Saponins as Insecticides: a Review

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ABSTRACT

Chaieb, I. 2010. Saponins as insecticides: a review. Tunisian Journal of Plant Protection 5: 39-50.

Saponins are heterosides (substances containing in their structure one or more sugar molecule) of plant origin. This type of molecules has an interesting pesticide potential and this review constitutes an inventory of principal researches realized in this direction. In the first part of this review, saponins are defined and their different structural families are presented. The biological significance and principal sources of saponins were also outlined. The second part of this review focused on insecticidal activities of saponins. In fact, these substances are known by their toxicity to harmful insects (anti-feeding, disturbance of the moult, growth regulation, mortality...); the insecticidal activity of saponins is due to their interaction with cholesterol, causing a disturbance of the synthesis of ecdysteroids. These substances are also protease inhibitors or cytotoxic to certain insects. The third part of the review gave an idea on the limits which can slow down the use of saponins as insecticides: saponins have a strong toxicity to mammals because of their cytotoxic and haemolytic activities. The second constraint is the loss of molecule activity due to degradation of sugars associated with the aglycone. The hydrophilic nature of saponins limits their penetration through the lipophilic insect cuticle. The structural complexity of saponins limits the exact identification and synthesis of active molecules.

Keywords: Cholesterol, insecticide, natural products, pest management, saponin, toxicity

Some substances synthesized by plants are necessary for their fundamental activities whereas others, called secondary metabolites, are involved in the process of co-evolution between plants and other organisms (10). The plant uses these secondary substances for two reasons, the first is a cooperation with other species, to attract the pollinating insects or the auxiliaries of the phytophagous insects (39) or antagonistic fungi (54); the second consists of a synthesis of dissuasive substances to resist to pest organisms such insects (65), pathogenic microorganisms (10), and competitive plants (24).

Among substances involved in plant defense, saponins which are heterosides synthesized by several plants were reported to have a defensive role which was highlighted for the first time by Appelbaum in 1969 (3). Saponins or saponosides set up a large and frequent group of heterosides in plants. Characterized by their surface-active properties, saponins dissolve in water by forming a foaming solution due to their tension-activity; hence, theses substances take their name from latin (*sapo, saponis*: soap). Saponins are used for industrial as well as for pharmacological purposes. Several saponosides are used by pharmaceutical industry for obtaining

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Accepted for publication 18 January 2010

drugs or by cosmetics industry for their detergent property (12).

In this review, our interest will be focused on use of these substances as insecticide molecules.

Saponin presentation.

Chemical structure of saponins. Saponins or saponosides are heterosides composed of two parts: a water-soluble glucidic chain and a generally triterpenic or steroïdic liposoluble structure (aglycone) (Fig. 1).

