*Pest Technology* ©2011 Global Science Books

# Effect of *Olea europea* and *Cestrum parquii* Leaves on the Cuticle and Brain of the Desert Locust, *Schistocerca gregaria* Forsk. (*Orthoptera: Acrididae*)

**Amel Ben Hamouda[1,2*] • Mohamed Ammar[1] • Mohamed Habib Ben Hamouda[2]**

[1] Faculty of Sciences of Sfax, route de la Soukra km 4, 3038 Sfax, Tunisia
[2] High Institute of Agronomy of Chott Mariem, Sousse, 4024 Sousse, Tunisia
[3] National Institute of Agronomy of Tunis, 43 Avenue Charles Nicolle, 1082 Tunis Mahrajène, Tunisia

***Corresponding author*: * ben.hamouda@yahoo.fr**

## ABSTRACT

*Olea europea* (Oleaceae) leaf powder added at 2% in an artificial diet resulted in a partial inhibition of cuticle genesis of the desert locust, *Schistocerca gregaria*. This inhibition was expressed by cuticuline and wax layers narrowing the epicuticle which may explain the soft aspect of integument and the persistence of juvenile colour until mature stage. Whereas, brain structure at this stage, does not show visible modifications. In the case of *Cestrum parquii* (*Solanaceae*) leaf powder at 2%, the cuticle structure of 5$^{th}$ instars larvae was highly modified. In the absence of exuvial space, the layers of new procuticle were partially developed and the old cuticle partially reduced. Regarding the brain cells of treated locust, we notice the occurrence of well developed vacuoles and its pycnotic nucleus. Between these cells, gigantic vacuoles flood the brain. These observations show the toxic effects of *Cestrum* leaf powder.

**Keywords:** brain, cuticle, locusts, plant extracts

## INTRODUCTION

Countries concerned by desert locust outbreaks have adopted, within a technical assistance program of FAO, a control strategy based on a permanent investigation of gregarious areas and a preventive control program in order to eliminate rapidly the early outbreaks. This strategy needs the use of high quantities of pesticides which results in environmental pollution and affects the ecosystem (Chara 1995).

Funds allocated by FAO for locust control for one year are around 350 millions US dollars, of which a great part was used for pesticides. In the case of *Schistocerca gregaria*, the survey of locust movements using satellites and the modernization of control methods resulted in new preventive strategy (Lecoq 2005; Ben Halima 2006). And it is time to improve locust control methods by integrating alternatives to chemical methods such as the use of plant extracts.

Barbouche *et al.* (1996) have reported an anti-feeding effect of olive leaf extracts on *S. gregaria.* Treated adults exhibit weak sclerotization of the cuticle which remains soft. Ammar *et al*. (1997a) have tested *C. parquii* leaves, which are palatable to locusts despite their toxic effect towards these insects. The effect of this extract was moulting difficulty at the end of each larval stage leading to the death of treated locust. The effect of this extract was moulting difficulty at the end of each larval stage leading to the death of treated locust. Its toxicity is concentrated in the crude saponic extract (Barbouche *et al.* 2001; Chaieb *et al.* 2007a, 2009). This extract contains an efficient insecticidal saponin on *S.gregaria* larvae and adults (Barbouche *et al.* 2001; Chaieb *et al.* 2007b).

The larval life of insects was characterized by a cyclic physiological activity which appears mainly in the integument. Under the influence of endocrine factors, hypodermic cells secrete periodically a new protective cuticle and release the old one (Bullière 1973). Both cuticles (old and new) were separated by a sub-cuticular space. The cuticle during imaginal moulting was constituted of epicuticle, exocuticle and endocuticle. The epicuticule was consisted of cement, wax layer, polyphenol and cuticuline layers (Wigglesworth 1964; Joly 1968).

During the moulting process, an exuvial liquid was secreted in the sub-cuticular space by the hypodermis which increases its surface by mitotic division. This liquid, highly concentrated with chitinase and protease, plays a role in the resorption and elimination of the old cuticle. The new cuticle constituted by epicuticle and procuticle was developed in the moulting liquid. The exuvia contains only a part of exo- and epicuticle (Wigglesworth 1964; Joly 1968).

