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Impact of environmental and chemical stress on the activity of the acetylcholinesterase and glutathione S-transferase during reproduction of *Scolopendra morsitans* (Myriapoda Chilopoda) in the north-east of Algeria

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Abstract
The aim of this study was to assess the terrestrial environment quality along the east of Algeria using an approach based on biomarkers in the Chilopoda *Scolopendra morsitans*. Individuals were collected from four stations in the two sites (Annaba and Tebessa) based on the level of pollution. The non-urbanized sites were considered as a healthy reference site. The biomarkers selected during this study were the activities of acetylcholinesterase as neurotoxicity marker, glutathione S-transferase as phase II enzyme of stress marker. The results show differences between sites compared with the reference samples. This approach confirms that individuals from the polluted stations have been submitted to highly polluted environment. Individuals collected from these stations show the highest reduction of the mean of body and ovarian weight as well as the highest inhibition or induction of enzyme activities indicating a highly contamination status. The same results have been observed after injection of the ecdysteroid analog (RH-0345).

Keywords: *Scolopendra morsitans*, Pollution, Biomonitoring, Biomarkers, Eastern Algeria.

1. Introduction
Soil is a vital resource for human societies and ecosystems to be protected in view of increasing degradation especially related to population growth or pollution. To implement, monitor and ensure the actions of protection and management should be to identify indicators that identify and quantify the disturbances, changes in the soil and ecosystem impacts [1].The soil contamination by various substances, including pesticides, has been recognized as one of the main threats to soil [2]. Pesticides in soil can come from agricultural activities but also maintenance activities of green spaces and gardens or weeding of road and rail networks. The pesticide infiltration rate in the soil depends on the soil (moisture, organic matter content, pH and pesticide [3]. Furthermore, there is no equivalent means to those related to water and air for the characterization of soil contamination by pesticides.
Pollution Bioindicators are organisms or groups of organisms used to determine the presence, abundance and bioavailability of environmental contaminants through concentrations in their bodies, in one or more organs or tissues [4]. The assessment of the level of pollution involves the use of biomarkers [5].
Biomonitoring of Environmental Quality is based primarily on the use of biomarkers [6]. Among these biomarkers, Acetylcholinesterase (AChE) is a vital enzyme whose major role is to hydrolyze acetylcholine (ASCh), an ester released into the synaptic space during transmission of nerve impulses from one cell nerve to another [7, 8]. This biomarker is commonly used to detect environmental pollution caused by neurotoxic compounds [9]. Glutathione S-transferase (GST) involved in the Phase II detoxification process is used as biomarkers of organochlorine pesticides and polychlorinated biphenyls (PCBs) in invertebrates [10].
The growing awareness of environmental hazards and the man of the misuse of insecticides has helped increase the interest in more specific and less toxic alternative compounds, insect growth regulators (IGRs). These derivatives because of their specific mode of action [11], and low environmental impact are becoming increasingly integrated into the fight against

mosquitoes programs [12]. Among these compounds (IGRs), the halofenozide (RH-0345) non-steroidal structure novel insecticides, very specific and it is not toxic to mammals that mimic the action of the molting hormone, the steroidal 20-hydroxyecdysone, are currently developed by the industry.

In this context, we evaluated the impact of environmental pollution on the species *Scolopendra morsitans* little known in Algeria, which is part of the soil fauna and belongs to the class of Millipedes and centipedes order considering morphometric study, weight and enzyme firstly at two sites in the north-east of Algeria and the other; these parameters were assessed after treatment of individuals by an analog of molting hormone (RH-0345).

2. Materials and Methods

2.1. Study sites

Sampling sites were chosen because of their geographical location in Easthern Algeria (Fig. 1). Site selection was based on the level of pollution as well as ease of access to the study area and abundance of species.

2.1.1. Annaba is located about 80 Km from the Tunisian border it is located geographically in the north-eastern part of the country is subject to a humid climate: 2 stations were chosen at this city: The Drean station (36 º 41´ 00"N and 7 º 45´ 00"E): is located about 25 km to the East of Annaba far from any human activities, and considered as a relatively clean station, this station away from any source of pollution. The Sidi Amar station (36 º 54´ 00" N and 7 º 46´ 00"E): is located Northeast of the region of Annaba near the steel complex of El Hadjar (Arcelor Mittal) this resort is fully open, is considered polluted station.

