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Larvicidal effects of aqueous extract from *Ricinus communis* L. leaves against mosquito *Culex pipiens* : mortality and histopathology of treated larvae

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Abstract

The present work is part of the control mosquito, it's of interest to study the biocidal action of the aqueous extract of *Ricinus communis* L. leaves against mosquito larvae. The toxic action of this aqueous extract, tested as larvicide, was studied on the larvae of the L2 and L4 stages of the *Culex pipens* mosquito. Mortality kinetics and larval histopathology were studied. Larvae of *Culex pipiens* exposed of *Ricinus communis* aqueous extract showed significant histological changes. After one hour of exposure, the cells of the middle intestine are deeply affected, the toxic substances ingested have manifested their activity on the intestinal epithelium. Hypertrophy and lysis of epithelium intestinal cells are the main changes observed. The toxic action of the aqueous extract is also manifested on the musculature and the external teguments of larvae. After 3 hours of exposure, total tissue lysisis observed. At low concentration, the toxic action of aqueous extract has effectively manifested as a biocide, by absorption on the digestive tractandby external contact on the teguments and the musculature.

1. Introduction

As is already known, mosquitoes, Culicidae, are vectors of many parasitic and also viral diseases. In many parts of the world, these insects can inoculate viruses that cause severe infections (chikungunya, dengue fever, Zika virus disease, yellow fever, encephalitis, West Nile fever ...). The chemical control of these nuisances based on the use of chemical insecticides poses problems for the environment and for human health. It also leads to the development of resistance in treated mosquitoes [1]. The systematic use of chemical insecticides to control mosquito larvae has led and still leads to the appearance of resistance phenomena which considerably reduce the effectiveness of the treatments [2]. In various species of mosquitoes, including *Culex pipiens*, these resistances have already been studied in Morocco for conventional insecticides [1, 3-5].

Taking these realities into account, it has become essential to respect the equilibrium of ecosystems by limiting chemical control as much as possible [6]. It's for these reasons that research has been oriented in search of biological alternatives, which are inexpensive and environmentally friendly.

Among the biological treatments used, we quotethe formulations of *Bacillus thuringiensis* (pathogenic bacteria for insect larvae), pathogenic fungi [7], larvivorous predators (batrachian and fish) and the release of males from sterile mosquitoes. However, these different means of control remain insufficient to ensure effective control on a large scale. For a long time, plant extracts have been the subject of several studies with the aim of isolating active substances against mosquito larvae.

In terms of toxicity, the aqueous extracts of the *Ricinus communis* leaves showed a significant effect on larvae of *Culex pipiens*, with low lethal LC50 concentrations[8]. In this work, our objective is to show the larvicidal activity of the aqueous extracts of *Ricinus communis* leaves on the fourth stage larvae (L4) of *Culex pipiens* which is the most abundant and most frequent species in the region of Mohammedia [9]. The kinetics mortality and the histopathological changes of the treated larvae were evaluated and compared with work on other mosquito species with other biocides.

2. Materiel and methods

2.1. Biological materials

Larvae used in this study belonging to the species *Culex pipiens*. They were collected from a littoral atlantic region of Mohammedia (Morocco) in breeding sites with polluted water rich in organic matter. They weremaintained in the water of their biotopes under laboratory conditions (75% relative humidity and 20 ° C).All 2ndand 4th instars larvae selected for larvicidal activity came from the same generation and from the same breading sites. The aqueous extract of *Ricinus communis* is prepared from the leaves recovered in the same region of Mohammedia, washed and dried in an oven for 24 hours at 40 ° C, then reduced to powder. An infusion is prepared by pouring 1L of boiled water on 100 g of this vegetable powder, with magnetic stirring for 30 min. After cooling and filtration on wathmann paper, dilutions were prepared from an initial concentration of 10%.

2.2. Toxicity tests

The methodology of the toxicity tests was based on the WHO standardized sensitivity tests [10]. Using the aqueous extract of *R. communis*, a series of concentrations ranging from 0.13% to 5% was prepared. Then, twenty 4th instar larvae were put into each one of the concentrations. Four replicates were performed for each concentration. In control experiments, larvae were placed into distilled water. The mortality kinetics was monitored over a 72 h period for the larvae of the second and fourth stages. Larvae were considered dead or moribund when they became unable plunging for a period and when they became unable to react after a light touch with a fine brush. Lethal concentrations were determined by mortality rates after 24 h of exposure. Means calculated were treated and compared by the Scheffe test at the threshold of $P = 0.05$ [11]. Lethal concentrations for 50 % mortality, LC50, were calculated using the Spearman software [12].

