also includes insecticides intended to control arthropods that are not insects (such as spiders or mites such as ticks) as well as sometimes repellents. We distinguish between contact products, “systemic” products, and

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intermediate position products, called "translaminar". From 1955 to 2000, Agricultural intensification has led to an increase of more than 75% in the production of insecticides. These are biopesticides intended to destroy insects that are widely used in agriculture and community health (vector control). Insecticides are actually highly toxic substances to the central nervous system, and their neurotoxicity explains their effectiveness on insects. And its toxic effects on humans. Recent regulatory developments have led to the recall of several active ingredients. A class that benefits from the best toxicity efficacy ratio, pyrethroids is the insecticides currently used most often in formulations for agricultural and veterinary use, but their use has been subject to significant restrictions due to presumed adverse effects on non-target groups as well as described toxic effects in humans. These products used for crop protection pose significant potential risks to non-target aquatic organisms due to their leakage and sedimentation in surface water and their inability to degrade, so they are transported to rivers and lakes to be polluted (Morsley et al., 2015). Therefore, the current study aimed to evaluate the effect of two neonicotinoid insecticides: the first is the commercial preparation Rustile of the insecticide acetamiprid, which is widely used in agriculture, and the second is Thiam, an insecticide from the Thiamethoxam chemical family, which works to eliminate insect pests and is considered an environmental pollutant. Alien. On non-target species and the biological indicator of the water mite (*Piona uncata*) collected from Lake Tonga located in the state of El Kala in the far north-east of Algeria, we concluded from the results that the two pesticides have a significant toxic effect on aquatic insects, especially water mites.

Material and methods

Presentation of the study area Lake Tonga

Our study was conducted within the El Kala National Park (PNEK), located in the far north-east of Algeria and covering approximately 80,000 hectares. The latter was established on July 23 under Description No.: 83-462 and was established as a Biosphere Reserve by UNESCO. On December 17, 1990, it is home to the largest wetland complex in North Africa (Elafri, 2017). It is bordered to the east by the Algerian-Tunisian border. To the north is the Mediterranean Sea, to the west is the end of the alluvial plain of Annaba and the hills of Jebel el-Kursi, and finally to the south are the foothills of abstract mountains (Loucif et al., 2020; Bayley et al., 2021). It is located between latitudes 36° and 51° north and 08° and 30° east in the far north-east of El Kala National Park (Tarf Province). Lake Tonga has been included in the Ramsar list since 1983 (Abulhawa and Cummings, 2017). It is located east of the city of El Kala, 70 km east of the city of Annaba. To the north, Tonga is bordered by coastal dunes that separate it from the sea. The site plays an important role in controlling winter floods, capturing sediments and materials removed by floods from upstream and carrying them towards the lake, and stabilizing coastal dunes. Considered among the wetlands of unique international importance in the Mediterranean region, Lake Tonga is the most important breeding site for waterbirds in eastern Algeria. It occupies a huge coastal depression covering an area of 2,600 hectares and is 7.5 km long and 4 km wide. Mount Arjoub Al-Rashid (167 meters long) to the north and northwest is a dividing line separating the basins of Lake Tonga and Obaira to the west, and the Algerian-Tunisian border to the east (Raachi, 2007). Lake Tonga is an expanse of freshwater, temporarily lawless due to various actions carried out over the past century. Our samples were taken during December 2019 (*Fig. 1*).