2.1.2. The city of Tebessa is located in Eastern Algeria near the Tunisian border. Is located in the pelvic area of the high plains and is subject to a semi-arid climate. The station Bir el Ater (34 º 44´ 55"N and 8 º 03´ 29"E) is located south of Tebessa at a distance of 87 Km it is far from any sources of pollution. The second station Elmaelabiod (35 º 12´ 20"N and 8 º 10´ 7" E): situated about 22 Km to the south of Tebessa city. This station is daily exposed by discharges of cement Elmaelabiod considered as the polluted area.

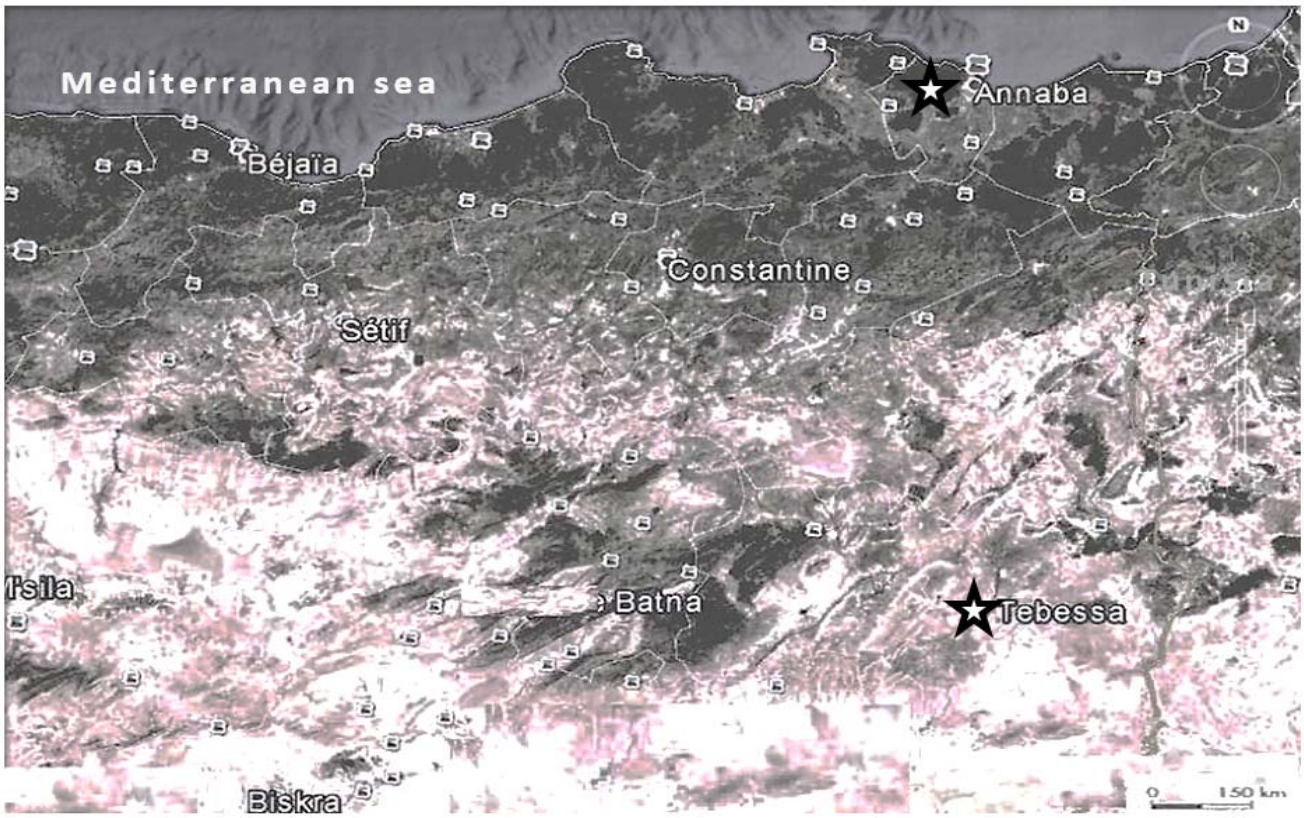

Fig 1: Geographical location of the study sites Annaba and Tebessa.

2.2. Collection of animals

Experiments were conducted on mature females *Scolopendra morsitans* collected during spring of 2012 (reproduction period of the species), from the two areas Annaba with two sampling stations (Drean and Sidi Amar), Tebessa with two stations (Birelater and Elmaelabiod) (Fig.1). Animals were maintained individually in plastic boxes of 500 ml containing humid earth and covered with moistened filter paper. They were fed with insects (cockroach, larvae, flies and mosquitoes) and spiders. Each female was measured and weighed. Livestock was kept at a temperature ranging from 15 to 18 °C, with relative humidity of 80%.