2.3. Histopathological study

Two samples of *C. pipiens* larvae are exposed to a concentration of 0.5% of the aqueous extract of the *Ricinus communis* leaves, the exposure time is one hour for the first sample and 3 hours for the second. A sample of control larvae is left in laboratory conditions in clean water with food. The treated larvae as well as the control larvae were subjected to the preparation steps applied in animal histology [13]. The histological sections are made using a microtome of the Minot type set at 5 µm thickness and then observed under an optical microscope. The pictures were taken using a digital camera placed on the eyepiece.

3. Results and discussion

3.1. Mortality kinetics of treated larvae

Larval mortality is observed from the first minutes of exposure of both larval stages L2 and L4 (Fig 1). The concentration of 5% caused a total mortality after one hour of exposure for the second stage (L2) and after two hours for the fourth (L4). The kinetics of this mortality also remains rapid for all concentrations on the two stages L2 and L4. The lowest used concentration (0.13%) in this biotest, caused 50% mortality larvae in less than 6 hours for L2 stage and less than 12 hours for L4. The aqueous extract at the lowest used concentration (0.13%) remains active even after 72 hours of exposure indicating its good remanence. This prolonged activity over time is one of the main characteristics of any product considered as an effective larvicide. In a similar study, using the aqueous extract of *Euphorbia guyoniana* [14], total mortality of *Culex pipiens* larvae (100%) of third stage (L3), was observed only at concentrations greater than or equal to 25%. The lowest concentration of this extract (1%) had caused a low larval mortality of 3.33 % ± 5.75 % during 72 hours of exposure.

The lethal concentrations LC50 calculated using the Spermann software are 140 ppm and 589 ppm for the L2 and L4 stages respectively. This shows that larval vulnerability is very important when the larval stage development is early. In accordance with this result, in another study using larvae of *Culex quinquefasciatus* [15], the aqueous extract of suneem was also more effective at the younger stages L1 and L2 as well as on eggs hatching. The toxic effect of the aqueous extract of the fruits of *Citrilus colocynthis* L. [16] was also found to be more toxic in terms of lethal dose on *Culicidae* larvae. The toxic effect of the aqueous *C.colocynthis* extract was also shown to be more toxic in low lethal concentrations on *Culicidae* larvae [16]. According to these works, the showed larvicidal activity may be due to the presence of active molecules belonging to the alkaloids which accentuates the severity of the aqueous extract.

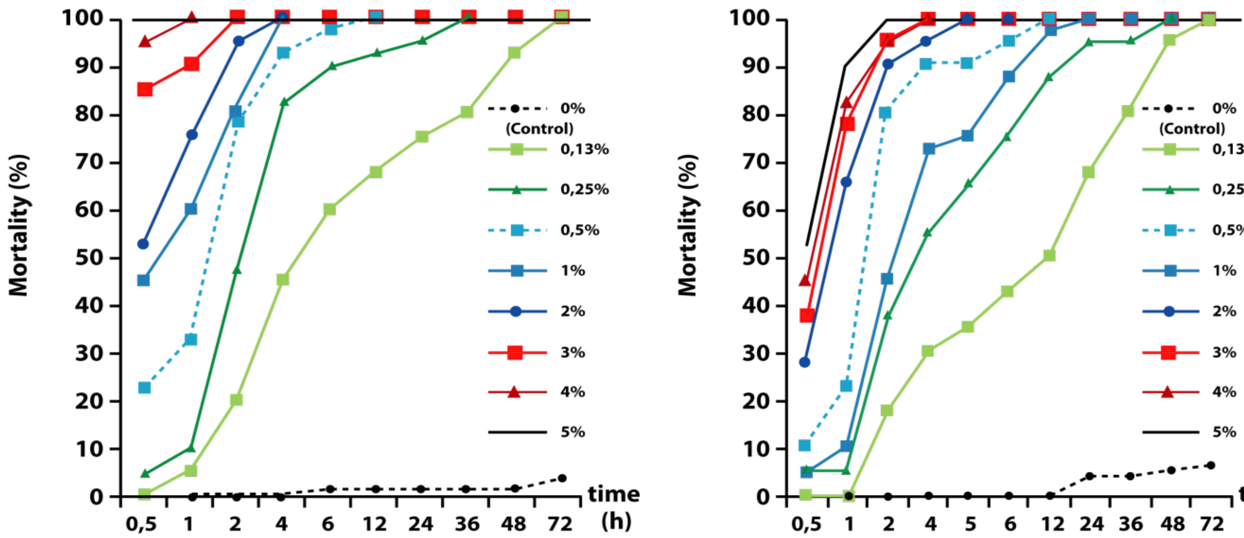

Figure 1: Kinetics of *Culex pipiens* larvae mortality of L2 stage (left) and L4 (right) exposed to aqueous leaves extract of *Ricinus communis*.

