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Acetylcholinesterase and catalase activities in several tissues of a bivalve mollusc (*Ruditapes decussatus*) fished from Mellah lagoon (North East of Algeria) after malathion exposure.

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ABSTRACT

This study has focused on the effect of malathion on acetylcholinesterase and catalase activities on several tissues in a sentinel specie Ruditapes decussatus (Mollusca, Bivalvia). Clams were fished from the lagoon of El Mellah (North-eastern Algeria) and then immediately transferred to the laboratory. Treatment with Malathion at 100 and 300 µg/l of water lasted 24h. We evaluated protein and lipid contents and activities of acetylcholinesterase and catalase in the digestive gland, gills, adductor muscle and mantle. Results showed that treatment with Malathion caused, in the four tissues studied, a decrease of lipid and protein levels. The enzymatic activity of acetylcholinesterase was strongly inhibited by Malathion with a dose-dependent manner, whereas that of catalase increased significantly with both doses. These results confirmed the toxic effect of malathion on clams and also the status of this species as sentinel species and bioindicator of pollution. Moreover, among the tissues studied, it appeared that the gills and digestive gland were more sensitive to pollution and seemed to be the most appropriate tissue to monitor littoral pollution by organophosphorus pesticides.

Keywords: Malathion, Ruditapes decussatus, Acetylcholinesterase, Catalase, Gills, Digestive gland.

INTRODUCTION

The presence of pesticides in the marine environment had effects on aquatic organisms; lethal effects resulted in serious physiological disorders or death, while sublethal effects were manifested by disturbances of metabolism. Malathion is an organophosphate parasympathomimetic which binds irreversibly to cholinesterase. It is mainly used in agriculture as a pesticide broad-spectrum, especially in the fight against sucking insects. The spraying of malathion to aquatic areas also contributes to the decline of amphibian populations [1].

During the last decade, various studies had shown that seawater and sediment of coastal industrial regions were significantly contaminated by several pollutants [2, 3]. The environmental risk assessment and ecotoxicological involved the use of biological markers to highlight an early stage of pollution [4]. Many biochemical and cellular biomarkers had been studied in aquatic organisms, and particularly in fish and bivalve molluscs. These biomarkers included those that were specific to oxidative stress, recommended for biomonitoring the quality of the aquatic environment, including malondialdehyde (MDA) which is derived from lipid peroxidation of polyunsaturated fatty acids in cell membranes during oxidative stress [5, 6, 7], reduced glutathione (GSH) involved in the antioxidant defense system [8] and catalase (CAT) which is the first line of defense against oxidative stress [9]. There are other enzymes that play no role in the detoxification in living things such as acetylcholinesterase (AChE), which remains, however, a good indicator of pollution by organophosphate pesticides. Some studies on pollution and biomarkers were devoted to bivalves such as clams *Ruditapes decussatus* [4, 10, 11, 12] and *Cardium glaucum* which are considered like organisms showing the correlation between contamination and accumulation of pollutants [9, 13, 14, 15]. Studied tissues were often the gills and digestive gland that are in direct contact with the pollutant [16] while few studies had examined the effects of pollutants on tissues with metabolic activity (mantle or muscle).

The present work aimed to assess the biochemical response of *R. decussatus* from exposure to malathion in the laboratory. Two biomarkers were used: Acetylcholinesterase (AChE) and catalase in various tissues (mantle, gills, digestive gland and adductor muscle) to assess the sensitivity of each tissue to exposure to malathion.

MATERIALS AND METHODS

Collection, treatment and dissection of bivalves

R. decussates 34-37 mm (n=60) were collected near the low water level during February 2008. At the time of sampling, water temperature varied between 16 and 18°C. The clam's samples were immediately transferred to our laboratory and allowed to acclimate to laboratory conditions in fiberglass tanks (30L) filled with continuously aerated water. During the acclimation period, half of the water in each tank was renewed with water every 3 days. After this period, clams were divided into 3 groups: A control group (n = 10) and two groups treated with malathion for 24 h (100 and 300 µg/l of water) (n = 10 for each group). After the treatment period, clams were quickly dissected; mantle, adductor muscle, gills and digestive gland were removed and weighed. Protein and lipid concentrations and enzyme activity of AChE and catalase were measured in tissue samples (n = 4 for each dose).

Quantification of proteins and lipids in tissue

Proteins and lipids of each tissue from control and treated-malathion clams were extracted [17] and evaluated [18, 19] respectively. Concentration of each metabolite was expressed as µg/mg of tissue.