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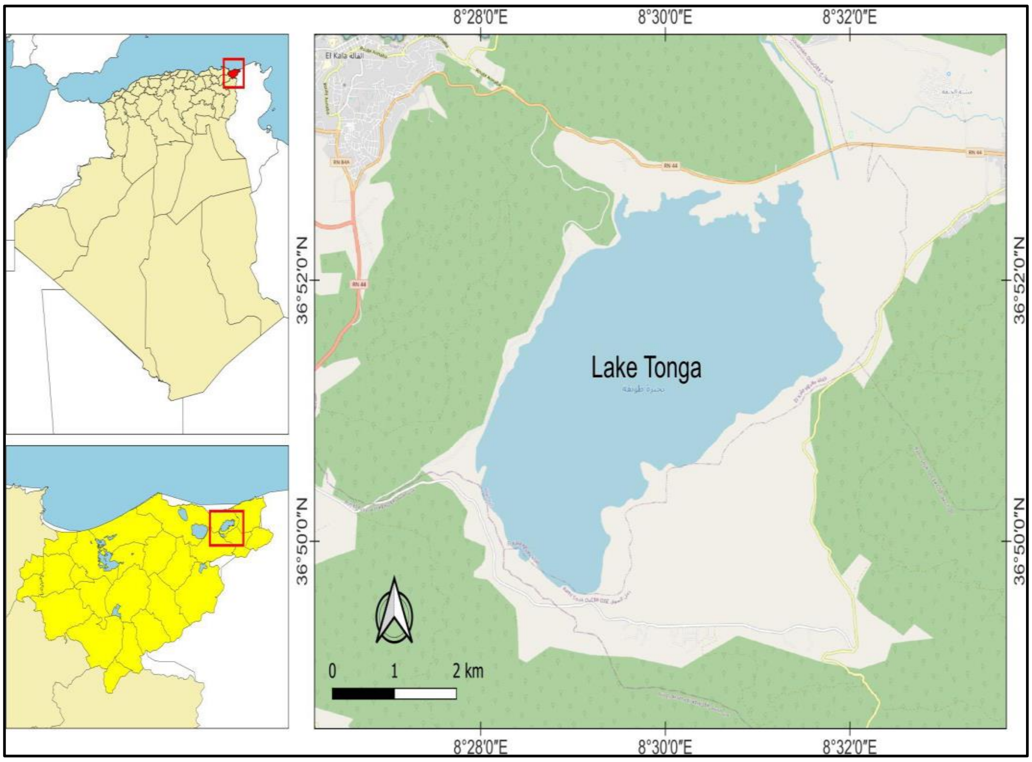

Figure 1. *Geographical position of Lake Tonga, Algeria (Gacem, 2022)*

Presentation of the species

Origins of Hydracarians Hypotheses regarding their origin generally assume an ancestral terrestrial parasite (Mitchell, 1957; Davids and Bellaire, 1979). A recently proposed alternative hypothesis (Wiggins et al., 1980; Smith and Oliver, 1986) suggests that the primitive stem parasite could be hydracariids resembling some extant hydracarinae. The parasite model represents a set of adaptations to exploit spatially and temporally patchy aquatic habitats (Cook, 1957; Poinar, 1985; Smith and Cook, 1991). Available morphological and behavioral data indicate that extant Hydracarians are monophyletic (Barr, 1972; Cook, 1974; Smith and Oliver, 1976, 1986). There are more than 5,000 species of Hydracarians currently recognized worldwide, representing more than 300 genera and subgenera in more than 100 families and subfamilies. In fact, the Hydracarian group appears to rival several orders of aquatic insects in diversity. Hydracarans are clawed arthropods of the class Arachnida and family Pionidae. They are purely aquatic moths with soft, brightly colored coverts. One of the most striking things about Hydracarans is their bright colors, often orange, yellow, and red (*Figure 2*), possibly due to noxious secretions from skin glands (Moor, 2019). Microscopic size ranges from 0.5 mm to 5 mm. The body is univentricular, with the prosoma and epistosoma closely fused with indistinct segmentation. The mouthpart consists of a pair of chelicerae and pedipalps (Franz-Guess, 2019). The most recent classification is presented by Walter and Proctor (1999). He divides mites into four groups: prostigmata, astigmata, gripattida, and mesostigmata. Systematic identification of collected species was performed using the binary keys of Smith et al. (2001). Hydracarids also exhibit effective biological control against Culicidae through their voracious predation on the larvae of these insects. When water mites attack mosquito larvae with two stingers in their mouths, called Chelicers, they inject a toxin that numbs them. When the larvae stop moving, water mites suck out their guts and kill them (Eiras et al., 2021; Gacem et al., 2022).