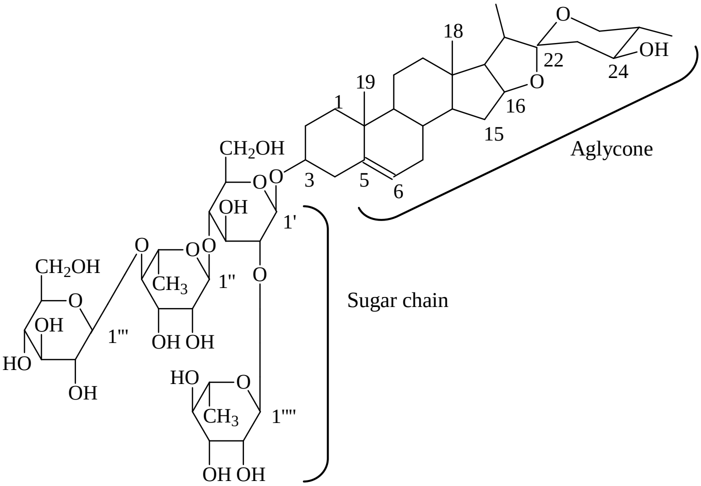
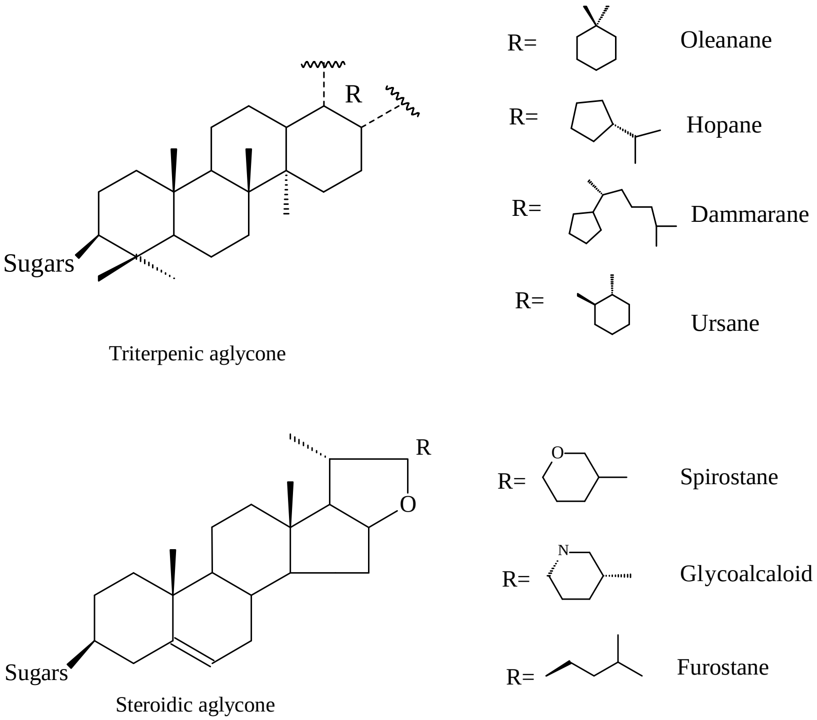


Fig. 1. Example of steroidic saponin with 4 sugar chains: Parquisoside 1 extracted from *Cestrum parqui* (7)

The sugars constitutive of the saponosides can be: D-glucose D-galactose, L-arabinose, L-rhamnose, D-xylose, D-fructose or D-glucuronic acid. Generally, the sugar part of heteroside consists of one or two linear or ramified oligosides. The molecule can include 11 sugars (but generally 3 to 5) (12).

Saponins are classified by the majority of the authors in two groups according to the nature of their aglycone (Fig. 2): (i) saponosides with steroïdic aglycone, (ii) saponosides with triterpenic aglycone. The steroidic aglycones represented in Fig. 2 have a whole skeleton with 27 carbon atoms. These molecules come from an intramolecular cetalisation which intervenes after oxidation in $C_{16,}$ C_{22} and C_{26} of a cholestanic precursor taking into account spiro-nature of C_{22}; this hexacyclic skeleton is usually indicated by the spirostane term. In fresh plants, it is not rare that hydroxyl in C_{26} is engaged in a connection with a sugar. The structure can be pentacyclic; it is called in this case furostane. Some authors include glycoalcaloides with saponins having steroïdic aglycone group (11). The glycoalcaloides have the same structure as a spirostanic steroidic aglycone, except the existence of an atom of nitrogen often on the level of the sixth cycle (12).

The triterpenic aglycones, come from the cyclization of the (3S)-2,3-epoxy-2,3-dihydrosqualene. This cyclization gives pentacyclic compounds like dammaranes, oleananes, ursanes, and hopanes. The majority of triterpenic sapogenins belong to these four basic skeletons (Fig. 2) (12).



Triterpenic aglycone

Steroidic aglycone

Fig. 2. Different possible structures of saponin aglycones (11, 12)

Origin of saponins. Several saponosides substances are extracted from *Glycyrrhiza glabra*, *Agave attenuata*, *Panax ginseng*, *Saponaria officinalis* (20), *Allium sativum* (22), *Medicago sativa* (43), and *Cestrum parqui* (18). In addition to their plant origin, saponins can be obtained from some marine animals. Some saponins are isolated from Antarctic starfish belonging to *Asteriidae* family; triterpenic saponins are also isolated from marine sponges (*Ectyoplasia ferox*) (13).

