Journal of Entomology

ISSN 1812-5670

Academic Journals Inc.

www.academicjournals.com

Journal of Entomology 4 (2): 113-120, 2007
ISSN 1812-5670

Toxicity Experiments of the Saponic Extract of *Cestrum Parqui* (*Solanaceae*) on Some Insect Spices

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Abstract: The goal of present study is to undertake various experiments to explore this entomotoxic activity of this extract on various harmful insects in order to determine the best mode of application of these natural biopesticides. Several insects were tested in these experiments: Two phytophagous insects: desert locust (*Schistocerca gregaria*) and the cotton noctuid (*Spodoptera littoralis*), a stored products devastating insect (*Tribolium confusum*) and a health harmful hematophagous insect (*Culex pipiens*). Several biological toxicity assays were elaborate according to the species and the stage used. Experiments utilizing injection, grafting, forced ingestions, experiments of contact toxicity or toxicity by incorporation to the food substrate were employed. All these experiments enabled us to conclude that the application by contact of the CSE is generally ineffective because of the impossibility of passage of saponins (hydrophilic molecules) through the cuticle of the insect. The saponic extract must be introduced by the insect to express a toxicity from where the idea to design toxic soft foods based on extract saponic of *Cestrum*.

Key words: *Cestrum parqui*, crude saponic extract, toxicity tests, insects

INTRODUCTION

Certain plants when consumed by phytophagous insects react by secreting defences substances such saponins. These substances are heterosidic molecules studied in several works for their insecticidal activity (Oleszek *et al*., 1999; Puszakar *et al*., 1994; Adel *et al*., 2000; Harmatha *et al*., 1987). Among rich saponins plants we find *Cestrum parqui* a decorative shrub which we showed its toxicity for certain insects (Ammar *et al*., 1995; Chaieb *et al*., 2001). Barbouche *et al*. (2001) showed that the active fraction of this plant is the Crude Saponic Extract (CSE), this extract was tested by the same author on the desert locust (*Schistocerca gregaria*) by injection.

The goal of present research being to construct a whole of experiment of toxicity of this active extract on various harmful insects by using various techniques of biological tests, be experiments aim at determining the means possible of application of the extract in question and on the manner with which these substances act. For that various kinds of treatment are carried out applications by contact, ingestion or artificial incorporation of substances to the body of the animal.

MATERIALS AND METHODS

Saponins Extraction

The saponin extraction is made like is described by Barbouche *et al*. (2001). The leaves of *C. parqui* obtained from the garden of National Tunisian Agronomic Institute (INAT) are dried in a steamroom at 40°C during 4 days, the dried leaves are finely grounded. One hundered grams of the

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powder washed with petrol 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 (Crude Saponic Extract). Experiments were realized in the Entomological Laboratory of the High school of Horticulture (Tunisia) in 2004.

Animal Rearing

Schistocerca gregaria

The insect eggs are brought from a breeding of desert locust *Schistocerca gregaria* at the gregarious state maintained in the insects physiology and physiopathology laboratory of INAT. This breeding is maintained since the last great invasion of the locust in 1988-1989. The adults and the larvae undergoing of the experiments are maintained in laboratory conditions.

Spodoptera littoralis

L_5 larvae were obtained from a rearing maintained at the Entomological Laboratory of the High School of Horticulture (Chott Mriem; Tunisia). The caterpillars kept individually in petri disches and fed on simplified artificial substrate according to the formula of Poitout and Bues (1974). Larvae are reared in culture rooms under a temperature of 25°C, with a relative humidity of 70% and 8 h photoperiod of illumination.

Tribolium confusum

The adults of *Tribolium* were also obtained from a rearing maintained at the Entomological Laboratory of the high school of horticulture. Raising was made in 10×30×10 cm plexiglass limps containing 500 g of semolina and wheat bran. In a conditioned temperature room under 30°C in total obscurity.