2.3. Chemical and toxicity test

RH-0345 (halofenozide) was developed by Rohm and Haas Company (Pennsylvania, USA) it was a gift by Prof. G. Smagghe (Laboratory of Agrozoology, University of Gent, Belgium). RH-0345 was dissolved in acetone to prepare a concentration of 10 µg/µl for experimental use 3 µl were injected by means of a micro syringe between the third and the fourth dorsal segment toxicity test were performed during a 15-day period, were measured 5, 10, and 15 days after injection. 100 females between control and treated were used in this study. 50 Control animals were maintained in the same conditions and injected with 3 µl of acetone.

2.4. Weight study
After extraction the ovaries of females *S. morsitans* (controls, treated) and also of females collected from different sites, the ovarian weight was considered.

2.5. Biochemical analysis
2.5.1. Acetylcholinesterase activity
Determination of AChE activity was estimated according to Ellman and al. [13] with the use of acetylthiocholine (ASCh) as a substrate. The activity rate was measured as a change in optical density (OD/min) at 412 nm and results were expressed as (µM/min/mg protein).

2.5.2. Glutathione-S-transferase activity
(GST) activity was determined accordingly to [14], using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate and glutathione (1 and mM final concentration, respectively) in 100 mM sodium phosphate buffer, pH 7.5. The activity rate was measured as change in optical density (OD/min) at 340nm the final activity of AChE and GST was expressed as (µM/min/mg protein). Protein content was quantified by the coomassie blue method [15], using Bovine Serum Albumin (BSA) as standard.

2.6. Statistical analysis
The results were expressed as means ± standard deviation (S.D). Comparison of mean values was estimated by student's t-test. The effects of time, sampling sites were tested by a two-way analysis of variance (ANOVA). All statistical analyses were performed using Minitab Software (Version 14, PA State College, USA). The level of significance was set at $p < 0.05$.

3. Results
3.1 Impact of environmental stress on the ovarian weight
3.1.1. Analysis of ovarian weight

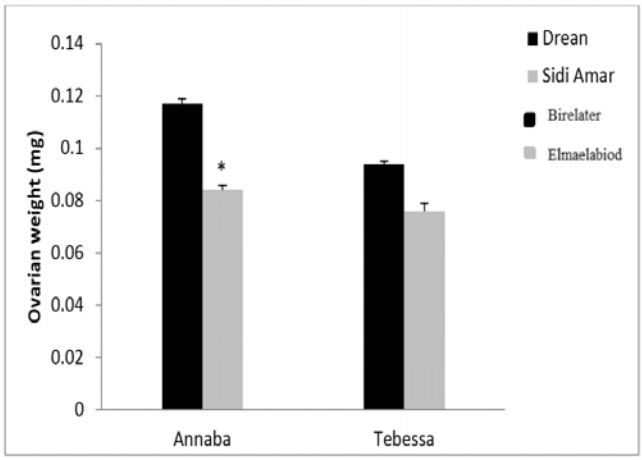

Fig 2: Evaluation of the ovarian weight (mg) of female *S. morsitans* (m ±s, n = 4), collected at the two sites (Annaba and Tebessa).

* Very significant (P <0.05); ** Highly significant (P <0.01); *** Very highly significant (P <0.001)

The results which were obtained show that the ovarian weight is significantly lower than the level of polluted stations (Fig. 2); however, it is very significantly under the level of the polluted station Annaba (Sidi-amar). Furthermore, there is no significant difference between the stations of Tebessa (Birelater and Elmaelabiod).

3.2. Biomarker analysis
3.2.1. Change in acetylcholinesterase activity

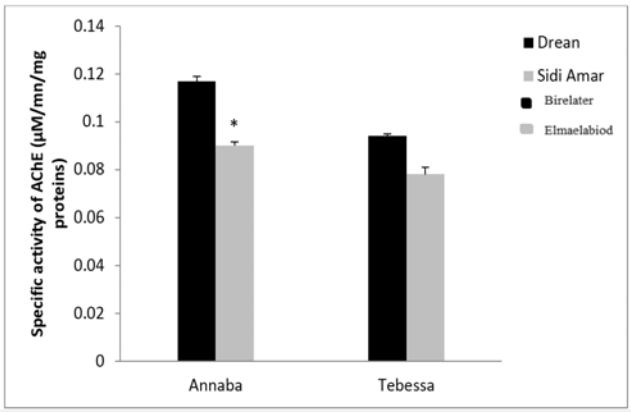

Fig 3: Specificactivity of AChE (µM/mn/mg proteins) in female Scolopendra *morsitans* in both sites (Annaba and Tebessa) (m±s; n= 5).