Saponins are also found in defensive secretions of certain insects. Triterpenic saponins are isolated from Chrysomelidae especially the *Platyphora* genus (41). Species of this genus sequester saponins from their plant hosts to use them for their own defense (53).

Biological significance of saponins. The various structures of saponins are involved in several biological activities with some beneficial or toxic effects. These molecules have a nonspecific but enough significant activities to control the interaction existing between plants and associated organisms (28, 37).

Several authors have already shown the defensive role of saponins. In fact, these substances protect plants from phytopathogenic microorganisms, phytophagous mammalian and insects (28, 34, 37, 42).

Moreover, saponins are known for their detergent properties, i.e. they have the possibility of forming micelles with lipids. They can also interact with cholesterol to form insoluble complexes.

The majority of the biological properties of saponins rise from these fundamental characteristics (26, 35).

Insecticidal activity of saponins.

Researches concerning the interaction between plants and phytophagous insects are multiple particularly those focused on toxicity of certain substances toward insects. This toxicity appears primarily in the three following ways.

Interference with the feeding behavior. Some saponins have antifeeding activity as is the case of saponins extracted from *Ilex apocea* which inhibit the food uptake of *Limantria dispar* (8). These saponins are antifeeding for a mite species (*Oligonichus illicis*) and for two caterpillar's species (*Hyphantria cunea* and *Malacosoma americanum*) (33, 37). Discoraceae plants shows antifeeding activity to *Acromynes octospinosus* ant (14, 25).

Rich saponin alfalfa varieties applied on flour worm larvae *Tenebrio molitor* cause a decrease of dry food quantity metabolized by this insect (42). The incorporation of saponins of alfalfa in the artificial diet of *Ostrinia nubilalis* increases the larvae weight loss (36). Similar results were reported on *Spodotera littoralis* larvae treated by fifteen various purified saponins obtained from several plants (1). Agrell *et al*. (2) also noticed that *S. littoralis* larvae consumed less significant quantities of damaged alfalfa leaves than those of control leaves; this phenomenon was explained by the increased synthesis of two triterpenic saponins by the plants under biotic stress.

In the same way, the addition of saponins of certain leguminous plants (chickpeas, garden peas, broad beans, haricot beans, lentils, peanuts) in the artificial diet of *Callosbruchus chinensis* inhibits its food uptake; this inhibition is stronger when saponins used originated from different host plants (3).

Pluetella xylostella is a phytophagous specific insect consuming plants belonging to Brassicaceae family. It was noticed that the larvae are unable to attack one Brassicaceae species (*Barbarea vulgaris*) (45). The separation of the fractions of this plant revealed the involvement of triterpenic saponin, with two sugars in C_3 position, in the important inhibition of the food uptake activity (46).

A spirostanic saponin isolated from Solanaceae (*Solanum laxum*) showed an antifeeding activity against *Schizaphis graminum* aphid on artificial diet (48). Saponins extracted from *Blanites roxburghii, Agave cantala* and *Phaseolus vulgaris* were tested for their antifeeding activity on *Spilosoma obliqued* larvae. Monodesmoside saponins are shown to be more active than the bidesmoside ones. Saponins having the least significant number of sugar chains were most active (31).

Glycoalcaloids extracted from the genus *Solanum* species inhibit the weight increase of *Tribolium castaneum* and *Manduca sexta.* In these compounds, neither the aglycone alone nor when associated with sugars present this inhibitory activity (55).

Works on *Cestrum parqui* saponins show a repulsive activity against the caterpillar of *Pieris brassicae*, as well as a moderate antifeeding activity for *Spodoptera littoralis* and *Helicoverpa armigera* larvae (15).