Figure 2. *Vue ventral de Piona uncata Koenike 1888. Agr.15, 8× 10^3 (Gacem, 2015)*

Insecticide and treatment

Thiam insecticide

THIAM is a very powerful insecticide, with rapid and long-lasting poisoning activity. It is particularly effective in controlling mites and has low toxicity to beneficial insects (Rezende-Teixeira et al., 2022). Thiamethoxam 25% WDG, water-dispersible granules, is a broad-spectrum systemic insecticide on contact and ingestion that is readily absorbed into plant tissue (Jijisha and Jacob, 2014). It is effective against many sucking and chewing insect pests and is used to control Lepidoptera, Coleoptera, Thysanoptera and leaf miners and to prevent insects such as mosquitoes, aphids, sand flies, fleas, whiteflies and white grubs. This herbicide helps to maximize crop potential and allows for healthier and stronger plants, improved quality of harvested fruits and grains with higher yields. Characteristics of Thiam, which is a highly specific systemic insecticide that is known to be applied foliarly or by irrigation (Li et al., 2022)

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, and 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).

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Method of taking samples

Samples were taken from several villages during the month of December 2019. We need plastic boxes to place our water samples with the hydrangea samples. Before sampling, we spend a lot of time carefully monitoring the water. You should know that they are living organisms. They crawl on plants and small rocks and rise to the surface to breathe. Organisms need food, and the best places to host them are areas where there are aquatic plant remains and mosquito larvae. To achieve this, we used a large container with a long line to catch hydracaries from the various stations we chose on Lake Tonga. After catching the hydracariens in a container, we place them in plastic bottles with the hydracariens water that will be used for reproduction. Material from each sample is collected in a large container (Bendali, 2006) and sorted in the laboratory.

Bioassays

To determine the lethal concentrations for the tested species, we tested the two pesticides on the L_4 mosquito larvae, the target group, where the lethal concentration of 50% was determined, as well as the lethal concentration of 90% of the mosquito population from it the lethal concentrations of the species tested in this study were determined. The test hydracrian is harvested from Lake Tonga. The various tests were performed in 500 ml bottles in triplicate in 200 ml of filtered lake water. The first test included 10 individuals of water mites in each replicate, where the CL_{50} = 1.45 ml/L of the insecticide “RUSTILE” and the CL_{50} = 1.90 ml/L of the insecticide “THIAM”. The second test included 10 individuals of water mites with a lethal concentration of CL_{90} = 1.90 ml/L for the insecticide “RUSTILE” and a lethal concentration of CL_{90} = 4.22 ml/L for the insecticide “THIAM.” Tests were conducted on non-target species treated with two doses of the two pesticides. The tests included three repetitions and a control experiment and were conducted in plastic boxes designated for storing food. Mortality was recorded after one day, two days, and three days (Gacem, 2023). There are different international guidelines for risk assessment of non-target aquatic organisms and we took EPA as the criterion for study design. In short, the chosen concentrations affect the rate of field application, and the recommended concentrations ensure the safe and effective use of the two insecticides.

Statistical analysis

The results were expressed as mean ± standard deviation (SD). Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene tests, respectively. Two-way analysis of variance (ANOVA) was tested, followed by Tukey's post hoc test. All statistical analyzes were performed using GraphPad.Prism.v6. A significant difference was defined at $P < 0.05$.

Results

Results according to the standard and average deviation of the effect of the two insecticides “THIAM and RUSTILE”.

In order to compare the effect of “Thiam pesticide” and “Rustile” at the same doses (Cl_{90} and Cl_{50}) on a non-target species of water mite “*Piona uncata*”, we studied the mortality of these species. In this sense, three repetitions were performed for 72 hours.

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We conducted a statistical analysis, showing that there is a difference between the effect of the two pesticides on the treated species, and the values are summarized in *Table 1*.