This phenomenon was controlled by the ecdysone secreted by ventral glands which activated by other hormones secreted by the brain neurosecretory cells (Raccaud 1980).

In the order to clarify the moulting difficulty induced by C*estrum parquii* leaves for locust larvae and the low cuticle sclerotization induced by olive leaves for locust adults, brain and cuticle structure of treated insects were studied by adding 2% leaf powder of these two plants to an artificial diet supplied to crowded locust colony.

## MATERIALS AND METHODS

### Plant material

Fresh alfalfa (*Medicago sativa*), olive tree (*Olea europea*) and *Cestrum parquii* leaves were dried at 37°C then grinded. The powder or "plant flour" obtained was kept in sealed flasks.

### Locust rearing on artificial diet

Insects were obtained from the locust crowded colony conducted in our laboratory according to Ammar *et al*. (1997b). To observe the effect of our plants powder in the laboratory, locusts were reared in a group of 100 individuals in a wood-framed medium

**Received: 7 October, 2010. Accepted: 25 January, 2011.**

**Original Research Paper**

sized cage (40 × 40 × 40 cm) at a photoperiod of the light-dark 12:12 and 28-30°C. They were fed on fresh alfalfa or bersim.

24 hrs after hatching, eight groups of 20 hatchlings were maintained until the last instar nymphs. They were fed an artificial diet according to Tira (1975): [sodium caseinate (4 g), saccharose (10 g), cholesterol (0.3 g), ascorbic acid (0.2 g), benzonic acid (0.15 g), nipagin powder (0.11 g), agar (3 g)] were purchased from Sigma Chemical Company (St. Louis, MO, USA), plant flour (0.2 g), bran (4 g), wesson salt (2.5 g), yeast (6.96 g) and distilled water (70 ml).

The plant flour was alfalfa leaves for the control (3 groups) or olive (3 groups) and *Cestrum* (2 groups) leaves respectively and separately for the treated insects.

Each group received 20 g of the artificial diet which was renewed each 1-3 days according to the stage and the age of insects until the end of the essay.

## Brain histology

Brains of 7-day-old 5th instar larvae (about 20 for each type of artificial diet) were dissected, fixed in Bouin-Holland fixative for 24 hrs, sliced at 4 µm, stained with paraldehyde-fuchsin (Ramade 1968) and examined under a LEIKA DMLB microscope (Leika Microsystems) (about 100 for each type). Photos were taken using a LEICA WILD MPS32 camera.

## Ultra and semi-thin cuts of the tegument

Around 20 tegument sections in the case of each type were taken at 4th and 5th abdominal tergites of 7-day-old 5th instar larvae for C*estrum* tests and for control, and of mature adults for olive test and for control. All sections were fixed in 4% osmium tetroxide and 2% glutaraldehyde solution in cacodylate buffer (0.05M and pH 7.2 with 4% of sucrose) (Sigma Aldrich Chemie, Switzerland).

The semi-thin cuts (1 to 2 µ) were stained either with 1% thionine and methylene blue or with 0.1% toluidine blue (about 100 slides for each type). Observations and photos were obtained as above.

The ultra-thin cuts were contrasted with uranyl acetate (Sigma-Aldrich Laborchemikalien, Germany) and lead citrate then observed under a JEOL JEM 1010 microscope (Jeol Korea Ltd) (about 5 grids for each type).

## RESULTS

## Effect of olive tree plant

### *1. Adult colour*

At age maturity, 100% of adults fed with olive tree leaf flour were red brick colour and smooth that indicates a juvenile character which persists in treated insects whereas in control, this colour changes and adults become yellowish and hard (**Fig. 1**).

### *2. Structure and ultra-structure of the cuticle*

Thionine and methylene blue stain the cement in green, the wax layer dark in blue, polyphenols and exocuticle in red, and cuticulin and endocuticle in blue.