Growth Regulation. Several researches show that saponins are able to regulate the growth of many insect species. These studies resumed in Table 1 concern purified or crude saponins extracted from several plants. The effect of saponins is generally characterized by developmental stages duration disturbance and moulting failure. The mode of action of "Insect Growth Regulator's" activity is discussed below.

Table 1. Growth regulation effects of saponins on some insects

Insect species	Saponins	Effects	Reference
Ostrinia nubilalis	alfalfa saponins	Lengthening of the larval stages	(36)
Spodoptera littoralis	alfalfa saponins	Lengthening of stages, delay of time necessary to reach the maximum size in last larval stage, delay of the interval separating the last larval stage and the nymphal moulting, and delay of time necessary for the emergence of the adults	(1)
Culex fatigans	commercial saponins	Larvae show more pronounced pigmentation and deterioration of the head and abdomen shape	(50)
Acrolepiosis assectella	*Allium porrum* saponins	Larvae present ecdysial disturbances, which often finish by characteristic malformations: larvae with double head	(5, 28, 29)
Acrolepiosis assectella	commercial digitonin	Ecdysial failure	(6)
Collosobruchus chinensis	Fabaceae saponins	Reduction in the rate of adult emergence	(55)
Spodoptera littoralis	*Cestrum parqui* saponins	Impossibility to get free from the old cuticle during the molting process	(16)
Shistocerca gregaria	*Cestrum parqui* saponins	Ecdysial disturbances	(9)

Entomotoxicity. The crude saponins extracted from *Cestrum parqui* injected to the L_5 *Schistocerca gregaria* larva increase insect mortality (9). In the same way, the spray of tomato leaves by 0.1 to 0.2% of an aqueous solution of alfalfa saponins reduces the number of *Tetranychus urticae* mite and *Pharodon* sp. aphids by 85 and 90%, respectively. Saponins of alfalfa can also cause mortalities on eggs of *T. urticae* (37).

The introduction of alfalfa saponins into the food of *Ostrinia nubilalis* cause larval mortalities reaching 100% for the L_2 larval stages. Mortalities were also recorded for the nymphal stage; moreover, only 60% of the treated chrysalis emerge (36). Treated by 100 ppm saponin of alfalfa leaves, *Spodoptera littoralis* shows a cumulative mortality of 90% at the larval and the nymphal stages (1). Various forms of chronic toxicity as a reduction in the fertility of the females and the blossoming eggs rate are observed in the same insect species (1). The saponins extracted from the leaves and the roots of the alfalfa are toxic for *Leptinotarsa decemlineata* larvae (49).

The addition of aginoside 1 (steroidic saponin) to the artificial diet of *Acrolepiosis assectella* larvae with an amount of 0.9 mg/g, causes 56% of mortality (29). The commercial saponins extracted from *Quillaja saponaria* have a larvicidal activity against the mosquitos larvae of two species *Aedes aegypti* and *Culex pipiens*; 100% of mortality is obtained by using amounts of 1000 mg/l during 5 days (40).

Crude saponins of *Cestrum parqui* showed a variable toxicity on various tested insects (*Schistocera gregaria, S. littoralis* and *Tribolium confusum*) but the most significant toxicity was observed on the larvae of the mosquito *Culex pipiens* (14).

Forming insoluble complexes with saponins, cholesterol is not absorbed any more by the digestive system of various animal species. The mechanism of formation of the cholesterol/saponin complexes is still unknown. Certain authors suggest a chemical reaction between the saponic aglycone and the lipophylic sites of cholesterol (51); Mitra and Dungan (35) show that there is a formation of micelle or spheres structures between cholesterol and saponin molecules.

The hypocholesterolemic activity of saponins was largely studied in many mammals (20, 34). Is such cholesterol/saponin interaction possible in insects? Theoretically yes, since insects, while being unable to synthesize cholesterol, they use this substance in the biosynthesis of the ecdysone (moulting hormone) and various other ecdysteroids. This hypo- hypocholesterolemic mechanism, similar to that observed in the mammals following the action of saponins, could interfere with the biosynthesis of the ecdysone and explain the disturbance of moulting process often observed following ingestion of *Cestrum parqui* leaves (9) or by the incorporation of extracts in the insect diet (15).