* Very significant (P <0.05); ** Highly significant (P <0.01); *** Very highly significant (P <0.001).

The specific activity of the (AChE) was determined during the period of sexual activity spring from the heads of female *S. morsitans* collected at the two study sites, in Annaba (Drean Sidi Amar) and Tebessa (Birelater and Elmaelabiod). The results showed that there was an inhibition of AChE activity at stations affected by pollution thus indicating neurotoxicity (Fig. 3). However, there is a very significant difference between Drean station and Sidi Amar, but no significant difference was detected between the two stations of Tebessa (semi-arid station).

3.2.2. Change in glutathione-S-transferase activity

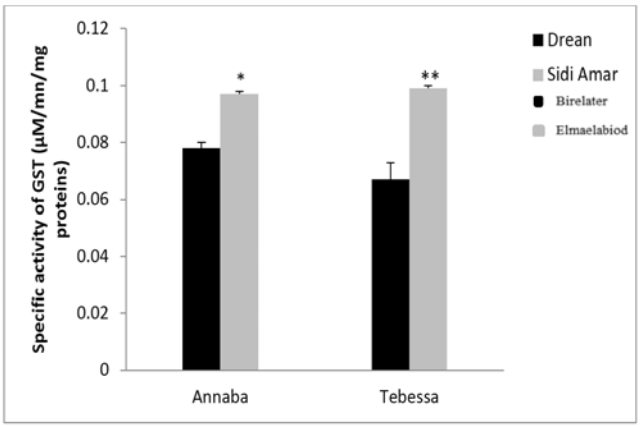

Fig 4: Specific activity of GST (µM/mn/mg proteins) in female *Scolopendra morsitans* collected at both sites (Annaba and Tebessa) (m ± s, n = 5)

* Very significant (P <0.05); ** Highly significant (P <0.01); *** Very highly significant (P <0.001).

The GST activity was determined during the spring period of sexual activity from the digestive tract of female *S. morsitans* collected at the two study sites, namely, Annaba (Drean, Sidi Amar) and Tebessa (Birelater and Elmaelabiod). The results showed a very significant increase in the GST at the Sidi Amar station over Drean (Annaba site) and highly significant at the Elmaelabiod station relative to Birelater (Tebessa site) (Fig. 4).

3.3. Impact of an analog hormone (RH-0345) on the ovarian weight

3.3.1. Effects of RH- 0345 on the ovarian weight (mg)

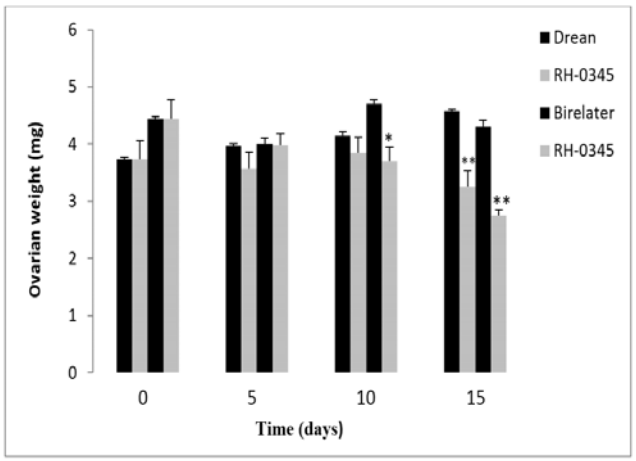

Fig 5: Evolution of ovarian weight (mg) of the control females and processed RH-0345, *S. morsitans* (m ±s, n = 5), collected at the Drean station and Birelater.

* Very significant (P <0.05); ** Highly significant (P <0.01); *** Very highly significant (P <0.001).

After applying injection of RH-0345 *S. morsitans* females, it caused a very significant decrease in ovarian weight of collected females at Drean the fifth day and highly significant in the fifteenth day of treatment; However, there was a very significant reduction in 10 and 15 days after treatment of females collected at Birelater (Fig. 5).

