Journal of Entomology

ISSN 1812-5670

Academic Journals Inc.

www.academicjournals.com

Journal of Entomology 4 (2): 121-128, 2007
ISSN 1812-5670

Development Perturbation of Cotton Leave Noctuid with Green Cestrum Extracts

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Abstract: The green cestrum (*Cestrum parqui*) is a *Solanaceae* which provoke toxicity on cotton leaves noctuid (*Spodoptera littoralis*, Noctuidae). This toxicity is demonstrated by some larval mortality caused by exuviation difficulties for *Spodoptera*. We observed some nymphal mortalities caused by desiccation of the chrysalis or by emergence impossibility of adults, these mortalities are due to different types of malformations. Some rare case of adults mortality are observed, these mortalities are due to the difficulties to redject the nymphal integuments. The toxicity could be also chronicle: An elongation of the larval stadiums until development stopping, the reduction of nymphal weight, of the adults wing-span and of their reproductive potential are observed.

Key words: *Spodoptera littoralis*, *Cestrum parqui*, insecticide, toxicity, saponins

INTRODUCTION

The massive use synthetic pesticides causes many human and animal health problems, pollution and disturbance of ecological balances. For this reason other alternative methods of pest management were developed. The use of bio-insecticides of vegetal origin is one of them. These insecticides have the advantage of being less phyto-toxic and more degradable in the enviroument (Cooping and Meun, 2000). Research in the field of insecticide of plant origin offers a very promising ground and especially going in the wake of the new concepts of development of sustainable agriculture and the protection of the natural resources.

Cestrum parqui is a solanous shrub used in Tunisia as ornamental plant, this species is shown to be toxic to desert locust (*Schistocerca gregaria*) (Ammar *et al*., 1995), the toxicity of this plant comes from the crude saponic extract (CSE) (Barbouche *et al*., 2001). The encouraging results obtained with *Cestrum* lead us to undertake similar work on other plant pest like Noctouid Lepidoptera. The toxicity of this plant was studied with the aim to explore the physiological and biological perturbations caused by *Cestrum* extracts on *Spodoptera littoralis*. In this work we try to identify any acute (mortality) or chronical (fitness) toxicity caused by *Cestrum* crude extract and to verify that the saponic extract is effectively the toxic fraction of the plant on cotton leave noctuid.

MATERIALS AND METHODS

Insect Rearing

Adults of *Spodoptera littoralis* are captured using a luminous trap. These latter are put in Plexiglass boxes and are fed by a 30% honey solution containing 1% of Mimosa pollen.

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The laying is carried out directly on the walls of the box. The adults are then remouved troughs of limp in order to ensure the hatching and the breeding of the young caterpillars. The larvae of 3rd stage are reared individually in *Petri* dishes on a simplified artificial diet of Poitout and Bues (1974).

All the biological stages of *S. littoralis* are maintained in culture rooms at 25°C, with one of relative humidity of 70%, under a 8 h photoperiod illumination. Insect rearing was maintained since 2001 and all experiments were realized in 2004.

Preparation of the Cestrum Dry Extract (CDE)

The leaves of *Cestrum* are collected during September from the garden of the INAT (Tunisian natioual agronomic institute). The leaves are subjected to a drying at 40°C for 72 h. Thereafter, they are finely crushed and the powder obtained is preserved in boxes safe from moisture.

Preparation of the Crude Saponic Extract (CSE)

One hundred grams of *Cestrum* leaves powder were washed with petroleum ether, then extracted three times with 300 mL methanol. After filtration the methanol is evaporated with rotary evaporator At 40°C. We obtained a dry residual weighing 6 g, the dissolution of 1 g of this residual in 100 mL methanol then the addition of 100 mL of ethylic ether permits to get 0,06 g of a brown precipitate symbolized (CSE).

Biological Tests

The toxicity of different extracts is studied on of L_3 recently moulted larvae, the extracts are incorporated in various amounts in the artificial diet at the time of its preparation. Control insects are reared on free extract diet. Different biological and physiological parameters where measured.