Various natural or synthesized insecticidal substances affecting the biosynthesis or the mechanisms of action of ecdysone, have a disturbing effects on insect growth and moulting (5, 6). In fact, saponins are substances often cited in the literature as provoking difficulties of exuviations and malformations of various insect species. Some of these works evoke the possibility of interaction of saponins with cholesterol but no demonstration was made until now.

Some experiments (Table 2) showed an Insect Growth Regulator activity of *Cestrum parqui* saponins. Indeed, insects consuming saponins supplemented with cholesterol support better the toxic effect of saponins; this fact is in favor of an antagonistic effect of cholesterol and consolidates our assumption concerning the mode of action of saponins (17).

Table 2. Effects of cholesterol addition in the diet of some insects treated with different saponins

Insect species	Saponins used	Effects of cholesterol addition	Reference
Acrolepiopsis assectella	Aginosid	Reduce the larval mortality from 56% to 22% and moulting failures from 19 to 8%	(29)
Acrolepiopsis assectella	Digitonin	Reduction in the death rate from 62 to 27%	(5)
Acrolepiopsis assectella	Digitonin	Removes completely the toxicity	(6)
Tribolium confusum	*Cestrum parqui saponins*	Reduction of larval mortality from 95 to 45%	(17)
Tenebrio molitor	Alfalfa saponins	Elimination of the saponin toxicity	(43)
Tribolium castaneum	Solmargine, Solasonine, Tomatine	Increase the viability of treated larvae	(55)

Several authors (29, 43, 55) suppose a possible interaction saponin/cholesterol causing cholesterimic deficit in insect, disturbing the ecdysone synthesis. This complexation can occur in food, hemolymph, or inside the insect cells. Studies trying to react in vitro cholesterol with saponin remained unfruitful although the use of various methods and solvents (14), whereas certain works reported formation of a precipitate with similar reactions (26, 51).

The mechanisms of interaction of saponins with cholesterol are still unknown and according to certain authors, there is no formation of an intermediate compound but a spherical structure, intercalation between saponin molecule and cholesterol, called micelle (35) or tubular structures (32) may be involved. Consequently, saponins do not block cholesterol or other phytosterols in the food, but this reaction could take place later inside insect body where other conditions are satisfied (pH, enzymatic arsenal).

Other scientific attempts to proportionate cholesterol in insects consuming saponins did not lead to reliable results because undoubtedly of methodologies used which would be unsuited to very low circulating cholesterol rates. Cholesterol is not in majority in the phytophagous insect food because plants contain other types of sterols as sitosterol and sigmasterol. It is possible that this interference between saponin and cholesterol would take place inside insect cells (17). Some authors suppose the possibility of interaction of saponin with ecdysteroid receptors (22, 23).

With the injection of crude saponins of *Cestrum* to *S. gregaria* locust, some necrotic symptoms appear at the injection site. In the same way, a forced ingestion of crude saponins has, as a consequence, a softening of the consistency of the digestive tract of *S. gregaria* adults. A pickling of the fat body of *Spodoptera littoralis* in saponins increases its tanning (14).

Histological studies revealed structural modifications at the fat body of *S. littoralis* as well as on the foregut and the gastric caeca of *S. gregaria*. These modifications were due to the cytotoxicity effect of *Cestrum parqui* saponins (19). Similar effects are obtained by treatment of *Culex pipiens* mosquito larvae by *Cestrum parqui* saponins (18).

The microscopic observations of treated insect tissue cuts show smaller size cells than the control at the fat body of *Spodoptora* as well as at the digestive tract of *Schistocerca*. In addition, the cells of the fat body appear darker due to the loss of their contents probably caused by the modification of their membrane permeability, and even with the disorganization of their molecular architecture (19).