Table 1. *The results of the effect of the two doses of the THIAM and RUSTILE insecticide during 72h; M±S*

CL_{50}+CL_{90}	**Dose**	**Mean**	**SD**
	CL50	25.56	5.83
	CL90	30.56	6.35
CL_{50}	**Temps**	**Mean**	**SD**
	H24	21.67	2.89
	H48	23.33	5.77
	H72	31.67	2.89
CL_{90}	**Temps**	**Mean**	**SD**
	H24	36.67	2.89
	H48	30	5
	H72	25	5

After 24 hours and 48 hours of treatment with “Dose Cl_{50}” and “Dose Cl_{90}”, the results showed that the average death rate in the first dose is less than the average death rate in the second dose. After 72 hours of treatment with the “two doses”, the results show us that the average mortality rate in the first dose is greater than the average mortality rate in the second dose. It appears that the second dose may be associated with an increased risk of mortality within 24 hours compared to the first dose, but over a period of 72 hours the ratios change so that the mortality from the first dose becomes higher. This change may indicate that deaths associated with the second dose occur more quickly after the dose, while the effect of the first dose may be late, but it is more sustainable in the longer term.

Kruskal-Wallis test analysis

Time effect

We used test statistical analysis (KW) to determine and prove the presence or absence of a difference between exposure periods to the two chemical pesticides “THIAM” and “RUSTILE”. We found a clear difference in the mortality rate between the first day and the third day, as shown in the results of *Figure 3*.

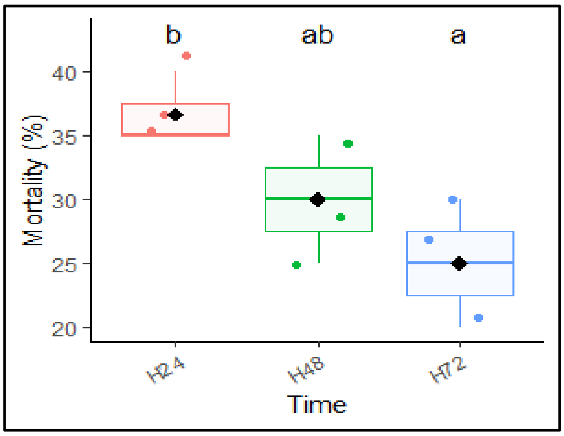

Figure 3. *Time effect on the mortality by KW*

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We used test statistical analysis (KW) to determine and prove the presence or absence of a difference between the effect of the two doses of insecticides “THIAM” and “RUSTILE” on water mites during the three times. The results shown in *Figure 4* show that there is a difference in the percentage of mortality rate between CL_{90}_H24 On the one hand and CL_{50}_H24; CL_{50}_H48 ; CL_{90}_H72 on the other hand.

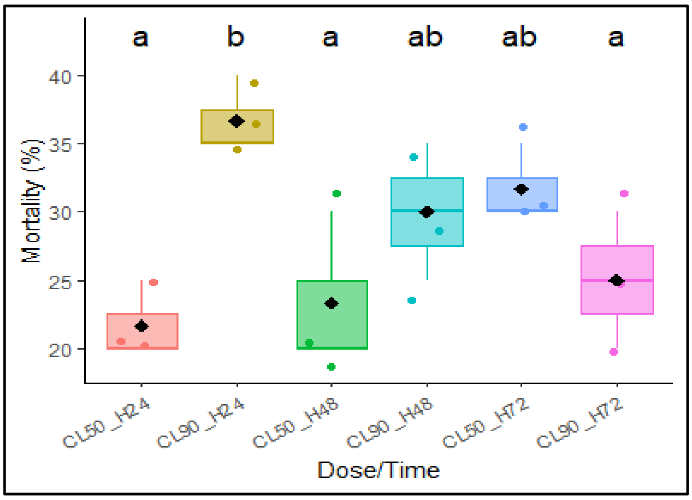

Figure 4. *Time and Dose effect on the mortality by KW*

Figure 5 shows that the two doses of the two chemical pesticides have a significant effect, as they kill water mites, which translate into a high mortality rate. It was noted that the dose of CL $_{90}$ has a greater effect than the first dose, CL $_{50}$. Experiments show that using the second dose had a greater effect in killing water mites, and this indicates that increasing the dose increases and enhances the effectiveness of insecticides in eliminating water mites.