In the control adults, the epicuticle consisted top-down of cement, wax layers, polyphenols and cuticulin (**Fig. 2**). In contrast, in treated insects, we noted the disappearance of the blue layer corresponding to the cuticulin. The exocuticle and the endocuticle had shrunk.

Electronic microscopy was carry out on the cuticle has to compare the thickness of the epicuticle layers of the treated and control insects. In fact, the epicuticle of the treated insects showed a very thin cuticulin and a thinner wax layer and seemed to be less organized in comparison with the controls (**Fig. 3**).

### *3. Brain structure*

No differences were found between the treated and control

**Fig. 1 Persistence of juvenile colour at maturity age in the treated insect fed with olive leaf powder (left) in comparison with control (right).**

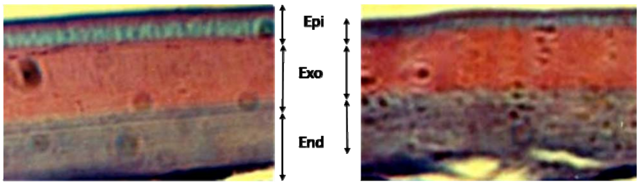


**Fig. 2 Cuticle structure in the control mature adult (left) and treated (right) by coloration with 1% thionine + methylene blue (Gx 400).** End: endocuticle; Epi: epicuticle; Exo: exocuticle.

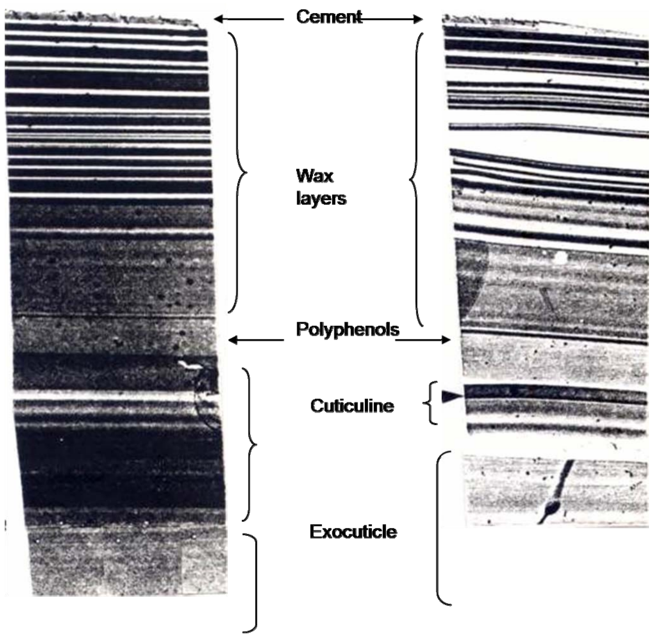


**Fig. 3 Ulrastructure of epicuticle mature adult: control (left) and treated (right) (X 1100), the head of arrow show the first cuticuline layer.**

insects in terms of brain structure (**Fig. 4**). The neurosecretory cells of the *pars intercerebralis* were similar in the treated and control insects in spite of their polymorphism and their sizes. In each brain section, the globuli have a light nucleus with condensed chromatin and a reduced cytoplasm in both treated and control insects.

## Effect of Cestrum parquii

### *1. Moult*

An effect of C*estrum* leaf flour was observed at the end of each instar. Changes were manifested as moulting difficulty