In addition to the moulting disturbance and the cytotoxic activity, certain authors evoke an inhibitory activity of the digestive proteases of saponins involved in the entomo-toxicity recorded (9). Another work concerning the effect of food treated by *Cestrum parqui* leaves on *S. littoralis* larvae shows a deficit in the digestion of proteins and a decrease of the protein rate in the hemolymph and the cuticle (16).

Limits of the use of saponins in phytoprotection.

Stability problems. Saponins are relatively big size molecules which contain sugars whose degradation is easier under certain conditions (pH slightly acid or basic, presence of hydrolysis enzymes...). This degradation leads to the loss of activity which enormously depends on the water-soluble sugar chains. The modification of the structure of *Cestrum parqui* saponins (14) by the acetylation of sugars hydroxyls or the separation of the aglycone by hydrolysis led to a loss of the insecticidal activity of the molecule, which confirms results obtained by various authors (4, 9, 30, 32, 51).

Barbouche (9) already reported that sapogenins of *Cestrum parqui* are less active than saponins; this demonstrates the loss of saponin’s activity following their hydrolysis. Indeed, it has been shown that the aglycone obtained was inactive by grafting of these crystals in *S. gregaria*, just like acetylated saponins. It seems that the various structural modifications are involved in the hydrophily loss; the molecule needs the sugar chain for its solubility in the hemolymph and for its activity (14).

Moreover, various authors report the loss of the biological activity of saponins by structural modifications. Indeed, Keukens *et al.* (32) showed that a reduction of the chain of α-tomatine or of α-choacine increased the total loss of activity due to the membrane rupture. In the same way, a study of the digitonine/cholesterol interaction shows that analogues of digitonine could be associated with cholesterol. Various degrees of glycosylation of the digitonine are used: two, four or five sugars are associated to the aglycone, the results show that this complexation increases when the number of associated sugars increases (51).

Hu *et al.* (30) then Armah *et al.* (4) confirm these results by using similar saponins having the same triterpenic aglycone and by showing successively that the nature of sugar influences little on the molecule activity, but that, on the other hand, the hydrolysis of one, two or three sugars increases the total or partial loss of activity.

Antifeedancy. There is another problem which makes delicate the practical application of saponins as insecticide; it is the repulsive or antifeeding activity of saponins to several pest insects. Indeed, it was noticed that saponins decrease very appreciably the quantity of food consumed; this phenomenon seems to be a defense reaction of the animal against these toxic substances; this have as consequence the reduction in the quantity of active molecules introduced by ingestion and then reduction of the activity (14).

Problems of application. The insecticidal activity of saponins of *Cestrum parqui* is interesting in experiments of injection and forced ingestion. Death, in these cases, is observed after a few hours. The problem is that these experimental methods are practically not applicable. It is necessary to develop simpler and more effective techniques. Treatments by topic application do not give the anticipated results because of the impermeability of the cuticle to saponins. Some researches tried to associate saponins with abrasive insecticides (diatomous earth) which can cause wounds on the cuticle; this association remains also unfruitful (14).

Synthesis difficulty. Saponins are molecules characterized by a heavy molecular weight and an important structure complexity; this reduces their chance to be used like model to synthesize insecticidal molecules. Most works undertaking the synthesis of these products do it only partially (28).

Toxicity. Saponins have a cytotoxic (27) haemolytic (52) effects and are able of inhibiting the proteases activities (56); this represents a constraint if we attempt to apply these substances as agricultural products. These saponins are, in fact, rather as toxic for pests as for human.

Conclusion.

Secondary substances in plants are known for a long time for their medicinal and pharmacological properties. These substances are necessary for the plant to evolve in a hostile environment. The plant can indeed use its secondary metabolites to be protected against several pest animals and pathogenic microbes.

Saponins present one of these substances of large action spectrum broad, because of their toxicity to various insects. The mode of action of saponins seems in relation to the property of these molecules to be interacted either with structural cholesterol (membrane) or with metabolic cholesterol (food).

