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Evaluation of crude chitinases extracted from marine biomass (stomach of fishes) on the feed insect: *Callosobruchus maculatus* (Coléoptera: Bruchidae)

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Abstract

In order to develop a marine waste insecticide to protect our environment, this study aims to assess the potential of crude chitinases (specific enzymes hydrolysis of chitin) extracted from marine biomass as a biological insecticide at different doses 3, 12 and 21%, on the larva feed of the insect, a destroyer of stored products.

The unexpected results show that the amount of food ingested in the control batches (3.7g) is higher than in treated ones by these crude chitinases (3.02g), (0.9g) and (0.34g) respectively.

The dose of 21% produced a very significant larvicidal activity (97.12%) by adults rate emerging at only (2.88%) compared to the reference sample (100%).

Histological sections of the digestive tube of treated larva at the same dose (21%) show a destruction of different cell types after the death of larva. This feed inhibition is explained by the effect of the extract crude chitinases on peritrophic membrane of the stomach of this bio aggressor (incorporated mainly of chitin), that prevents it to reach the adult stage.

Keywords: Fish waste; environment; chitinases; chitin; larvicide

1. INTRODUCTION

The marine biomass and their diversity are set of exploitable and very valuable resources, useful to humans, food and health sectors. The biomass produced by the ocean is much greater than the one issued from dry land, but only a small part, resulting from fishing activities is directly used.

In Algeria, despite the weakness of the fishing industry, it would be desirable to invest in the marine resources exploitation, allowing in one hand to help clean the shoreline other to produce active substances such as chitinases.

The literature data indicate that these chitinases are insecticide enzyme that can be used in insect pest's eradication [1, 2]. [3, 4], reported that once ingested by insect. Chitinases will hydrolyse the membrane of the insect gut that consists of 12% chitin. The enzymatic attack would lead to a lethal effect resulting from a severe abrasion of a portion of the unit tract

The chickpea (*Cicer arietinum*) (Fabales: Fabaceae) is one of the most important legume crops in the word. As all countries, Algeria is facing a very high rate of destruction of crops chickpea during storage mainly because of *Callosobruchus maculatus* (Coleoptera: Bruchidae) (Minister of Agriculture of Algeria, 2011).

C maculatus infestation begins in the fields. Propagation over several generations of insects from the primary infestation(infestation in the fields) combined with infestations during storage by

insects from outside leads to a pronounced proliferation dynamics of *C* maculates [5,6].The eggs laid by adult females develop into larvae that penetrate in to the seeds and consume the cotyledon to grow. After the pupation period has ended, the adult chews through the seed coat and emerges from the chickpea, which results huge losses during storage [7]. In untreated, it is recorded that 50% loss in seed weight and 70% loss in protein content of pulses is due to infestation caused by these insects [8].

The chemical control of stored products by insecticides or fumigants presents problems such as handing hazards, residues, development of resistance resurgence and environmental pollution [9, 10], intoxication and ecological disorder [11].

Many chemical fumigants, commercially is use, are considered as carcinogen, mutagenic agents and they contain carcinogen, mutagen and hepatoxins.

It is within this framework that is inscribed the theme of our work to extract the chitinases from marine waste (offal fish: Scorpion fish) and test their potential as an insecticide against a pest stored products: *C maculates.*

2. MAERIALS AND METHODS

Several criteria are taken into consideration in the choice of biological material. In our case the animal biological that was chosen is the *C. maculatus*. The chickpea weevil for several reasons: the species is cosmopolitan and infests stored products of economic importance in Algeria. This species breeds readily in the laboratory which allows us to test our crude chitinase extract a large number of individuals. The mass rearing of insects has been achieved in jars of 18cm and 11cm in diameter on the chickpea. The jars are kept in the dark in an oven set at a temperature of 28°C and a relative humidity of 75%. The same conditions of temperature and humidity were selected to perform our experiments.

As for the second biological material, we choice chitinases extracted from the gut of fish offal: Scorpion fish. These are recovered from restaurant's wastes. The extracts were obtained by soaking in a buffer citrate 1:6 (W/V) [12], centrifuged at 6000g; the supernatant was recovered as a crude extract chitinases. The insecticidal properties of these enzymes are described in the literature.

