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Pesticidal Potentialities of *Cestrum parqui* Saponins

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Abstract: *Cestrum parqui* is a shrub used in Tunisia as ornamental plant. The insecticidal activity of the leaves of this plant was demonstrated the first time on desert locust (*Schistocerca gregaria*); this toxicity comes from the saponic fraction of the plant. The aim of present research is to explore the pesticidal toxicity of *Cestrum parqui* saponins on some plant pest organisms like insects, fungus, mollusks and nematodes. The results show that saponins are toxic to some other insects like *Spodoptera littoralis* and *Tribolium confusum*, this toxicity is probably due to cytotoxic activity of *Cestum parqui* saponins, this was demonstrated histologically on the fat body and the gut of *Schistocerca gregaria* and *Spodoptera littoralis*. This toxicity can be also the result of interference with ecdysone metabolism by the obstruction with dietary cholesterol; this hypothesis was proved biologically on *Tribolium confusum*. The molluscicidal activity of *Cestrum parqui* saponins was observed on *Theba pisana* snails. Our investigation on the mode of action of these substances shows that saponins interfere with the water retention mechanisms witch causes mortality by dehydratation. Saponins are especially toxic for the eggs of *Meloidogyne incognita* nematode. This was illustrated *in vitro* experiments and confirmed *in vivo* assays on tomato plants infected with the nematode pest. *Cestrum parqui* saponins are, on the other hand, totally ineffective against phytopathogenic fungi (*Fusarium solani*, *Botrytis cinerea*). This is probably due to the secretion of detoxifying enzymes. *Cestrum parqui* represents a high potential for isolating natural pesticide molecules. More studies are necessary to purify and identify pesticidal products from *Cestrum parqui* saponic crude extract and to confirm physiological action of these compounds.

Key words: *Cestrum parqui*, saponins, insecticidal, nematicidal, molluscicidal, fungitoxic

Introduction

To circumvent the problems of human and animal health, pollution and disturbance of ecological balances generated by the massive use of synthesis pesticides, other alternative methods of pest management are developed. The use of bio-pesticides of plant origin is one of them. These pesticides have the advantage of being less phytotoxic and more degradable in the environment (Cooping and Menn, 2000). Research in the field of pesticide from plant origin offers a very promising ground and especially going in the wake of the new concepts of sustainable development and the protection of natural resources.

The toxic activity of *Cestrum parqui* was demonstrated the first time on desert locust (*Schistocerca gregaria*) (Ammar *et al*., 1995). This toxicity was also remarked on other insects like *Spodoptera littoralis*; *Helicoverpa armigera*; *Pieris brassicae* (Chaieb *et al*., 2001; 2004). Barbouche *et al*. (2001) have established that the toxicity of *Cestrum parqui* comes from the Crude

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Saponic Extract (CSE). The saponins are heterosid molecules well known for their role in plant defense mechanisms. The aim of this study is to explore this natural phenomenon and to use saponins as plant pest control product. In this research we try to make different sorts of biological tests in the objective to study the effect of these substances on several plant pests.

Materials and Methods

Extraction of Saponins

The saponin extraction is made like is described by Barbouche *et al*. (2001). The leaves of *C. parq*ui obtained from the garden of National Tunisian Agronomic Institute (INAT) are dried in a steam room at 40°C during 4 days, the dried leaves are finely grounded. One hundred 8 grams of the 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).

Animal Rearing and Biological Tests

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.

Insecticidal tests are carried out using injection and forced ingestion experiments; a saponic solution is injected (5 to 20 μL) with Hamilton syringe under the cuticle of the insects. The injection can also be done on the esophagi of the animals and this constitutes a forced ingestion experiment. Mortality is controlled after 24 h.

Spodoptera Littoralis

L5 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.

Experiments on *Spodoptera* are done like those practiced on *Schistocerca*, injection and forced ingestion experiments are employed.

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.

A saponic solution was mixed with semolina (1 mL g^{-1} of semolina), this diet was dried in a steam room. A 2 mm length larvae were deposit on this diet in the same conditions, mortality was controlled when all leaving larvae reached the nymphal stage.

Meloidogyne Incognita

The nematode eggs of *M. incognita* were collected during the period of May-June, on roots of pimento obtained from the plantations under greenhouse among agriculturists of the region of the Sahel (Tunisia). The eggs masses are cleared from the roots with the help of a fine tweezers and conserved in a salted solution until use.

Egg masses are incubated in laboratory conditions in different concentrations of CSE solution. Dead eggs are counted after 48 and 96 h under photonic microscope after coloration with Meldolat blue (a dye coloring dead cells).

Theba Pisana

This snail is collected on some wild plants like *Lycium arabicum* and *Retama retam* around agricultural parcels in Chott Mariem (Tunisia). Animals are washed with current water and then dried on filter paper. The animals are put in 10 cm diameter plexiglass limps, under fixed temperature (27°C) and photoperiod (16/8 h light). These molluscs are fed on wheat bran moistened with water or on cabbage leaves. A period of one week is necessary before beginning tests to eliminate dipteral parasitic molluscs.