Culex pipiens

The L_3 and L_4 larvae are collected from larval lodgings area in Chott Mariem (Tunisia), these larvae are sorted according to their stage in different cups and maintained in water distilled in condition of laboratory until the beginning of experiments.

Artificial Introduction of Substances into the Body of the Insect

Injection Assays

The desert locust is an insect which supports the biological tests particularly utilizing injections. That's why we must provide a syringe being able to manage small volumes. we can use either Hamilton micro-syringes or insulin syringes. In all the cases in this kind of experiments we should not exceed 20 μL of volume injected.

The needle of the syringe is introduced usually between the 2nd and the 3rd tergites of the ventral abdominal segments. The needle is introduced paralleling with the cuticule to not touch the internal bodies. The needle is introduced with a depth of 3 to 5 mm into the direction of the Thorax, the injection is done on the level of the first abdominal segment (Fig. 1).

The injections can also be carried out on larvae of *S. littoralis* we generally use the 4th or the 5th caterpillar stage. This spice remains sensitive to this kind of experiment considering the significant loss of hemolymph, due to the frequent contractions observed during handling.

Forced Ingestions Assay

These tests consist in an artificially introduction of an aqueous solution of the extract to be tested in the digestive system of the insect. An insulin syringe provided with a point on which we fixed a 10 cm length and 0.5 mm of diameter catheter, the catheter being stretched at its end.

The catheter is introduced into the oral part of the insect until reaching the oesophagus, the substance is thus injected (Fig. 2). we can inject relatively significant quantities (50 μ L). The same experiments are carried out on larvae L_5 of *S. littorlis*.

Grafting Assay

This operation consists in introducing the CSE in solid form under the cuticle of the insect, this technique is practised when we cannot dilute the substance in question in water, since the organic solvents are toxic for the insect.

Male adults of *S. gregaria* are incised laterally on the level of the 3rd abdominal segment. The incision is done all along the segment (3 mm approximately). We raises the cuticle and we insert a crystal of CSE of 1 mm of diameter in the incision, this crystal is deposited 3 to 5 mm upstream of the wound (Fig. 3). A paraffin drop is deposited on the level of the incision, to obliterate the wound and to thus prevent the loss of the hemolymph. The experiment is generally done on at least 5 individuals and the control group are only incised as described before.