2.1 Evaluation of the biocidal action of chitinases raw biology of *C maculatus*

2.1.1 Bio essay of crude chitinase on larval feeding

We used the method described by reconstituted seed [13] in order to measure chitinases effect on the insect feeding. 50g chickpea flour mixed with 14 ml of the solution chitinase for making meatballs the size of a chickpea. The seeds used for the control are made with flour and chickpea buffer. The reconstituted seeds are dried for 24 h at a temperature of 30 ° C (to avoid distortion of chitinases and protein fractions) and a humidity of 70-80%. Chickpeas well prepared are placed in Petri dishes of 14cm diameter with 10 couples of insects to obtain the eggs. The eggs laid were counted daily until the death of the females and placed in an oven to assess fertility. The experiment is repeated four times for each dose and for the control. The rate of emergence (that represents the number of insects emerging on the number of eggs spawned) is calculated as follows:

$$Re = Ne/Ne'.100 \quad (1)$$

Ne: Number of insects emerging
Ne': Number of eggs spawned

2.1.2 Bio essay of crude chitinases on the digestive tract of C maculatus

Larvae treated with 21% (lethal dose after 1 h of treatment contact) have left the reconstituted seeds after a week of lying. These larvae were immediately fixed in 10% formalin for 72 hours. The control larvae have also been removed and fixed. After dehydration in ethanol baths of increasing concentration of 70°, 90° and 100° with one hour for each bath, the pieces were placed in butanol

for 24h, then impregnated and embedded in paraffin. The sections with a thickness of 2µm were stained by the method of [14].

3. RESULTS AND DISCUSSION

3.1 Toxicity of crude chitinases on larval feeding

To evaluate the effect of chitinase on larval feeding, we made sure that five days after treatment all eggs laid entered reconstructed seeds. The results for this bio test are reported in Table 1.

Table 1. Ingered food quantity by larvae of *C maculatus* treated by crude chitinases (IW: initial weight of seeds, FW: final weight)

Repetition	R1		R2		R3		R4		Mean		Ingired quantity (g)
Doses (%)	IW	FW	IW	FW	IW	FW	IW	FW	IW	FW	
Control seeds	9.6	6.24	9.7	5.89	9.1	5.85	9.2	4.75	9.4	5.69	**3.70**
3	5.35	2.10	4.46	1.80	4.64	2.3	5.30	1.5	4.94	1.92	**3.02**
12	9	8.1	9	8.2	8.9	7.75	9.3	8.95	9.05	8.15	**0.9**
21	5.75	5.55	5.65	5.35	5.62	5.02	5.80	5.50	5.70	5.36	**0.34**

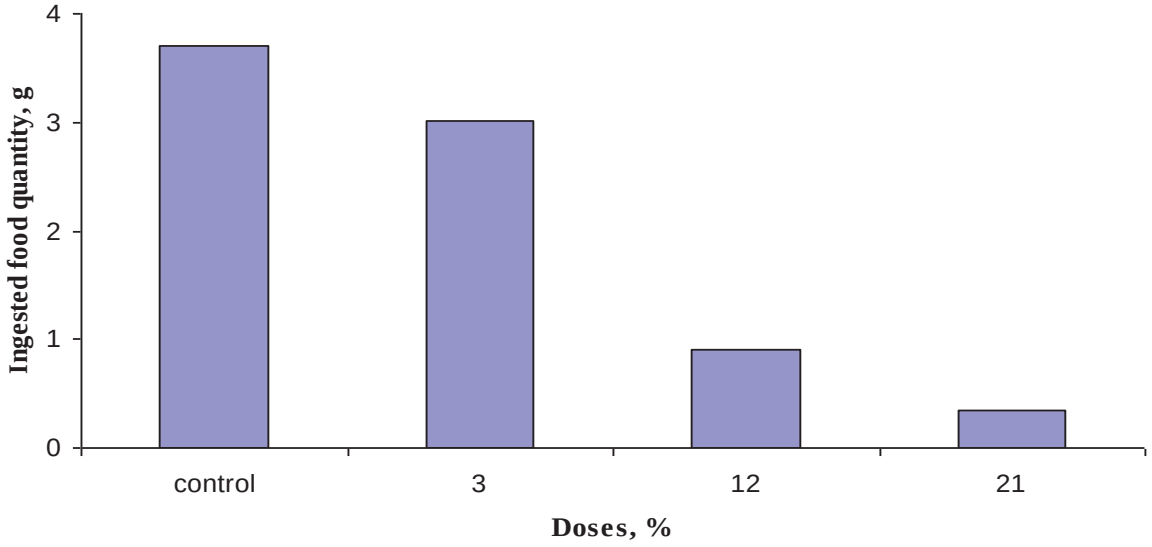

Figure 1. Effect of crude chitinases on the feed of *C maculatus*

As it is represented in Table 1 and Figure 1,the decrease in food intake explains the larvae die soon as they feed the contents of this seeds is processed, we assume that chitinases are equipped with a toxic by ingestion. This possibility is not ruled out, the effect of chitinases as organic insecticide is reported by some authors. According to [15, 16] once ingested by insects, chitinases will hydrolyse the membrane of the insect gut. The enzymatic attack would result in serious abrasions of part of the digestive system, leading to a lethal effect (Figure 2).