Homogenous size snails where put upside down and undergo an application of 5mg of powder or a crystal of CSE on the obvious part of their bodies (the foot). Mortality is controlled after 24 h.

Fungi Species

The fungi strains used are maintained at the phytopathological laboratory of the High school of Horticulture. Strains are conserved in petri dishes containing PDA medium, these last are inoculated with these purified strains and preserved at +4°C, subcultures are realized regularly.

On a PDA medium, inoculums are deposits (disks of 6 mm of diameter). The substance to be tested is diluted in suitable solvent and is deposited on a filter paper disc (8 mm of diameter). After drying under the hood, the disc is deposited close to the inoculum the whole is put in culture in a drying oven at 22°C during 48 h, a control disc is soaked with the solvent. In the case of fungitoxic activity we have a zone of inhibition which appears around the filter paper.

Results

Insecticidal Tests

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

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

Dissection of treated animal reveals a necrosis of the tissues closer the zone of injection. Histological investigation of these zones demonstrates a cytotoxic activity on the fat body and digestive system (results not shooed).

Effect of Cholesterol Addition on Insecticidal Activity

Larvae of *Tribolium confusum* are separated in three batches of 20 larvae, a first control batch is nourished on corn semolina; the second batch receives the same food added with 10 mg g^{-1} of CSE and

Table 1: Mortality of *Schistocerca* imagos and L_5 *Spodoptera* larvae in injection and forced ingestion experiments

Parameters	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 ingestion	1	0/5	2/5
	5	0/5	4/5
	10	2/5	5/5
	20	5/5	5/5

Table 2: Mortality rate of *Meloidogyne* eggs treated with CSE *in vitro* assay

Treatments	24 h	96 h
Control	77.86±10.61^{a}	170.43±57.56^{a}
2 mg mL^{-1}	47.71±15.10^{a}	115.10±20.93^{b}
4 mg mL^{-1}	44.00±13.71^{b}	85.86±22.02^{bc}
8 mg mL^{-1}	34.71±10.44^{b}	71.29±19.79^{c}

Data with same letter have not significant difference (Duncan)

Table 3: Weight and weight rate loss of saponin treated *Theba pisana* snails

Parameters	Little size	Big size
Loss weight (g)	0.428±0.06	0.396±0.11 (ns)
% of loosed weight	35.9±7.5%	14.2±4.6% (s)

s: Means significant difference with little size data; ns: Not significant difference with little size data

the third batch consumes the same food as the second added with cholesterol 10 mg g^{-1}. this cholesterol was added to demonstrate our hypothesis concerning the interaction of saponin with dietary cholesterol.

Control is carried out at the end of 80 h by counting survival insects at the imago stage. The results shows a mortality of 3 adults for 20 tested in control patch, 19 mortality for 20 tested in CSE treated group and only 9 mortality on 20 for CSE and cholesterol treated group (Table 2).

We noticed that cholesterol decreases the toxicity of saponins. Indeed, cholesterol improves, clearly, the number of individuals leading at the imago stage at *Tribolium confusum* nourished on semolina added with CSE.

Nematicidal Tests

Egg masses of *Meloidogyne incognita* are placed in dishes containing saponin solutions with 2, 4 and 8 mg mL^{-1}. The control egg masses are placed in distilled water. Seven repetitions of 10 masses each one are used for each treatment. The hatched nematodes are then counted under magnifying binocular after 24 h and 48 h of incubation. The results expressed by the Table 3 show that:

- Saponins inhibit the hatching of *M.incognita*
- This inhibition is dose dependent

In order to verify if this inhibition is due to a delay of hatching of eggs or to a lethal action of saponins on those eggs, we concept the following experiment: egg masses having remained 48 h in a saponic solution with 8 mg mL^{-1} was washed then placed in distilled water. These eggs loss totally and definitively their ability to hatch, this means that saponin kills eggs and not delay their hatching.

Molluscicidal Activity

Molluscs undergoing an application of 5 mg of CSE on the obvious part of their bodies begin an instantaneous defense reaction against these substances. The animal rejects its mucus and its

excrements, it doesn't even try to flee and retract in its shell. The animal body shrinks considerably and the dissection after 24 to 48 h reveals the death of the animal.

The snails losses much mucus and this can be the reason of their mortality by dehydratation. The excessive excretion of mucus can be a defense method of animals against toxic substances, this same phenomena is observed when using the principal mollusiscidal commercial molecule: metaldehyde.

We notice that the toxicity of saponins is more important in the case of snails having little size, to explore this phenomenon we concept another experience.

We included the first experiment on two snail batches, ones have a small size (weighing 1.3 g on average) and the others have big size (weighing 2.8 g on average).These snails received a dose of 5 mg crystal deposited on their foot. We carried out the evaluation of the consecutive mucus loss due to the treatment.