The practical application of this type of substances remains difficult because of easy degradation of these substances, the impossibility of acting by contact, the difficulties of their synthesis and their toxicity to mammals.

Saponins present an excellent model of study of natural substances with insecticidal effect due to their large spectrum of action and to the multitude of their physiological effects. It is, however, early to recommend application of saponins as insecticides. Thorough studies of their modes of action and application should be done firstly.

RESUME

Chaieb I. 2010. Les saponines comme insecticides: revue de synthèse. Tunisian Journal of Plant Protection 5: 39-50.

Les saponines sont des hétérosides (molécules ayant au moins un sucre dans leur structure) d'origine végétale. Ce type de molécules présente un potentiel insecticide faisant l'objet de cette synthèse. Dans la première partie de notre étude, nous avons essayé de les définir et de présenter leurs différentes familles structurales. Un aperçu sur la signification biologique et les principales sources de saponines est donné. La deuxième partie de cette synthèse s'intéresse aux principaux travaux réalisés sur les différentes activités insecticides. Ces substances occasionnent plusieurs formes de toxicité à l'encontre des insectes nuisibles (anti-appétence, perturbation de la mue, régulation de la croissance, mortalité…); l'activité insecticide des saponines proviendrait de leur interaction avec le cholestérol causant une perturbation de la synthèse des ecdysteroïdes. Ces substances possèdent également des propriétés inhibitrices de protéases et cytotoxiques. Dans la troisième partie de ce travail, nous avons donné une idée sur les contraintes qui peuvent freiner l'utilisation des saponines comme insecticides: les saponines présentent, en effet, une forte toxicité à l'égard des mammifères à cause de leur activité cytotoxique et hémolytique. La deuxième contrainte est la dégradation facile des sucres associés à la génine entraînant souvent la perte d'activité de la molécule. Le caractère hydrophile des saponines limite leur pénétration à travers la cuticule lipophile des insectes. La complexité structurale des saponines est une barrière à l'identification exacte des molécules actives et à leur synthèse.

Mots clés: Cholestérol, insecticide, lutte, saponines, substances naturelles, toxicité

ملخص

الشايب، إقبال. 2010. الصابونيات كمبيدات حشرية: مراجعة . Tunisian Journal of Plant Protection 5: 39-50

الصابونيات هي مواد تحتوي في بنيتها على سكر واحد أو أكثر من مصدر نباتي. أظهرت عديد الدراسات أن هذه المواد تتمتع بإمكانيات إبادية هامة وقد حاولنا في هذا العمل القيام بجرد لأهم الأعمال العلمية المنجزة في هذا الاتجاه. يهتم الجزء الأول من العمل بدراسة تقديمية للصابونيات حيث حاولنا إعطاء تعريف لهذه المواد ودراسة عائلاتها البنيوية. أعطينا كذلك خلاصة حول الأهمية البيولوجية وأهم المصادر لهذه المواد. يمثل الجزء الثاني من هذه الدراسة جردا تصنيفيا للفاعليات الإبادية للصابونيات، إذ تتمتع هذه المواد بفاعلية سمية ضد الحشرات الضارة حيث تسبب عندها تقليل الشهية وتعطل عملية طرح الغشاء الخارجي وتأخر مراحل النمو وموت الحشرة. إن آلية عمل هذه المواد تكون عن طريق التفاعل مع الكوليستيرول الذي يستخدم في إنتاج هرمون الاكديزون الذي يتحكم في عملية طرح الغشاء الخارجي للحشرة. كما أن هذه المواد تسبب تثبيطا لإنزيمات الهضم أو تسمما للخلايا. في الجزء الثالث من هذا العمل وجهنا الاهتمام إلى الصعوبات التي تعترض استعمال الصابونيات كمبيدات طبيعية، حيث أظهرت تلك المواد في عديد من الأحيان سمية للثدييات من خلال مقدرتها على حلّ الكريات الحمراء وتثبيط بعض الإنزيمات. كما أنه يصعب على الصابونيات اختراق غشاء الحشرات هذا ولا سيما أن السكريات المكونة لهيكل الصابونيات سريعة التدهور مما يفقد هذه المواد فاعليتها. أضف

إلى ذلك أن التركيبة الهيكلية المعقدة للصابونيات تقف عائقا أمام دراسة التشخيص الدقيق لجزيئاتها الفعالة وإنتاجها اصطناعيا.