Acetylcholinesterase activity

For AChE activity, tissues (mantle, adductor muscle, gills and digestive gland) were homogenized in Tris buffer (0.1 M, pH = 7). Obtained homogenate was centrifuged at 9000 g for 20 min. An aliquot of the supernatant obtained was used to evaluate the activity of AChE by the method of ELLMAN [20]. The extract was incubated in the presence of substrate (acetylthiocholine) and 5.5-dithiobis-2-dinitrobenzoic acid (DTNB). The reaction is performed at 25 ° C and the absorption measured at 412nm. Each enzymatic reaction was quantified against a

blank without substrate. The AChE activity is expressed as nmol of product released per min/mg of protein.

Catalase activity

Catalase activity was measured using colorimetric method [21] based on the dismutation of H_20_2 with catalase. This activity is estimated at 240 nm for 1 min at 25 ° C and expressed in tissues as µmole of H_20_2 used per min/mg of protein.

Statistical Analysis

Differences between control and treated-malathion groups were evaluated by an analysis of variance (one-way ANOVA) with significance level of 0.01 using the MINITAB software. Normality and homogeneity of variances were verified and a parametric one-way analysis (ANOVA) was performed on data.

All results are expressed as mean ± standard error.

RESULTS

Protein and lipid contents

Protein and lipid contents analysed in four tissues of *R. decussates* control and malathion-treated are presented in Tab.1 and Tab.2 respectively. Malathion exposure reduced significantly ($p < 0.01$) protein concentrations in all tissues studied.

Table 1: Effect of malathion on protein concentration (µg/mg of tissue) in mantle, adductor muscle, gills and digestive gland of *R. decussatus*. (mean ± s, n = 4). For the same tissue, averages followed by the same letter are not different at 1% level.

Tissues	Control	Malathion 100µg/l	Malathion 300µg/l
Mantle	13.148 ± 1.546a	5.883 ± 0.828b	5.166 ± 0.738b
Adductor muscle	15.574 ± 3.106a	3.291 ± 0.374b	2.902 ± 0.718b
Gills	3.759 ± 0.509a	1.442 ± 0.244b	1.342 ± 0.074b
Digestive gland	2.564 ± 0.264a	1.932 ± 0.767b	1.744 ± 0.275b

Lipid concentrations was significantly reduced in clams treated with the two doses with a dose-dependent manner ($p<0.01$) in all tissues studied.

Table 2: Effect of malathion on lipid concentration (µg/mg tissue) of mantle, adductor muscle, gills and digestive gland of *R. decussatus*. (mean ± s, n = 4). For the same tissue, averages followed by the same letter are not different at 1% level

Tissues	Control	Malathion 100µg/l	Malathion 300µg/l
Mantle	4.04 ± 0.698a	0.291 ± 0.066b	0.046 ± 0.002c
Adductor muscle	1.610 ± 0.419a	0.259 ± 0.068b	0.244 ± 0.079b
Gills	1.418 ± 0.100a	0.196 ± 0.044b	0.034 ± 0.001c
Digestive Gland	0.831 ±0.052a	0.282 ± 0.014b	0.029 ± 0.004c

AChE activity after malathion exposure (Fig. 1)

AChE activity in tissues (mantle, adductor muscle, gill and digestive gland) after treatment with malathion, reveals an inhibition after 24 hours of treatment with a dose dependant manner (100 and 300 µg/l) in the 4 tissues studied. This decrease was more apparent in the mantle and adductor muscle (due to the presence of neuromuscular synapses).