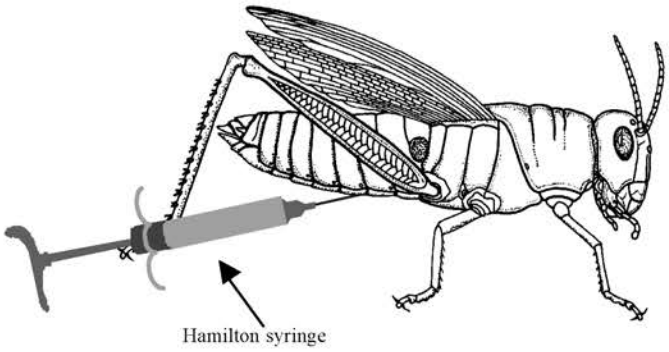

Fig. 1: Injection assay on *Schistocerca gregaria*

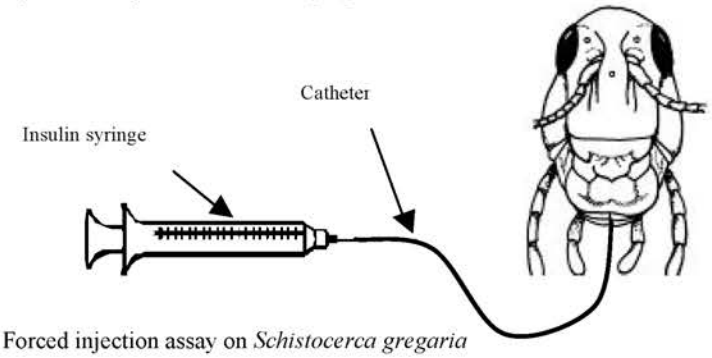

Fig. 2: Forced injection assay on *Schistocerca gregaria*

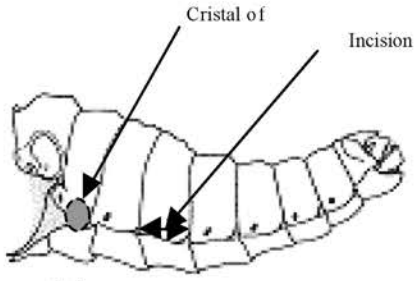

Fig. 3: Grafting injection assay on *Schistocerca gregaria*

Diet Addition Toxicity Experiments

Leaves Discs Assay

The substances to be tested are dissolved in an aqueous solution or another solvent. Discs 2 cm of diameter are cut out in cabbage leaves. These discs are soaked and rubbed in the solution containing CSE. These discs are rubbed to allow the elimination of the wax which prevents the substances from adhering to surface sheets. The control discs are rubbed in water and are dried during 1 to 2 h with the free air. The discs are weighed then deposited in limp of plexiglass of 5×10×15 cm. *Shistocerca* L_5 larvae are introduced individually and mortalities are controlled after 24 h.

A similar technique is used for the caterpillars of *S. littoralis* but we use a *Ricinus communis* leave discs treated in the same way that previously. In this case we measure the surface consumed using a planimeter, or using a graduated transparent plate. L_4 or newly molted L_5 Caterpillars are generally used.

Artificial Diet Incorporation Assay

This technique is generally applied for the larvae of *Spodoptera*. The extract tested can be mixed with the artificial diet of Poitout (Poitout and Bues, 1974). Mortalities was observed every 48 h.

In the case of *Tribolium* we treat the food of the insect consisted corn semolina by a solution containing the extract tested dissolved in methanol (Fig. 5). The solvent is then dried during 24h under the environmental conditions. Five larvae a length from 2 to 3 mm are introduced into one gram of semolina. Control group will have semolina treat only by solvent.

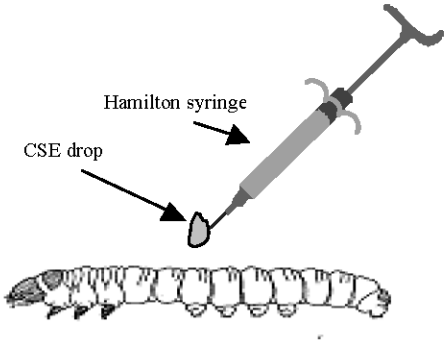

Fig. 4: Topic application assay on *Spodoptera littoralis*

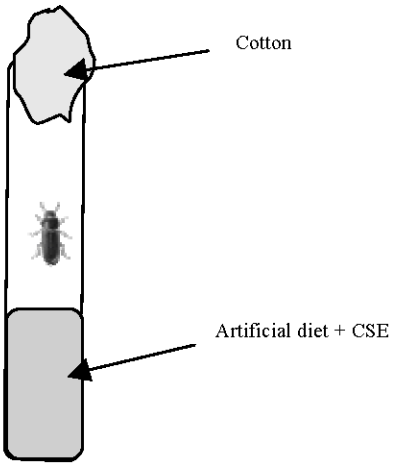

Fig. 5: Diet treatment assay on *Tribolium confusum*

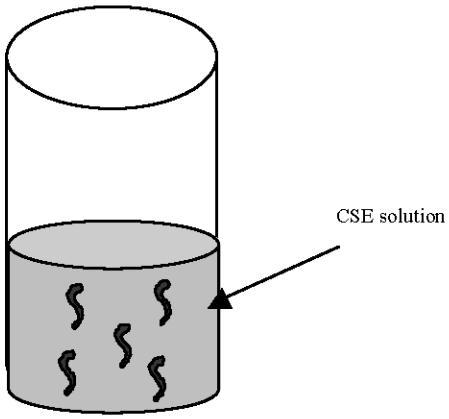

Fig. 6: Life environment treatment assay on *Culex pipien*

Contact Toxicity

Topics Application Toxicity Assay

The extract tested is deposited either in the form of powder or of aqueous solution on the insects cuticle. This is practised on the larvae and the adults of *Schistocerca* or on the larvae of *Spodoptera* (Fig. 4). For the larvae and the adults of *Tribolium* the test was carried out by depositing larvae or adults in Petri dishes papered of Watman paper soaked with a 2% CSE solution, possible mortalities are noted after 24 h.