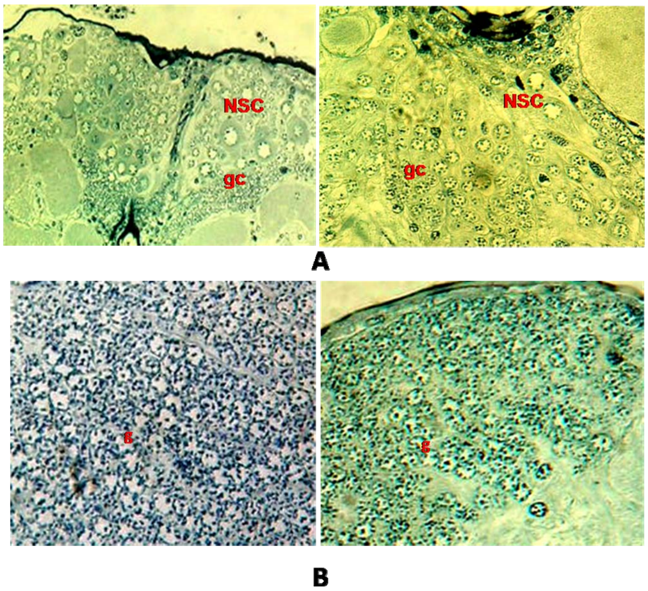


**Fig. 4 Brain structure of 5th instar larvae (7 days old) fed with olive leaf powder (left) and control (right).** (**A**) At *pars intercerebralis* level; (**B**) at protocerebron cortical area level. gc: glial cells; NSC: neurosecretory cells; g: globuli. Coloration with paraldehyde fuchsine. x400.

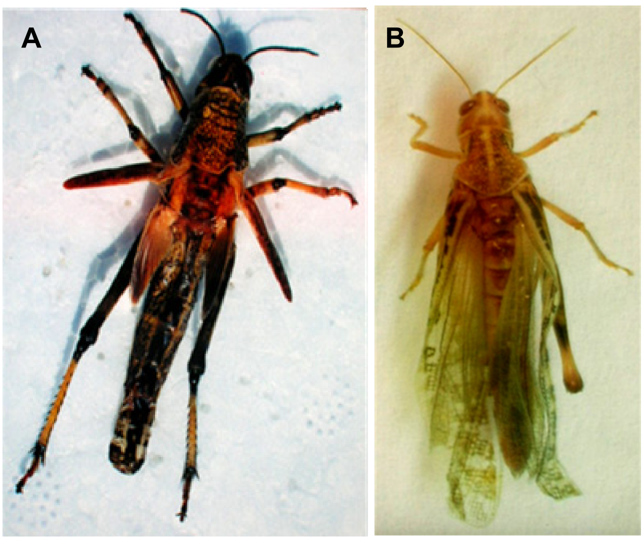


**Fig. 5 Moult difficulty and mortality of 5th instar larvae fed since the 4th moult with an artificial diet containing *Cestrum*.** Beginning (**A**) and end (**B**) of exuviations.

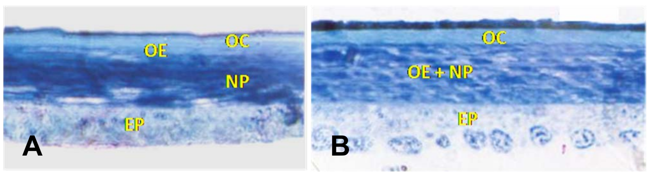


**Fig. 6** Tegument structure of 5th instar larvae locust (7 days old) fed with alfalfa (**A**) and with cestrum powder (**B**) by coloration with 0.1% toluidine. OC: old cuticle; OEND: old endocuticle; ES: exuvial space; EP: epithelium; NP: new procuticle. x 400.

leading to the death during the exuviation process; at the beginning (**Fig. 5A**) or at the end (**Fig. 5B**).

## *2. Cuticle structure and ultra-structure*

Using semi-thin cuticle cuts of samples at 7-days-old 5th instar larvae; we compared the moult process during the 5th larval stage of locusts fed with *Cestrum* and that of the controls (**Fig. 6**).

The exuvial space appears regular in control insects showing a new procuticle developed below instead of the old endocuticle which was resorbed (**Fig. 6A**). In contrary, treated locusts show the disappearance of exuvial space and the new procuticle mixed with endocuticle not well resorbed (**Fig. 6B**).

In fact, the ultra-structure observation shows that the cuticular layers were not synthesized to give rise to new cuticle in the controls (**Fig. 7A**). These latter showed a periodic typical structure corresponding to a deposition in light and dark layers phases of the diurnal and the nocturnal cycles. In the treated insects, the process of endocuticle resorption was incomplete and the layers of the new procuticule were less thick and visible than in controls (**Fig. 7B**).