These results (Table 3) shows that the same quantity of mucus (0.4 g on average) was lost as well for the small as for large snails ones. No significant difference was detected in the quantity of lost mucus. Thus It is followed from there that the small snails lost 35.9% of their weight whereas the large ones lost only 14.2% of them and this difference is highly significant.

It is probable that the excessive loss of mucus provokes the death of the animal. This is not possible even if this loss reaches a critical level. Also it is obvious that the mortality of the treated individuals is correlated with the rate of lost liquid compared to the weight of the animal.

Fungitoxic Activity

It seems that the synthesis of saponin by the plant is done exclusively to defend itself against certain phytopathogenic fungi, this led us to test their fungitoxic activity. The test carried out concerns the development inhibiting activity for two phytopathogenic species: *Fusarium solani* and *Botrytis cinerea.*

On a PDA medium a disc of medium containing an inoculum is deposited. Around this inoculum we deposits filter paper discs treated by 25 μL methanol solution containing 0, 1, 10 and 100 mg mL^{-1} of *Cestrum* saponin methanol is evaporated before deposit.

After 48 h of incubation no zone of inhibition around the discs was observed, saponins do not seem to have any activity on these two fungi.

Discussion

Saponins are substances known for their toxicity to insects, Many works demonstrate that saponins of several plants could perturb the insects physiology. 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). In addition the alfalfa Saponins added in the food of *Ostrinia nubilalis* cause larval mortalities going up to 100% for the young larval stages. Moreover these mortalities touch the nymphal stage, only 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). For the same insect various forms of chronic toxicity like a reduction in the fertility of the females and rate of eggs hatching are noted (Adel *et al*., 2000). Saponins extracted from the leaves and the roots of the same plant are toxic for the *Leptinotarsa decemlineata* larvae (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 for this insect (Harmatha *et al*., 1987).

The molluscucide activity of saponins of *Cestrum* was noted on a phytophagous mollusc. This activity which appears by contact offers more promising possibilities than the insecticidal activity.

The majority of works concerning the molluscucide activity are generally undertaken on watery molluscs secondary vectors of human diseases. Two mollusc species are always used: *Biomphalaria glabrata* and *Bulinustruncatus* (Treyvaud *et al*., 2000; Lemmich *et al*., 1995; Bekkouche *et al*., 2000; Aklilu, 1982). Very few work related to the effect of saponins on terrestrial molluscs (Smith *et al*., 2001). In all recent work which studied these phenomena, no mechanism was proposed. In present research we proposed and discussed a mechanism of action based on the phenomena of excessive loss of mucus by gastropods treated by *Cestrum* saponins.

Several plants were studied from the point of view their toxicity to the nematodes, indeed certain plants secretes root exudates which inhibit the hatching of the nematode eggs, poison their larvae and have repulsive or masculinisant properties against these parasites (Djian-Caporalino *et al*., 2002). Several works undertook on extracts or substances obtained from plants against certain nematodes, usually experiments are carried out *in vitro* (Oka *et al*., 2000). No work was made on nematicidal saponins, but certain plants known for their nematicidal effects are naturally rich in saponins, like the *Trigonella foenum graecum* (Zia *et al*., 2001).

The majority of works on the biological activities of saponins cover primarily the fungitoxic activity. It seems that this one is their principal function in the vegetal world (Oleszek *et al*., 1999). It is probable, once again, that this activity is explained by the interference of saponins with membrane cholesterol, causing a formation of the complex saponin/cholesterol, which generates a phenomenon of formation of pores and cellular loss of integrity. It even seems that saponins with alcaloidic genine can not only bind to cholesterol but straightforwardly extract it from the membrane (Morrissey and Osbourn, 1999).

In spite of several works highlighting the defensive role of saponins against the mushrooms phytopathogenes (Morrissey and Osbourn 1999), our saponins remain inactive against fungi tested, undoubtedly thanks to a development of a system of detoxification; certain fungi can indeed either reduce cholesterol in their membranes or synthesize detoxifying enzymes.

Conclusion

In this research we have demonstrated that *Cestrum parqui* saponins have a large action spectra. Cestrum CSE is active against several insects tested like *Schistocerca*, *Spodoptera* and *Tribolium*. These substances seem to act by interfering with cholesterol.

In addition CSE have the possibility to perturb the mucus retention of *Theba pisana* snails witch provokes the dead of these molluscs per dehydratation. *In vitro* assays permit to remark the ovicid activity of CSE against *Meloidogyne incognita* eggs.

On the other hand *Cestrum* saponins seem to have not activity against phytopathogenic fungi, *in vitro* assays shows that these substances are inefficacy against *Fusarium solani* and Botritis cinerea. Saponins present an excellent study model of natural substances at pesticide effect thanks to the extent of their spectrum of action and at the multitude of their physiological effects. However it is too early for 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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