Treatment of the Live Environment Assay

This test was realized on *Culex pipiens* L_3 larvae, different amounts of CSE in geometric progression (31, 2; 62,5; 125; 250; 500 ppm) are obtained on the basis of an aqueous solution of 1000 ppm, these different concentrations are applied to 80 L_3 *Culex pipiens* larvae for each amount, divided into four groups of 20 larvae in 30 mL of solution each one (Fig. 6). Control group is maintained in 30 mL of distilled water. Observation of mortalities is carried out after 24 h.

RESULTS

Artificial Introduction of Substances into the Body of the Insect

The saponic extract is tested with the concentration of 5 mg mL^{-1} on imagos of *Schistocerca gregaria* and on L_5 larvae of *Spodoptera littoralis*. Batches of 5 individuals of each species received an injection by oral way (forced ingestion) or in the hemocoel of 1, 5, 10 and 20 μ L of this extract (Table 1). Significant mortalities were thus noted for the two species a few hours after the injection in particular for the concentration of 20 μ L. Nevertheless, in all the cases, death occurs within a time which hardly exceeds 48 h.

The larvae of *Spodoptera* seem to support better the dose of 1 and 5 μ L managed by oral way, undoubtedly because of a possible decomposition of saponins on the level of their foregut.

Tropic Application Toxicity

The topics application of saponins on 5 *Schistocerca* imagos and 5 L_5 *Spodoptera* larvae does not give any result, even with strong amounts (5%). That is undoubtedly due to the impossibility of passage of saponins through the cuticle. The same results are obtained with the addition of detergents or organic solvent (methanol). Only the application of saponins to strong amount on artificially caused wounds could result in the death of the insect.

Table 1: Mortalities of *Schistocerca* adults and L_5 *Spodoptera* larvae in injection, grafting and forced ingestion tests

Assay	Dose (µL)	*Spodoptera*	*Schistocerca*
Injection	1	3/5	2/5
	5	5/5	5/5
	10	5/5	5/5
	20	5/5	5/5
Forced ingection	1	0/5	2/5
	5	0/5	4/5
	10	2/5	5/5
	20	5/5	5/5
Grafting	5 mg/insect	Not tested	5/5

Table 2: Mortalities of *Spodoptera littoralis* larvae nourished with artificial diet supplemented with 10 and 20 mg g^{-1} of CSE

Assay	10 mg g^{-1}	20 mg g^{-1}
L_1	10/10	10/10
L_3	2/10	5/10

Table 3: Mortalities of *Tribolium confusum* larvae nourished with artificial diet supplemented with 1, 10 and 100 mg g^{-1} of CSE

	Control	1 mg g^{-1}	10 mg g^{-1}	100 mg g^{-1}
Mortality	2/20	7/20	18/20	20/20

Table 4: Mortalities of *Culex pipiens* larvae treated with CSE different rates

Dose (ppm)	% of mortalities
1000	100.00%
500	88.75%
250	75.00%
125	58.75%
62.5	36.25%
31.2	13.75%
Control	1.25%

Ingestion Toxicity

The evaluation of the acceptability of CSE incorporated in the food would make it possible to conceive a method of control of Noctuid pest by means of poisoned soft foods. Our tests using the incorporation of saponins with of 10 and 20 mg g^{-1} doses in artificial medium of two batches of 10 L_1 larvae and L_3 10 larvae per dose. The results obtained are presented in Table 2. This reveals that the saponin powder added with the food provoke 100% mortality with the L_1 caterpillars stage (10 mg g^{-1}) and with less degrees for the L_3 larvae (50% with 20 mg g^{-1}).