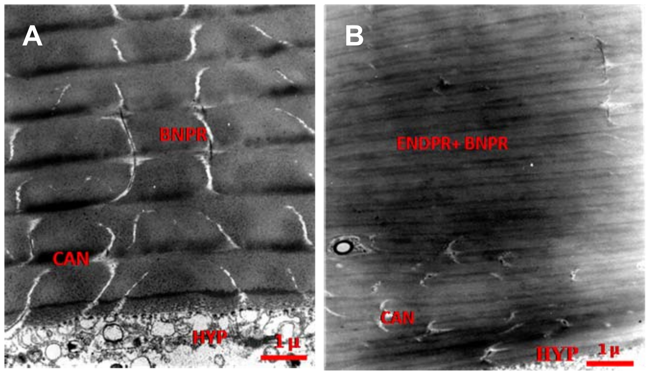


**Fig. 7 Cuticle ultra-structure of 5th instar larvae (7 days old) of *Schistocerca gregaria*.** Treated (**A**); control (**B**). BNPR: base of new procuticule; CAN: canalicule; ENDPR: endocuticule partly resorbed; HYP: hypoderm. x 8000.

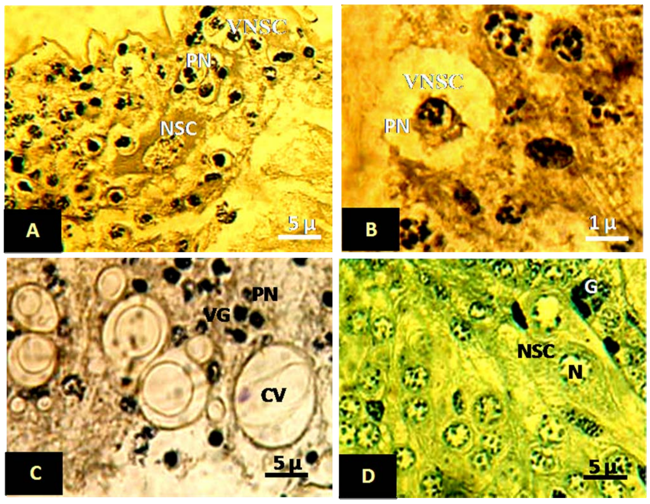


**Fig. 8** Brain structure of locust 5th instar larvae (7 days old) fed with *Cestrum* leaf (**A**, **B** and **C**) and control insects (**D**). NCS: neurosecretory cell; VNSC: vacuolar neurosecretory cell; G: globuli; VG: vacuolar globuli; N: nucleus; PN: pycnotic nucleus; CV: crystallised vacuole. Coloration with paraldehyde fuchsine. **A**, **C** and **D** = x400; **B** = x1000.

## *3. Brain structure*

The brain of the control shows the presence in the *pars intercerebralis* of neurosecretory cells with a lightly-staining nucleus and condensed chromatin (**Fig. 8D**). In the treated insects, most of neurosecretory cells show a pyknotic nucleus with dense chromatin and crystallized, vacuoled cytoplasm (**Fig. 8A, 8B**). The same appearance was observed in globuli (**Fig. 8C**). We notify also the presence of large and crystallized vacuoles that occupy whole the brain (**Fig. 8C**).

## DISCUSSION

Cuticulin with the exocuticular sclerotin and the endocuticular chitin contribute to the hardening of the cuticle (Beaumont and Cassier 1978). Therefore, the cuticulin layer shrunk at the epicuticle level in the adults treated with olive-leaf powder contributes to keeping the tegument smooth and juvenile at the mature adult stages.

Barbouche *et al.* (1996) reported the anti-feeding effect of *Olea europea* on *Schistocerca gregaria* and Ammar *et al.* (1997a) gave an explanation about this finding and reported that olive tree leaves induce a deficiency in protein in the insect haemolymph. The rate of soluble protein decreases in the adult cuticle because a large part of these proteins were used in the sclerotization process of the cuticle. The low doses of cuticular soluble proteins do not contribute to this process in the case of olive tree leaf treatment and these proteins remain unpolymerized.