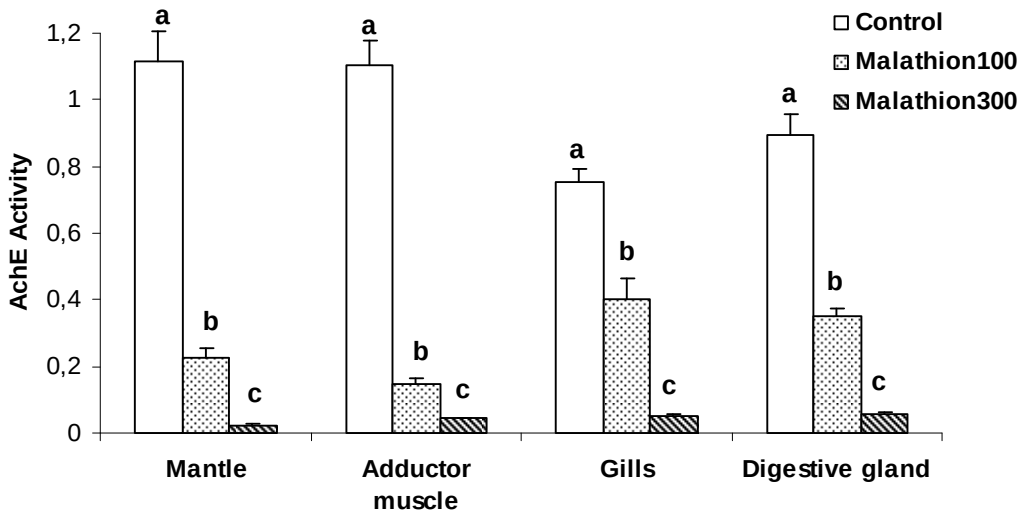

Figure 1: Effect of malathion on acetylcholinesterase activity (nM/min per milligram of protein) in the four tissues (mantle, adductor muscle, gills and digestive glands) of *R. decussatus*. (mean ± s, n = 4). For the same tissue, averages followed by the same letter are not different at 1% level.

Catalase activity after malathion exposure (Fig. 2)

Results showed a significant increase in catalase activity in *R. decussatus* for both doses (100 and 300 µg/l) compared with controls. This increase can be explained by the stimulation of antioxidant defense system in all tissues studied. This defense seems to be more expressed in the gill and digestive gland, two tissues that are in direct contact with malathion.

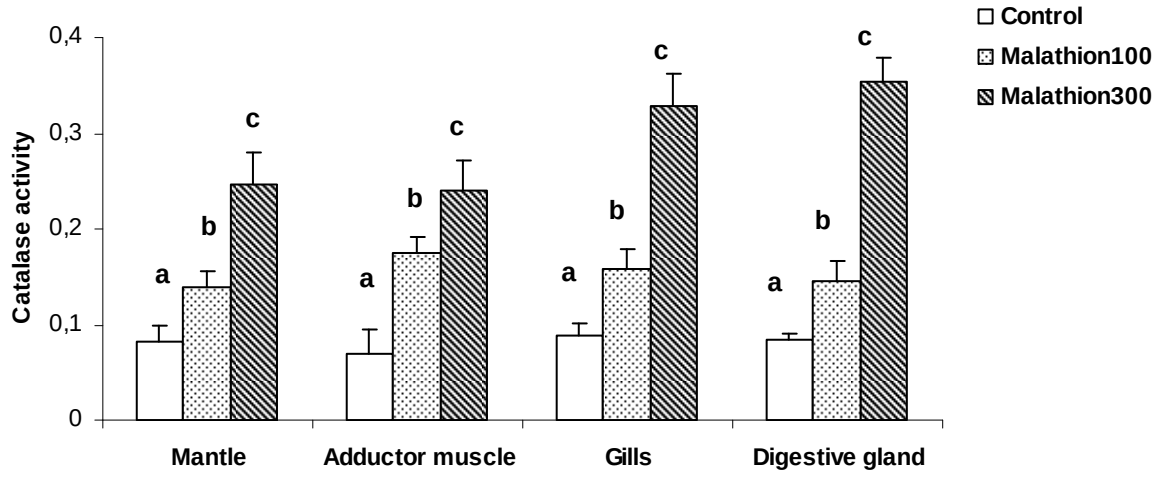

Figure 2: Effect of malathion on the activity of catalase (µM/min per milligram of protein) in the four tissues (mantle, adductor muscle, gills and digestive glands) of *R. decussatus*. (mean ± s, n = 4). For the same tissue, averages followed by the same letter are not different at 1% level.

DISCUSSION

Industrial discharges and waste disposal in urban estuaries and coastal regions are the main sources of water pollution [3]. The clam *Ruditapes decussatus* strongly accumulated hydrocarbons, metals, organophosphorus compounds (PCBs), pesticides and herbicides [22, 23].

The Gulf of Annaba is the most important tourist attraction and economic installed on the east coast of Algeria. Its fisheries resources are threatened by pollution-related economic activity

booming. In this context and within the coastal biomonitoring, we evaluated the effects of malathion exposure in laboratory on the responsiveness of the clam by the measurement of two biomarkers (Acetylcholinesterase and catalase) in various tissues and to assess sensitivity of each tissue.
Marine bivalve such as molluscs and mussels were appropriate sentinel species [24] for most of the biomarkers studies because they can accumulate a significant amount of pollutants in their tissues [25]. The environmental risk assessment and ecotoxicology involved the use of biomarkers designed to highlight an early pollution [4].