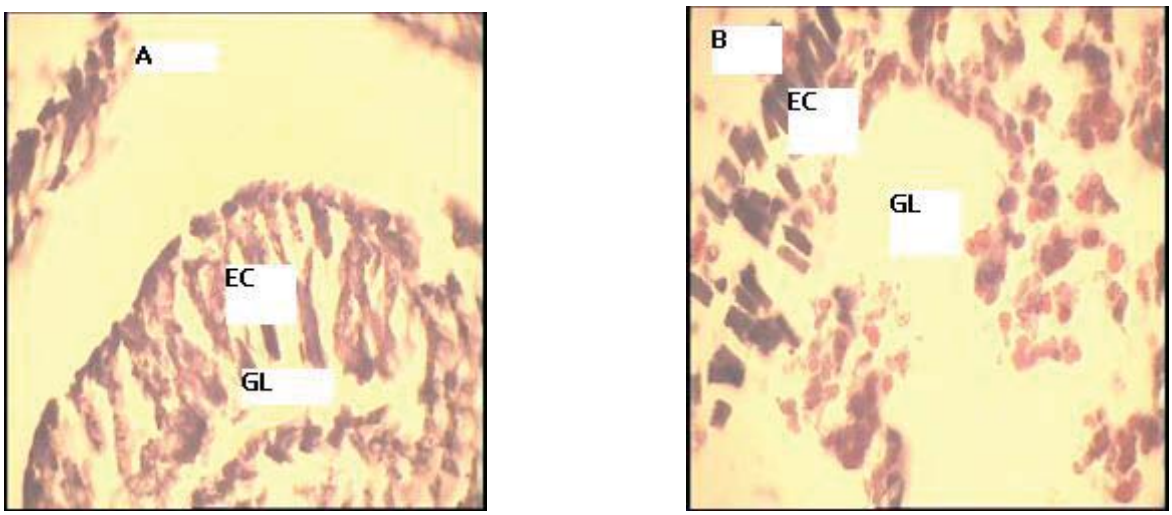

Figure 2. Histology of gastrointestinal tract of larvae of *C maculatus* Gr x100
A: control, B: larvae of insect treated (12%)
EC: Epithelial Cells, GL: Gut Lumen

Histology of the gastrointestinal of the larvae control, shows that it is consists of epithelial cells of cubic form. These cells possess a highly developed brush border (Figure 2 A), against, the larvae treated with crude chitinases to 12%, cellular hypertrophy is observed, the cell forms are hardly more perceptible. Cytoplasm debris is present in the lumen gut (Figure 2 B).

3.2 Effect of crude chitinases on emergence insects

The toxicity of crude chitinases on emergence insects is illustrated in Figure 3. As it is shown in this Figure, the number of insects emerging decreased with increasing doses 92.46, 18.75 and 2.88% respectively compared to the control 100%. These chitinases exert a toxic effect on larval development. This toxicity observed between the different doses is caused to the concentration of active chitinases in the crude extract.

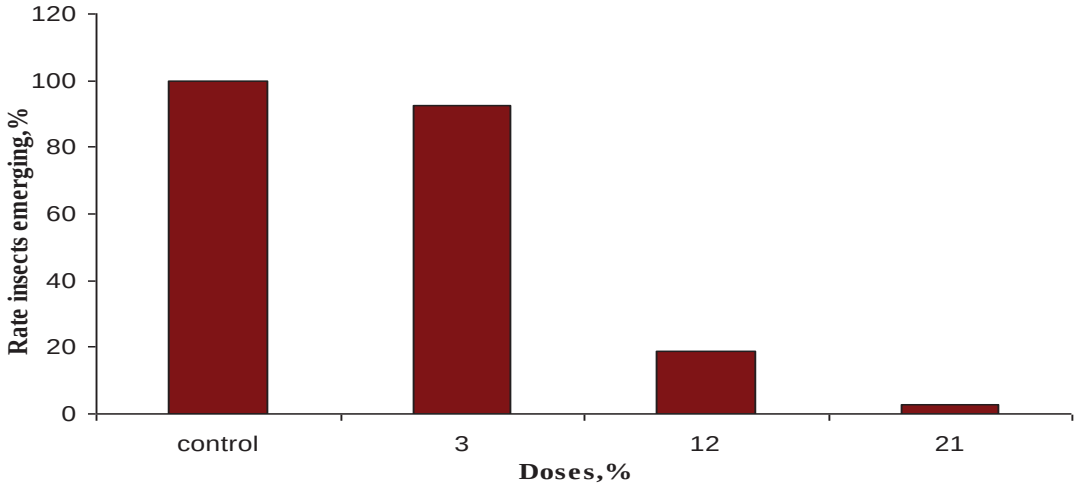

Figure 3. Effect of crude chitinases on the rate insects emerging

4. CONCLUSIONS

The efficiency of chitinases extracted from fish offal: Scorpion fish against the insect *C. maculatusn* was verified. Indeed, they affect populations of insects pests by reducing the rate of emergence. The use of chitinases able to control the pests in developing countries could be an alternative approach to complement the conventional insecticide treatment. In our study, we used three doses, it would be interesting to test other doses on the same insect to verify the toxicity of our biological

insecticide (unclear meaning please rephrase). Preservation of the nutritional quality is very important,therefore any attempt to study this area by using biological substances is justified.

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