RESULTS

Acute Toxicity

Larval Mortality

Dry extract of *Cestrum parqui* leaves was incorporated in different amount on the artificial diet of *Spodoptera* larvae, the concentrations used are 2, 4, 8, 16 and 32% the control insects have a normal artificial diet. 32 new molted L_3 larvae were used for each concentration.

The analysis of the Fig. 1 shows a high larval mortality with concentrations of 32% of dry extract. These mortalities are due maiuly to difficulties of moult. The difficulty in the rejection of the cuticule is undoubtedly in relation to structural and biochemical modifications in the cuticular components. The larva is thus with two cuticules, the old one is narrow and rigid, strangles the caterpillar and disturbs the functions of digestion, excretion, breathing and probably the hormonal functions. It is interesting to announce that only the concentrations of 32% of dry extract involve larval mortalities of *Spodoptera*.

Nymphal Mortality

Figure 2 shows a nymphal mortality with all the concentrations of CDE added. These mortalities are closely related to malformations affecting the chrysalis. Indeed the percentage of malformation is proportional to the concentration of *Cestrum* added in the medium and inversely proportional to the rate of emergence (Fig. 2). Two great types of nymphal mortalities are observed:

- A mortality by desiccation (Fig. 3a) appears at the beginning of the nymphosis. The larval exuvia becomes more difficult to eliminate during the nymphal moult that generates an separation of the nymphal teguments and the appearance of nude zones. These phenomena expose the chrysalis to desiccation. Moreover the naked zones of cuticle constitutes probably an entered doors for pathogenic micro-organisms.

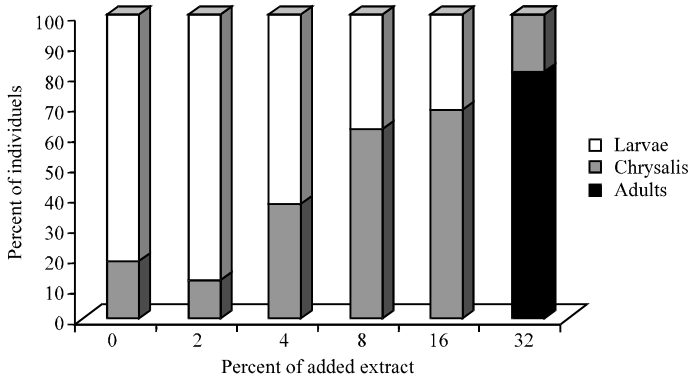

Fig. 1: Final stage of development reached by *Spodptera littoralis* larvae (n = 32)

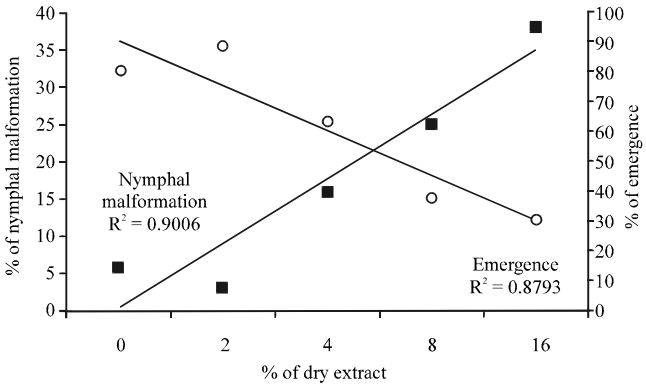

Fig. 2: Percentage of emergence and of nymphal malformations of *Spodoptera* according with the concentrations of additioned *Cestrum* dry extract in alimentation (n = 32)

- The second type of mortality is observed at the moment of the emergence of the adults coming from the malformed chrysalis. The butterfly is unable to break the exuvial lines, owing to the fact that the former part of the chrysalis is generally doubled by old larval cuticle (Fig. 3b).