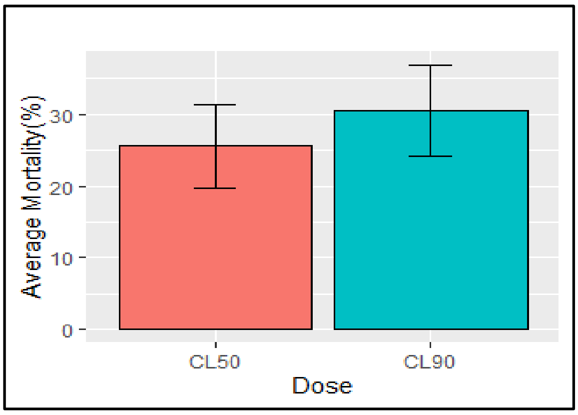

Figure 5. *The effect of the Cl $_{50}$ and Cl $_{90}$*

The results of *Figure 6* show that the first dose of CL $_{50}$ of the two chemical pesticides has a long-term effect, as it kills water mites for three days, knowing that the mortality rate is high on the third day, that is, after 72 hours. When the dose is effective in the long

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term, we expect that the number of water mites will gradually decrease after giving the dose, with the death rate increasing significantly over time, especially on the third day. We also expect that the water mites will show signs of weakness and a decrease in activity and movement before they die. The effect of the dose will be sustainable. Which leads to continued deaths over a long period as the environment stabilizes after the disappearance of infected individuals. While the results shown in *Figure 7* show that the second dose, CL $_{90}$, has a significant and high effect on the first day, that is, after 24 hours.