In fact, the comparison of cuticular protein content of treated locusts with olive tree leaf and control insects shows a decrease of protein content in the treated all larval stages and an increase in the adult stage (Barbouche *et al.* 1996). According to these authors, the highest level of these contents was observed in the control insects. In contrast, the hydrosoluble protein content of cuticle decreased continuously in all larval and adult stages in both treated and control insects. In this case, the highest level of these contents was observed in treated insects (Barbouche *et al.* 1996; Ammar *et al.* 1997a).

The moult process was initiated by a prothoracotrope hormone released by the neurosecretory cells of the protocerebron. This neurohormone stimulates the moult gland which produces the ecdysone (Wigglesworth 1964; Raccaud 1980).

Our study shows that the shape of neurosecretory cells remains normal in both olives treated and control brains. Consequently, the anti-feeding effect of olive tree leaves does not affect the structure of neurosecretory cells of the *pars intercerebralis* of 5$^{th}$ instars larvae that explains why moult process was normal for this treatment. In contrast, in the case of C*estrum*, the alteration in neurosecretory cells may be explained by the cells intoxication, as reported by Ramade (1967) in house fly in which neurosecretory cells was intoxicated by lindane.

Food provides raw materials and energy for protein synthesis which were necessary for an organism's growth (Carlisle *et al.* 1987). The glycosides soluble in the locust haemolymph which were related to *Cestrum* saponins inhibit proteases (Barbouche *et al.* 2001). Therefore, the leaf powder of *C. parquii* added to an artificial diet led to larval mortality following moult difficulties.

Effectively, the insect intoxication induces severe physiological disturbance manifested by a disorder of central and a peripheral nervous system. Some aspects of this intoxication syndrome suggest that the effect on nervous system was accompanied by a disorder in the endocrine system (Moreteau 1991).

The disorder observed by Ramade (1967) on the house fly and the cytopathological lesions observed by Moreteau (1991) in the endocrine gland of *Locusta migratoria* following lindane intoxication prove that our observations on *S. gregaria* were due to an intoxication induced by *Cestrum* leaf powder.

In the brain of old house fly (10 days and more), the *pars intercerebralis* includes numerous cells with very visible vacuoles in the central and deep part of cerebral cortex. Some cells show a large and single vacuole distributed on the whole of cytoplasm (Ramade 1968). With regards to these results, the occurrence of similar vacuoles in the brain of locust fed with *Cestrum* cannot be related to ageing but to the presence of toxic materials in food.

## CONCLUDING REMARKS

Brain intoxication and moult inhibition by *Cestrum* leaf powder on *Schistocerca gregaria*, as well smoothness of the cuticle in the case of olive tree leaf powder show that these plant extracts could be tested in a preventive control method of the desert locust.

## REFERENCES

**Ammar M, Barbouche N, Ben Hamouda MH** (1997a) Cuticle alteration and death of *Schistocerca gregaria* by difficulty in moulting under food effect of *Cestrum parqui* and *Olea europea*. In: *Sixth Arab Congress of Plant Protection*. October 27–31, 1997. Beirut, Lebanon, 504 pp

**Ammar M, Ben Hamouda MH, Barbouche N, Moumène K** (1997b) Apparition de la phase solitaire chez le Criquet pèlerin, *Schistocerca gregaria,* par simple isolement des oeufs 3 jours après la ponte. *Medlinden Faculteit Landbouww Universiteit Gent* **62**, 339-348

**Barbouche N, Dhouib S, Ammar M, Ben Hamouda MH** (1996) Action de 3 substrats alimentaires : bersim, faux poivrier et olivier sur le développement de la cuticule chez le criquet pèlerin *Schistocerca gregaria*. *Annales de l'Institut National de Recherches Agronomique de Tunis* **69**, 131-146