This second type of mortality can occur even in absence of the persistence of the larval cuticles. The butterfly cannot emerge because probably of a harder nymphal cuticle. We observed that the most chrysalis resulting from caterpillars nourished on 32% of CDE can't carry out their emergence. The dissection of these nymphs shows the presence of butterflies completely formed and stuck to the nymphal integuments.

Adults Mortality

Lethal malformations appear in the adults of *S. littoralis*, these malformations are due to the difficulty of getting rid of the nymphal cuticle. This difficulty is observed in particular on the level of the cephalic part of the exuvia which remains attached to the head. It seems that this type of malformation are related to the concentrations of *Cestrum* added to diet. However a manpower of 3% of these malformations in the total population does not allow to confirm this.

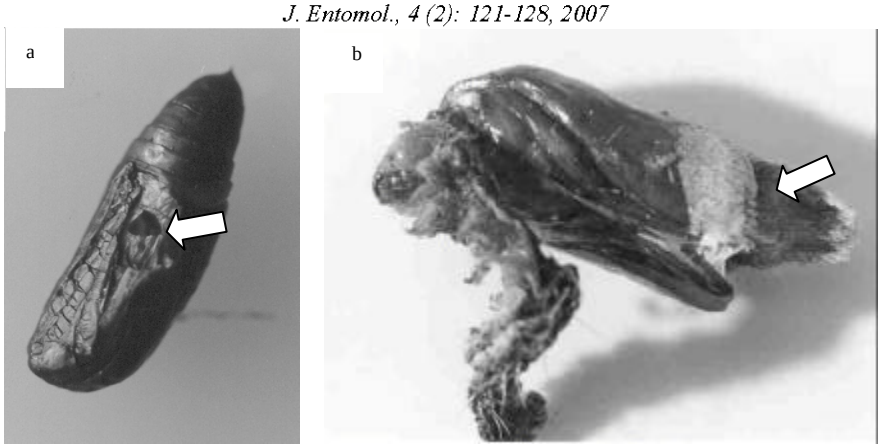

Fig. 3: Chrysalis mortalities (a): Mortality per desiccation: presence of nude zones causing chrysalis desiccation (b): Mortality per emergence impossibility: The presence of old cuticle on the former part of chrysalis perturb their emergence

Chronical Toxicity

Effect on Larval Alimentation

A reduction in the weight of the caterpillars and a delay of the larval stages was observed for larva consuming *Cestrum* leaves powder. The reduction of weight increases for treated individuals and this is probably due to its antifeeding activity, this appears from the results of measuring of consumed diet ant larval excrements. We constates a significant reduction of larvae weight, of consumed diet and of rejected excrements (Table 1).

The majority of the caterpillars of *Spodoptera* nourished on a medium containing 32% of *Cestrum* powder present a blocking of development at the level of the stage L_3 or L_4 This blocking can last for two months without the caterpillars not dying, given on a medium witnesses, these caterpillars take again their development.

Impact on Chrysalis Weight

The chrysalis of *Spodoptera* treated with a diet containing 32% of CDE have a blade colour, a reduced size and especially are totally nonviable. For this reason we choose the concentration of 16% to study the effect of *Cestrum parqui* on some biological parameters of *Spodoptera*.

The analysis of Table 2 shows a significant difference in the size of the control chrysalis compared to those obtained on a medium containing 16% of extract. These results underline the possibility that the reduction of the size can be only the consequence of insufficient alimentation caused by anti-feedancy (Chaieb *et al*., 2001).

The Fig. 4 shows a sensible reduction in the size of chrysalis coming from larvae treated with 16% of *Cestrum* dry extract, theses lasts are less voluminous and less coloured than control ones.

Effect on Adult

The size of the adnlts is strongly related to the weight of the chrysalis. Thus Table 2 shows a depressive effect of *Cestrum* on the scale of the adnlts resulting from larvae treated with 16% of dry extract. The significant difference of the size of the adults will be reflected obviously on the biotic potential of the butterfly.