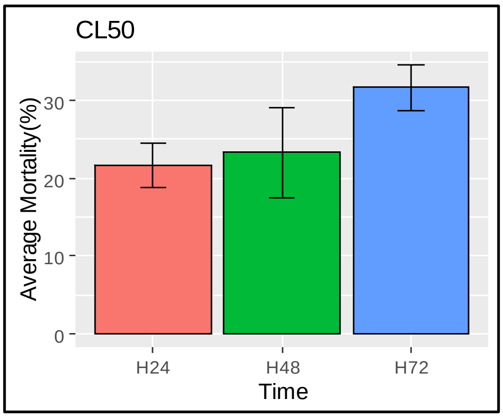

***Figure 6.** The effect of the Cl $_{50}$ for 3 days*

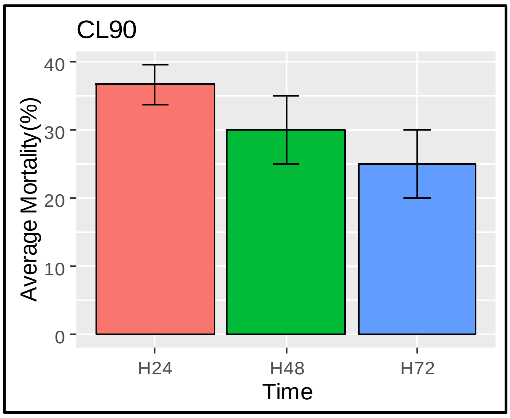

***Figure 7.** The effect of the Cl $_{90}$ for 3 days*

Discussion

Based on our results from studying the toxic effect of the pesticides “Thiam” and “Rustile” on water mites of the genus *Piona uncata* within 72 hours, there are clear differences in mortality rates. We found that using a dose of 4.22 ml/L of the insecticide “Thiam” is more toxic to water mites than a dose of 1.90 ml/L of the same insecticide. The insecticide "Thiam" is more toxic to water mites than Rustile. Another study titled “Effects of THIAM on Non-Target Aquatic Insects Implications for Risk Assessment of Pesticides in Aquatic Ecosystems” demonstrates that exposure to Thiam resulted in several negative effects on the survival, development and behavior of non-target aquatic insects. The researchers suggested that THIAM should be carefully evaluated for its potential risks to non-target species in aquatic ecosystems (Liu et al., 2019). Based on the results of a “comparative study between biological and chemical control against domestic mosquito larvae,” it was found that chemical treatment was more effective, as the results were excellent with the doses used. But indiscriminate use of chemical pesticides causes harm to the environment, while biological treatment using water mites is only effective for the target organisms and has no harmful effect on other organisms or the environment (Habiba et al., 2022). Hydracarids are ectoparasites of aquatic insect larvae while active post-larval hydracaria (second and adult instars) are entirely aquatic predators of small invertebrates (Furnham and Procter, 1989). Parasitological associations of larvae have been reviewed by Smith and Oliver (1976, 1986) and Smith (1988). The species that constitute the main host of hydracarian larvae are part of the aquatic or semi-aquatic insect class such as the family Culicidae, Tipulidae, Ptychopteridae, Chloropidae, and Empitidae (Lundblad, 1927). Some authors have given estimates of the location of attachment of larvae to their hosts. Hydracaria larvae parasitize mosquitoes and cling to various parts of the body: the chest, abdomen, trachea, at the level of the hairs on the tips

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of the abdomen, and on the neck of the female (Bendley, 2006). They puncture the tissue with their pen and suck out the liquid contents of the host (Lumdblad, 1927; Smith, 1976). Hydracarians, or water mites, Hydracanelli or Hydracanidae, are ecologically important and one of the dominant groups of freshwater arthropods. Their biological cycle consists of two stages, with larvae completing the aerial stage as ectoparasites of adult insects, such as Odonates (Baker, 2007; Baker et al., 2008). Diptera and adults have a free-living aquatic life and are voracious predators of immature aquatic insects (Gledhill, 1985) and small crustaceans. Since they present a specific preference and opportunistic prey selection, the genus Arrenurus followed by Piona are the most voracious towards the larval stages of *Culex pipiens*; *Anopheles maculipennis* and *Aedesa egyptie*, only the predation rate decreases with the development of the larval stage (Bendali, 2006). Interest in the larval stages of hydracariids has increased recently due to various aspects of their host relationships that influence distribution, dispersal, population size and development. Insecticide tolerance in some species represents the ability of populations to resist the introduction of foreign and sometimes contaminated products into the environment. The introduction of these substances enhances the stimulation of metabolic detoxification processes leading to increased activity of some enzymes, especially GSTs (Ishaaya, 2001; Wei et al., 2001; Enayati et al., 2005; Li et al., 2007). In fact, species groups appear to be related to the autumn-winter period. Moreover, its presence is associated with lower values of nitrite ion concentration, water temperature, air temperature, and alkaline pH in Lake Tonga (Messikh, 2016; Messikh et al., 2020). The study of the Hyrcanians in Algeria as well as in the world has not gained momentum, and no inventory has been made. A research program was established by our team, in order to carry out hydracaria inventories in eastern Algeria, and several species were identified (Bendali et al., 2014). The most abundant are *Eulais extedance Piona uncata*, *Arrenurus sp* and several other species.

Conclusion

The general objective of our work is to compare the effect of two insecticides on a non-target population *Piona uncata* collected from Lake Tonga located in the Kala region (far northeastern Algeria) after treating this species with lethal doses of the two insecticides Thiam and Rustile by subjecting it to toxicity tests for two chemical components: Acetamiprid used in the commercial formulation of the insecticide “Rustile” and thiametoxam 25% used in the commercial formulation “Thiam”. The results of the tests of the two insecticides obtained showed a variable sensitivity of the treated species, translated into mortality rates, which indicates that the insecticide “Thiam” represents the most toxic element compared to the second insecticide. Considering these findings and the importance of some non-target organisms such as aquatic hydracariens that are bioindicators of water pollution, the use of pesticides should be done with strict adherence to the preservation of biodiversity of some species that also play their role in the ecological balance.

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