Results obtained in this work showed that the rate of protein decreased in the 4 tissues studied and for both doses (100 and 300 µg/l). Same results were reported [26] on the study of effects of halofenozide (ecdysteroid agonist) on the hemolymph and ovary of the shrimp *P. kerathurus*. A decrease in protein levels was also reported in *P. kerathurus* treated with Dimilin (inhibitor of chitin synthesis) [27].

Evaluation of lipid levels in the 4 tissues showed a significant decrease in all tissues studied compared to that observed in controls. Lipid content may undergo significant changes under physiological and environmental conditions [28]. Previous studies showed that in aquatic organisms, lipids served as a reservoir to protect against the toxic effect of lindane and other pesticides [29]. Our results were consistent with those of [30] who assessed the lipid tissues of a bivalve, *Villorita cyprenoids var. cochinensis*, following exposure to organophosphorus pesticides (endosulfan, malathion and methyl parathion). They noticed a drop in blood lipid after 24 h of treatment and that for the three pesticides. This decrease in fat content depends on the toxicity of pesticides and the sensitivity of the test organism [30]. Organophosphorus pesticides are highly toxic to marine organisms [31].

To assess the impact of neurotoxic compounds on the marine environment, we had evaluated acetylcholinesterase activity, which, in marine organisms, was used as a biomarker of exposure to nerve agents such as organophosphate pesticides [32]. The inhibition of AChE has been widely used in aquatic ecosystems as an indicator of exposure to organophosphate insecticides (OP) and carbamate (CM) [32, 33].

Evaluation of AChE activity in tissues (mantle, adductor muscle, gill and digestive gland) after treatment with malathion, revealed an inhibition of AChE after 24 hours of treatment with a dose dependent manner in the 4 tissues studied. This decrease was more apparent in the mantle and adductor muscle due to the presence of neuromuscular synapses. Similar results had shown that treatment with two pesticides: carbofuron (carbamate) and chlorpyrifos (organophosphate) for 24 hours of exposure, inhibit AChE activity of *Gambusia Yucatan* [34]. In addition, AChE activity measured in the anterior adductor muscle of the mussel *Amblema plicata* was inhibited following exposure to various doses of organophosphate pesticides [35, 36]. This decrease was also recorded in the muscle and brain of *Anguilla Anguilla* treated with different concentrations of a herbicide, thiobencarbe [37], in gills of the clam *R. decussatus* exposed to lindane [38] and other bivalves such as oysters treated with carbofuron and malathion [39], mussels (*Amblema plicata*) treated by chlorpyrifos [36] and *Scrobicularia plana* fished in the Spanish coast [40]. The inhibition of acetylcholinesterase has been proposed as evidence of the presence of organophosphates and carbamates [41]. More generally, inhibition of acetylcholinesterase was linked to agricultural activities including the use of pesticides [42].

Catalase is an antioxidant enzyme acting against the toxicity of oxygen radicals [43].

Our results showed a significant increase in catalase activity in *R. decussatus* for both doses (100 and 300 µg/l) compared with controls. This increase can be explained by the stimulation of antioxidant defense system in all tissues studied. This defense seemed to be more expressed in the gill and digestive gland, two tissues that are in direct contact with malathion. Moreover, a study on *R. decussatus*, using treatment with hydrocarbons and pesticides (organophosphates and carbamates), showed that the digestive gland and gills expressed the highest catalase activity [44]. The increased activity of catalase was due to the presence of malathion and the production of free radicals, indicating an effective protection against H_2O_2 which is a powerful oxidizing agent and the main precursor of HO [45]. This increase of catalase activity was also observed in the muscle of *H. trunculus* exposed to cadmium and/or carbofuran and in the digestive gland of animals exposed to lindane. Several organic contaminants such as pesticides and fertilizers, had led to increase catalase activity in marine organisms [43, 46, 47, 48].

CONCLUSION

Results obtained in this study confirmed that the clams were rightly regarded as sentinel species in pollution by organophosphate pesticides. In addition, the gills and digestive gland appeared most sensitive to these pollutants than metabolic organs such as mantle and adductor muscle. Thus, evaluation of biomarkers in gills and digestive gland seemed to be the best solution for detecting agricultural pollution.

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