The action of the aqueous leaves extract of *Ricinus communis*, used in this work, was similar in terms of toxicity to that of essential oils of other plants. Thus, the minimum concentration used to cause total mortality on L3 and L4 *Culex pipiens* larvae were evaluated at 220ppm for the essential oil of *Thymus vulgaris* [17].
However, in a toxicity study [18], it was found that toxic litters act as xenobiotics which contribute in the appearance of resistance to certain insecticide products. It would therefore be interesting to plan, in a later work, a study of the resistance of the larvae to this *Ricinus communis* aqueous extract in order to valorize its toxic efficiency.

3.2. Histopathology of treated larvae
To justify the toxic efficiency of the aqueous leaves extract of *Ricinus communis*, which has been shown to be important with its rapid action and low lethal concentrations, the histopathology of treated larvae L4 stage has been studied on *Culex pipiens*. Compared to the control larvae (Fig 2), microscopic observations revealed significant lesions on larval cuts treated with aqueous extract. The morphology of normal healthy *C. pipiens* larvae is characterized by an alimentary column limited by a peritrophic membrane bordered externally by the intestinal epithelium, and in muscle tissue, the fibers appear associated with each other showing the muscles in their normal appearance. However, treatment of larvae by exposure to aqueous leaves extract of *Ricinus communis* induces histopathological changes at different levels of the body leading to disorganization of movements followed by immobilization and subsequent death.

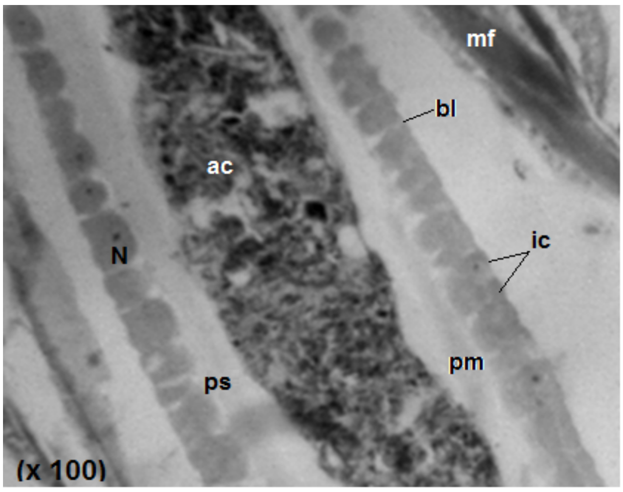

Figure 2 : Longitudinal section of the middle region of a control larva of *Culex pipiens* (stage L4).
ca : alimentary colomn ; ps : Peritrophic space; pm : peritrophic membrane N : nucleus ; bl : basal lamina; mf : muscle fiber; ic : Intestinal cells

Since mosquito larvae are considered to be non-selective detritivores, unable to select the food particles they ingest [19], profound changes have been observed in the gastrointestinal tract, particularly in the intestine median region. After 1 hour of exposure (Fig 3a.), a dislocation of the cells of the intestinal epithelium is observed accompanied by clear detachments of the basal lamina and the peritrophic membrane. A dislocation of the cells of the intestinal epithelium is observed accompanied by clear detachments of the basal lamina and the peritrophic membrane.

The epithelial cells have become necrotic and disorganized. In the same way, the external teguments of the larvae are not spared, they are also affected by the action of the used aqueous extract of the *R. communis*. The muscle tissue is also affected by the toxicity of the extract, the spaces observed between the muscle fibers reflect the attack of the musculature of the larvae, this is in agreement with the weakening of the larvae observed at the beginning of the exposure and which resulted in disorganized movements.