The enumeration of eggs in the ovaries of the females of *Spodoptera* after dissection shows a significant reduction in the number of eggs per female (Table 2). It is probably due to direct toxic action on the ovaries or to a reduction in the resources consequence of weak weight of the chrysalis which give smaller butterflies with reduced biotic potential.

Table 1: Effect of diet CDE addition on larval weight, consumed diet and rejected excrements of *Spodoptera littoralis*

% of added dry extract	Larva weight (g)	Consumed diet weight/larva/day (g)	Excrement/ larva/day (g)
Control	0.55±0.06[a]	0.51±0.06[a]	0.32±0.05[a]
2	0.52±0.14[a]	0.37±0.12[b]	0.22±0.07[b]
4	0.36±0.03[b]	0.24±0.04[c]	0.10±0.03[c]
8	0.36±0.12[b]	0.21±0.08[c]	0.07±0.04[c]
16	0.12±0.04[c]	0.11±0.06[d]	0.01±0.01[d]
32	0.03±0.01[c]	0.08±0.03[d]	0.003±0.002[d]

The values with same letter (a, b, c…) are not significatively different in a Duncan multiple range test

Table 2: Effect of 16% diet CDE addition on chrysalis weight, mouth spam and reproductive potential of *Spodoptera littoralis*

	Non treated	Treated (16%)
Chrysalis weight (g)	0.31±0.02	0.24±0.03**
Mouth spam (mm)	32.30±3.20	29.95±2.76*
Number of eggs by femal	205.00±30.28	181.00±17.29*

(*) means that value is significantly different from control at p<5% (**) means that value is signicatively different from control at p<1%

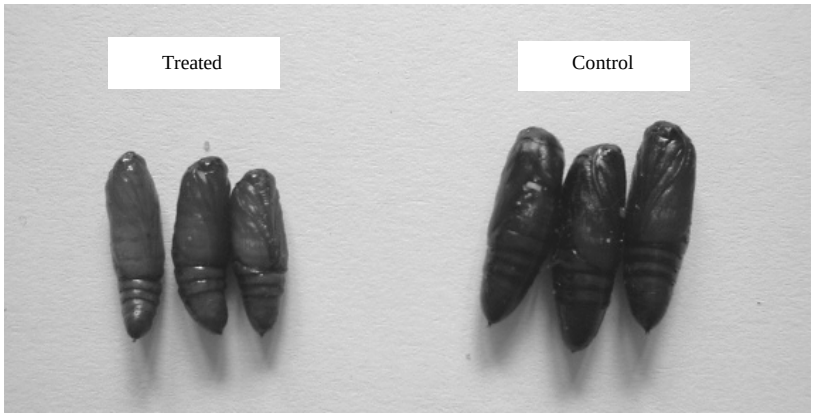

Fig. 4: Effect of 16% CDE diet addition on *Spodoptera littoralis* chrysalis morphology

Sponic Toxicity of the Extract

The addition of the CSE in the artificial medium of *Spdoptera littoralis* with a concentration of 1% cause the same types of toxicity seen below. The caterpillars present mortalities provoked by exuviation difficulties, the chrysalis shows a malformations increasing and reduction of reproductive potential of the treated individuals. Saponins are known for their affinity with cholesterol, their interaction with this substance causes its deficit. Cholesterol being raw material for the manufacture of the hormone of moult (the ecdysone). A deficit of this hormone would undoubtedly explain all the disturbances observed in the mechanisms of molt.

DISCUSSION

The larvae of *Ostrinia nubilalis* consuming of the saponins extracted from the leaves of alfalfa see their larval stages lengthening (Nozzolillo *et al*., 1997). Purified saponins of the alfalfa involve the larval lengthening of the stages, the lengthening of time necessary to reach the maximum size of the last larval stage, the lengthening of the interval separating the maximum growth and the nymphal moult and the lengthening of time necessary for the emergence of adults (Adel *et al*., 2000).