Tests are carried out on larvae L_1 the large majority of mortalities intervene at the stages L_3 and L_4. Toxicity appears in insects by softening of their bodies and a total loss of their mobility.

The results of incorporation of CSE in the diet of *Tribolium confusum* show that the strong rates of 100 mg g^{-1} cause a total mortality of the larvae (Table 3). Few control mortalities are probably due to an intra specific antagonism.

Toxicity by Treatment of the Live Environment

The results (Table 4) show a very strong toxicity of *Cestrum* saponins on *Culex pipiens* since first hours following the treatment. Indeed the mobility of the larvae decreases considerably and their movement becomes incoherent. They end up falling at the bottom of the container which cannot thus more go up on the surface to breathe. Mortalities increase following the CSE concentrations.

DISCUSSION

All experiments which we realized to determine the toxicity of CSE on the various species of insects are summarized in (Table 5).

Table 5: Summary of the toxicity experiment results of the CSE on various insect species

	Insect spices			
Assay	*S. gregaria*	*S. littoralis*	*T. confusum*	*C. pipiens*
Injection	(+)	(+)	(nt)	(nt)
Forced ingestion	(+)	(+)	(nt)	(nt)
Grafting	(+)	(nt)	(nt)	(nt)
Artificial diet incorporation	(nt)	(+)	(+)	(nt)
Treated leave disks	(-)	(-)	(nt)	(nt)
Topic application	(-)	(-)	(-)	(nt)
Environment treatment	(nt)	(nt)	(nt)	(+)

(+): active; (-): not active; (nt): not tested

In all results we notice that the saponic extract is almost active when applied by artificial introduction into the body of the insect. This type of technique is not applied in practice to control insects but realized only in laboratory to study the mode of action and the effectiveness of insecticidal substances. The same results are obtained by using the crude saponic extract of *Cestrum parqui* injected to L_5 *Schistocerca gregaria* larvae involves the mortality of these insects (Barbouche, 2001).

The toxicity by ingestion of the CSE is notable when the insect is treated with a sufficient quantity of extract, the same result occurs when incorporation extract in the diet of *Spodoptera* or *Tribolium*, on the other hand the treatment of the leaves does not seem to have any effect on the insects tested, which contrary with works previously carried out. Indeed the pulverization of the sheets of the plants by 0.1 to 0.2% of saponins of alfalfa allows the reduction of the number of *Tetranychus urticae* and of *Pharodon hop* respectively of 85 and 90%. Saponins of this plant can also cause eggs mortalities 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, only 60% of the treated chrysalis emerges (Nozzolillo *et al*., 1997). Treated by 100 ppm o leaves saponin, the cotton leave noctuid shows a cumulative mortality of 90% at the larval and nymphal stages (Adel *et al*., 2000). We attends in the same insect various forms of chronic toxicity like a reduction in the fruitfulness 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 semi artificial 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 treatment of the medium of life in the case of the larvae of mosquito to show an important toxicity, this was also observed in the case of the commercial saponins extracted from *Quillaja saponaria* which have a larvicide activity against the larvae of mosquito of two species *Aedes aegypti* and *Culex pipiens*. Hundred percent of mortality are obtained by using amounts of 1000 mg^{-1} during 5 days (Pelah *et al*., 2002).

CONCLUSIONS

Saponins of *Cestrum parqui* offer a strong pesticide potential thanks to their activity against several harmful insects, but in spite of the multitude of the results observed in experiments in laboratory, the practical application of saponins as pesticide remains problematic. Saponins of *Cestrum* cannot cross the cuticle of the insects, for applied it absolutely that they enter the body of the insect, which is possible only by ingestion.

Despite everything these problems of order practises saponins present an excellent model of study of natural substances for their pesticide effect thanks to the extent of their spectrum of action

and at the multitude of their physiological effects. It is however too early of speaking about application of saponins like biopesticides. Thorough studies relating to their mode of action remain to be undertaken; our results relating to this aspect are encouraging and deserve to be continued.

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