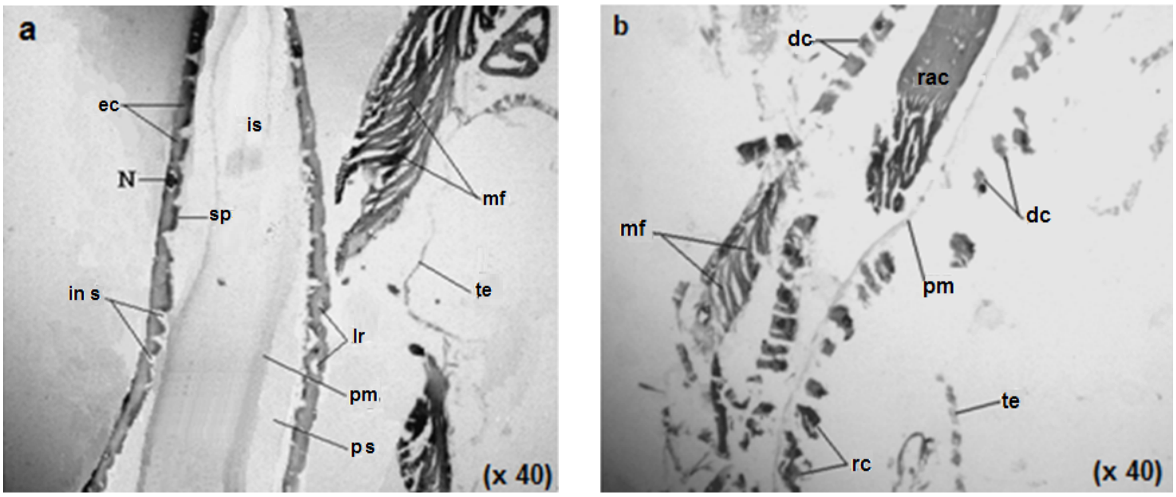

Figure 3 : Median region longitudinal section of the *Culex pipiens* larvae (stage L4) exposed to aqueous extract of *Ricinus communis*.a: after 1 hour of exposure b: after 3 hours exposure
ps: peritrophic space; is. intestinal space ; bm: basal membrane; N: nucleus; ec: Epithelial cells; sp: striated plate; Rca: rupture alimentary column; in s: intercellular space ; pm: peritrophic membrane; lr: lysis ranges; mf: musclar fibers ; cd: detached cells; te: tegument.

After 3 hours of exposure (fig 3b), the lesions become more pronounced in the gastrointestinal tract. The epithelial cells are completely detached from each other, the necroses have become more important until the rupture and even the enucleating of some cells and the completely destroyed peritrophic membrane. The muscular tissue is totally disorganized with detachment of the muscular fibers. The shredded appearance of the larvae reflects a complete tissue deterioration of the external tegument.

The pathologies observed in the digestive tract are very similar to those described in the work of Koua and al [20] on the larvae of *Anopheles gambiae* exposed to the *Persea americana* aqueous extracts, and also to those evoked in the works of Karch and al [21] on larvae of *C. pipiens* exposed to *Bacillus sphaericus* 1593-4 formulations. Moreover, in other studies [22], the toxicity of *Bacillusthuringienesis israeliensis*, which affects the entire digestive tract of larvae, remains more rapid on the cells of the median region where cell lysis is maximal. This same observation was made in this work, the cells most affected rapidly by lysis are those of the middle intestine. All the deterioration of the larvae observed in this histopathological study is the consequence of the action of the active substances in the extract by direct contact with the tissues of the exposed larvae.

However, in contrary the aqueous extract of *Persea americana* [20] and the formulations of *Bacillus sphaericus and B. thuringiensis* [21] and [22], which act mainly on the cells of the digestive tract, the *Ricinus communis* aqueous extract has proved its severity both on the cells of the digestive tract as well as on the cells of the musculature and external teguments. The active ingredients of this extract act on the external tissues even before their absorption. It is very probable that the *Ricinus communis* aqueous extract contains active molecules acting as endotoxins on cells of the intestinal epithelium and active molecules acting as exotoxins on the tissues of the outer skin and muscle tissue. All these results seem to be in line with the disorganization of larval movements and their immobilization before death.

Conclusion

Among the means of controlling mosquito larvae, bioactive substances of plant extracts are actively sought. The experimental study of the *Ricinus communis* aqueous extract larvicidal activity revealed a severe toxicity of this extract on the larvae stages L2 and L4 of the *Culex pipiens* mosquito. The speed of its action and its low LC50 testify to its toxic effectiveness. The histopathological lesions after exposure larvae to extracts are linked, on one hand, to the digestion of the extract components of the *Ricinus communis* leaves, on the other hand, to their direct contact with the external tissues of the teguments. The action mechanism in the gastrointestinal tract can be explained by the ingestion and absorption of the active ingredients of the aqueous extract used which penetrate the peritrophic membrane to invade all the cells of the intestinal epithelium. Thus, profound histopathological changes appear very early on the median intestine of the treated larvae. The separations of the peritrophic membrane, the dislocation of epithelial cells and their vacuolations as well as the fragmentation of the intestine are the main manifestations of digestive histopathology.

In addition, the action of the active ingredients by direct contact is manifested by the disorganization of the external teguments and the musculature. The severity of the active ingredients in the *Ricinus communis* aqueous extract leaves manifested by its low LC50 and by its speed of action, is confirmed by its histopathological impacts on the treated larvae.

Further research for isolation of these active ingredients as well as the study of their cytological mode of action could reveal more information on the possibility of using this extract as a biocide that could be exploited for mosquito populations management.

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