3.4. Biomarker analysis

3.4.1. Effects of RH-0345 on acetylcholinesterase activity

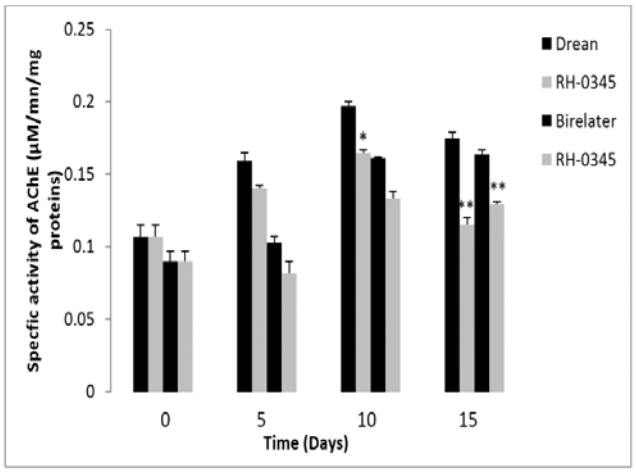

Fig 6: Effects of RH-0345 on the specific activity of acetylcholinesterase (µM /mn/mg proteins) in female *Scolopendra morsitans* collected at both sites (Annaba, Tebessa) (m ± s, n = 5)

* Very significant (P <0.05); ** Highly significant (P <0.01); *** Very highly significant (P <0.001).

After injection of the analog of the molting hormone (RH-0345), the results recorded a very significant inhibition of AChE activity at the tenth day compared to control (Fig. 6) represented by the individuals collected in Annaba (Drean) and highly significant at the fifteenth among those collected in the two reference sites (Annaba, Tebessa).

3.4.2. Effects of RH-0345 on glutathione-S-transferase activity

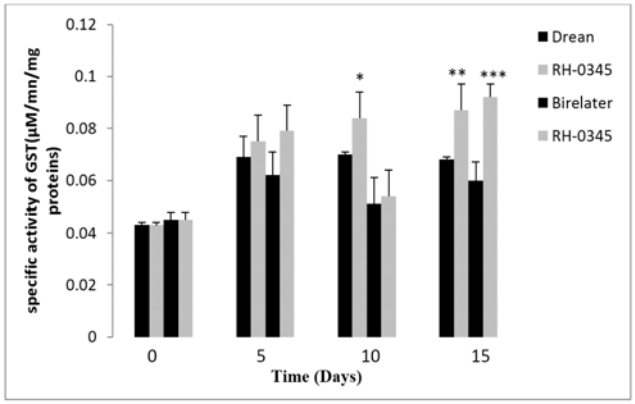

Fig 7: Effects of RH-0345 on the specific activity of glutathione-S-transferase (µM /mn/mg proteins) in female *Scolopendra morsitans* collected at both sites (Annaba, Tebessa) (m ± s; n = 5)

* Very significant (P <0.05); ** Highly significant (P <0.01); *** Very highly significant (P <0.001).

The results showed that treatment with halofenozide (RH-0345) caused very significant increase in the activity of glutathione-S-transferase from the tenth day in the individuals of Annaba (Fig.7); However, the fifteenth day of treatment, this increase is highly significant in individuals of Annaba; furthermore, it was very highly significant in individuals collected in Tebessa.

4. Discussion

Preserving soil quality has become a major concern of public authorities as well as the protection of aquatic and air environments. It is imperative to develop analytical techniques capable of detecting several pollutants [16]. Therefore, the use of biochemical parameters called biomarkers as indicators of ecosystem quality [17]. These biomarkers measure the interaction between a biological system and an environmental agent can be chemical, physiological or biological [18]; their validity due to three main characteristics: specificity, sensitivity and preciousness [19]. The inhibition or induction in vivo biomarker is a good tool to assess environmental exposure and potential effects of xenobiotics on organisms [20, 21]. Biomarkers can be grouped into three categories: biomarkers of exposure, effect and sensitivity.

4.1. Impact of environmental and halofenozide (RH-0345) stress on the ovarian weight

The results showed that the ovarian weight was significantly lower in the polluted stations, however, the latter decreased very significantly at the polluted station of Annaba (Sidi Amar). But, there was no significant difference between the two stations of Tebessa (Birelater and Elmaelabiod).

After injecting the RH-0345 to females *S. morsitans*, the ovarian weight decreased very significantly at the 5 day and highly significant at the 15 day after treatment of females collected from Dréan; however, there was a significant decrease at the 10 and at 15 day after treatment of females collected from Birelater.

Our results are consistent with the work of Maiza and al. [22] or disturbances oogenesis (low volume of the basal oocyte and the low number of oocytes per paired ovaries) are also observed in *Blattella germanica* after topical application of

various groups of pesticides such as carbamate, néomicotinoide, acetamiprid and RH-0345 [22].