**Barbouche N, Hajjem B, Lognay G, Ammar M** (2001) Contribution à l'étude de l'activité biologique d'extraits de feuilles de *Cestrum parquii* L'Herit. (Solanaceae) sur le criquet pèlerin, *Schistocerca gregaria* (Forskal). *Biotechnology, Agronomy, Society and Environment* **5**, 85-90

**Beaumont A, Cassier P** (1978) Biologie animale. Des Protozoaires aux Métazoaires Épithélioneurions (Tome I). Edit. Dunod, Paris, 459 pp

**Ben Halima T** (2006) Problématique du Criquet pèlerin et stratégie de lutte préventive. Commission FAO de Lutte contre le Criquet pèlerin dans la Région Occidentale (CLCPRO). In: *Congrès International d'Entomologie et de Nématologie*, April 17-20, 2006. El-Harrach, Algeria, 317 pp

**Bullière F** (1973) Cycles embryonnaires et sécrétion de la cuticule chez l'embryon de blatte, *Blabera craniifer*. *Journal of Insect Physiology* **19**, 1465-1479

**Carlisle J, Loughton BG, Ampleford E** (1987) Feeding causes the appearance of a factor in the heamolymph that stimulates protein synthesis. *Journal of Insect Physiology* **33**, 493-499

**Chara B** (1995) Concept et exécution de la lutte préventive contre le Criquet pèlerin. In: *Consultation Commission CLCPANO / FAO.* Du 18 Octobre au 2 Novembre 1995, Tunis, 13 pp

**Chaieb I, Boukamcha H, Ben Jannet H, Ben Halima M, Ben Hamouda MH, Mighri Z** (2007a) Purification of a natural insecticidal substance from *Cestrum parqui* (*Solanaceae*). *Pakistan Journal of Biological Science* **10**, 3822-3828

**Chaieb I, Trabelsi M, Ben Halima-Kamel M, Ben Hamouda MH** (2007b) Histological effects of *Cestrum parqui* saponins on *Schistocerca gregaria* and *Spodoptera littoralis*. *Journal of Biological Science* **7**, 95-101

**Chaieb I, Ben Hamouda A, Trabelsi M, Ben Halima M, Ben Hamouda MH** (2009) Toxicity investigation of *Cestrum parqui* saponins to *Culex pipiens* larvae. *Pest Technology* **3**, 73-75

**Joly P** (1968) *Endocrinologie des Insectes*, Edition Masson et Cie, Paris, 344 pp

**Lecoq M** (2005) Enseignement de la récente invasion du criquet pèlerin en France. In: *7ème conférence internationale sur les ravageurs en agriculture.* 26 et 27 Octobre 2005. Montpellier, France, 6 pp

**Moreteau B** (1991) Etude de certains aspects de la physiotoxicologie d'insecticides de synthèse chez le criquet migrateur: *Locusta migratoria* R. et F. La lutte anti-acridienne. Edit. AUPELF-UREF, John Libbey Eurotext, Paris, pp 167-178

**Raccaud J** (1980) *Les Insectes: Physiologie et Développement*, Edit. Masson, Paris, 288 pp

**Ramade F** (1967) Contribution à l'étude du mode d'action de certains insecticides de synthèse, plus particulièrement du lindane et des phénomènes de résistance à ces composes chez *Musca domestica* L. *Annales de l'Institut National Agronomique de Paris* **5**, l-268

**Ramade F** (1968) Sur la présence de cellules vacuolaires dans le cerveau de *Musca domestica* L. C. R. Acad. Sc. Paris, t. 266, Série D, pp 2437-2440

**Tira R** (1975) Elevage du Criquet Pèlerin, *Schistocerca gregaria* (Forskål 1775) sur milieu artificiel, son intérêt en entomologie appliquée. Thèse de docteur ingénieur, Université Paris Sud (Orsay- France), 45 pp

**Wigglesworth VB** (1964) *La Vie des Insectes: La Grande Encyclopédie de la Nature*, Edit. Rencontre Lausanne, France, 338 pp