The larvae of *Culex fatigans* consuming saponins have more pronounced pigmentation, a deterioration of the shape of the head and abdomen (Tabassum *et al*., 1993).

The larvae of *Acrolepiosis assectella* nourished on extracts of seeds and flowers of *Allium porrum* present ecdysial disturbances, which often finish by characteristic malformations: larvae with double head (Arnault and Mauchamp, 1987; Harmatha, 2000). The extraction and the purification of the active substances of this plant show that one deals with a steroidic, spirostanic saponin with 4 sugars in C_3 called Aginoside 1 (Harmatha *et al*., 1987). The commercial digitonine (steroidic saponin) built-in the artificial medium of these same larvae, produces the same symptoms (Arnault and Mauchamp, 1985).

In the same way the addition of various preparations of Leguminous plant saponins in the artificial diet of *Collosobruchus chinensis* can limit the development of the larvae and cause a reduction in the rate of adults emergence (Weissenberg *et al*., 1998).

The pulverization of the leaves of the plants by 0.1 to 0.2% of saponins of alfalfa allows the reduction of the number of acarian (*Tetranychus urticae)* and of aphids (*Pharodon humulus)* respectively of 85 and 90% saponins of this plant can also cause mortalities on eggs of *T. urticae* (Oleszek *et al*., 1999; Puszakar *et al*., 1994). Saponins of alfalfa in the food of *Ostrinia nubilalis* cause larval mortalities going up to 100% for the young larval stages. These mortalities also touch the nymphal stage, ouly 60% of the treated chrysalis emerge (Nozzolillo *et al*., 1997). Treated by 100 ppm of leaves saponin, *Spodoptera littoralis* shows a cumulative mortality of 90% at the larval and nymphal stages (Adel *et al*., 2000). One attends in the same insect various forms of chronic toxicity like a reduction in the fertility of the females and rate of blossoming of eggs (Adel *et al*., 2000). The saponins extracted from the leaves and the roots of the same plant are toxic for the larvae of *Leptinotarsa decemlineata* (Szczepanik *et al*., 2001).

The addition of Aginoside 1 (steroidic saponin) in the artificial semi food of the larvae of *A. assectella* at a rate of 0.9 mg g^{-1} involves 56% of mortality in this insect (Harmatha *et al*., 1987).

The commercial saponins extracted from *Quillaja saponaria* have a larvicidal activity against the larvae of mosquito of two species *Aedes aegypti* and *Culex pipiens* 100% of mortality are obtained by using amounts of 1000 mg L^{-1} during 5 h (Pelah *et al*., 2002).

The *Cestrum parqui* represents a high potential for isolating natural insecticide molecules, more studies are necessary to identify the active product and to explore it's physiological mode of action.

CONCLUSIONS

The Toxicity of *Cestrum parqui* for Studied Species of Noctuidae Presents Two Forms

An acute toxicity which involves mortality at more the share of the stages of development of the insects. A larval mortality caused especially by difficulties of exuviation, a mortality due to the prolonged blocking of the development, or a larval mortality due apparently to anti-feedancy.

Mortality nymphale related to malformations is caused by the difficulties in the achievement of the nymphal moult. These mortalities can occur at the beginning of the nymphal stadium (mortality by desiccation) or at its end (mortality by impossibility of emergence of the mouth).

Malformations occurring in the adults result in their death in the few hours which follow emergence. These malformations are very rare to be put on the account of Cestrum.

Toxicity can be also chronic, it can touch the various biological aspects of the insect. The incorporation of the dry extract of Cestrum in the food of *Spodoptera* causes a larval lengthening of the stages until blocking, reductions in the weight of the chrysales, scale of the adults and number of eggs in the ovaries of the females. All these chronic effects make it possible to reduce the devastating potential of the species.

Saponins of *Cestrum* can have a growth regulator effect by having an affinity with cholesterol, this last being used to synthesize the ecdysone. Work aiming to the demonstration of this assumption is in hand.

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