4.2. Effects of environmental and halofenozide (RH-0345) stress on the activity of the acetylcholinesterase (AChE).

The specific activity of AChE was determined during the period of sexual activity spring from the heads of female *S. morsitans* harvested at the two study sites, namely, Annaba (Drean, Sidi Amar) and Tebessa (Birelater and Elmaelabiod). The results showed that there was an inhibition of AChE activity at stations affected by pollution thus indicating neurotoxicity. However, there was one very significant difference between Drean station and Sidi Amar, but no significant difference was detected between the two stations of Tebessa (semi-arid station).

After injection the analog of the molting hormone (RH-0345), the latter results in a highly significant inhibition of AChE activity at day 10 compared to control represented by the individuals collected in Annaba (Drean) and highly significant at day 15 in those collected at the two reference sites (Annaba, Tebessa).

The sensitivity of acetylcholinesterase face insecticide is highly effective for the detection of pollutants in the environment like neurotoxic insecticides, organophosphates and carbamates [23]. Indeed inhibition of AChE was frequently used in toxicology, several authors confirm that this inhibition may be a metal contamination indicator [24].

The specific activity of AChE is also influenced by the insecticide treatment, this is related to the spring breeding season when the complex of the frontal lobes and neurosecretory cells play a major role on the development of the reproductive cycles. Our results are consistent with those made on other species such as *Helix aspersa* [25] *Achatina fulica* [26].

The inhibition of acetylcholinesterase may have effects on the behavior of living beings [27, 28] the analysis of the activity of acetylcholinesterase in different tissues terrestrial organisms is considered a biomarker of contamination of terrestrial [29, 30]. The inhibition of the activity AChE has also proved useful in the context of quality study of terrestrial environments [29].The variation between seasons can be explained by changes in environmental factors [31, 32]. Other studies also show inhibition of AChE in the El-Hadjar site may be due to the accumulation of ETMs by *Helix aspersa* from the Arcelor Mittal complexe [33, 34].Other studies, including those of Dolezych and al. [35] related to inhibition of the activity of AChE after exposure to carbofuran snail *Helix aspersa* (pesticide). The AChE activity measured gut level in *H. aspersa* exposed to Nickel is significantly reduced even at low concentration of the latter [36].

4.3. Effects of environmental and halofenozide (RH-0345) stress on the activity of glutathione-s-transferase

Glutathione-S-transferase (GST) is a multigene family of enzymes that play an important role in tissue protection against possible toxic and carcinogenic compounds of exogenous and endogenous origin [37]. These are iso enzymes II phase of detoxification mechanism, catalyzing the conjugation of glutathione with several electrophiles.

Our work showed that the GST has increased significantly over the two polluted sites and used treatment induced greater increases of GST in females collected from Annaba compared to those of Tebessa. This increase can translate into a huge pollution from the city of Annaba, which is surrounded by several industrial areas in relation to the town of Tebessa.

Wind direction plays an important role in the transportation of pollutants from industrial areas and also by the effect of fertilizers (Cd) including phosphate fertilizers [38]; atmospheric emissions from road traffic and the incineration of household waste [39]. The results of many experiments also show the increased activity of GST in response to the high amount of pollutants in the environment [39, 29]. The results of [40] found a decreasing gradient of the induction of GST at a terrestrial snails (*Theba pisana*) by the distance to the source of pollution (Egypt). Induction of the GST has been reported in the snail *Theba pisana* exposed to pesticides [41]. In a study of biomonitoring, induction of GST was observed in the snail *Helix aspersa* collected in polluted urban sites with heavy metals [42].

There was an increase in the GST rate in species that have been treated with pyrethrinoids and deltamethrin particularly in *Cydia pomonella* [43] and *Helicoverpa armigra* [44]. *Drosophila melanogaster* exposed to the phenol has a resistance to it by increasing the enzymatic activity of GST as well as *Anguilla Anguilla* which was exposed to naphthalene (NAP) at different concentrations and different exposure times [45].

5. Conclusion

Scolopendra morsitans was subjected to an environmental as well as a chemical stress represented by an agonist of ecdysteroids (RH-0345) during the spring season breeding in order to test the ovarian weight and to evaluate the activity two biomarkers. The inhibition of ovarian weight and the activity of AChE and GST females from the polluted site were respectively inhibited and stimulated. All these results can be attributed to the presence of contaminants at the site of Sidi Amar and Elmaelabiod, it is the same for the injected molecule that also is very toxic.

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