كلمات مفتاحية: سمية، صابونيات، كوليستيرول، مبيدات حشرية، مقاومة، مواد طبيعية

LITERATURE CITED

1. Adel, M.M., Sehnal, F., and Jurzysta, M. 2000. Effect of alfalfa saponins on the mouth *Spodoptera littoralis*. J. Chem. Ecol. 26: 1065-1078.
2. Agrell, J., Oleszek, W., Stochmal, A., Olsen, M., and Anderson, P. 2003. Herbivore-induced responses in alfalfa (*Medicago sativa*). J. Chem. Ecol. 29: 303-320.
3. Appelbaum, S.W., Marco, S., and Birk, Y. 1969. Saponins as possible factor of resistance of legume seeds to the attack of insects. J. Agr. Food. Chem. 17: 618-622.
4. Armah, C.N., Mackie, A.R., Roy, C., Price, K., Osbourn, A.E., Bowyer, P., and Ladha, S. 1999. The Membrane-permeabilizing effect of avenacin A-1 involves the reorganization of bilayer cholesterol. Biophys. J. 76: 281-290.
5. Arnault, C., Harmatha, J., Mauchamp, B., and Salama, K. 1987. Influence of allelochemical substances of the host plant (*Allium porrum*) on development and molting of *Acrolepiosis assectella (Lepidoptera*). Their role selective factors. Pages 249-255. In: Insects-Plants. V. Labeyrie, G. Fabers, D. Lachaise, Eds. W Junk Publischer, Netherlands, 448 pp.
6. Arnault, C. and Mauchamp, B. 1985. Ecdysis inhibition in *Acrolepiosis assectella* larvae by digitonin: antagonistic effect of cholesterol. Experientia 41: 1074-1077.
7. Baqai, F.T., Ali, A., and Ahmad, V.U. 2001.Two new spirostanol glycosides from *Cestrum parqui*. Helv. Chim. Acta 84: 3350-3356.
8. Barbosa, P., Gross, P., Provan, G.J., and Stermiz, F.R. 1990. Allelochemicals in foliage of unfavored tree hoss of the gypsy mooth *Lymantria dispar* L. seasonal variation of saponins in *Ilex opacea* and identification of saponin aglycones. J. Chem. Ecol. 16: 1731-1738.
9. Barbouche, N., Hajem, B., Lognay G., and Ammar, M. 2001. Contribution à l'étude de l'activité biologique d'extraits de feuilles de *Cestrum parquii* sur le criquet pèlerin *Schistocera gregaria*. Biotechnol. Agron. Soc. Environ. 5: 85-90.
10. Berenbaum, M.R. 1995. The Chemistry of defence: theory and practice. Pages 1-16. In: Chemical ecology: the chemistry of biotic interaction Thomas, E., and Meinwald, J., éd. National Academy of Science Washington DC, 224 pp.
11. Berger, J.M. 2001. Isolation, characterization, and synthesis of bioactive natural products from rainforest flora. Philosophical Doctorate. Virginia Polytechnic Institute and State University Blacksburg, Virginia, 210 pp.
12. Bruneton, J. 1999. Pharmacognosie phytochimie plantes médicinales. Lavoisier Eds., Paris, 1120 pp.
13. Cafieri, F., Fattorusso, E., and Taglialatela-Scafati, O. 1999. Ectyplasides A-B unique triterpene oligoglycosides from the Caribbean sponge *Ectyoplasia ferox*. Eur. J. Org. Chem. 193: 231-238.
14. Chaieb, I. 2005. Les saponines du *Cestrum parqui* nature chimique implications physiologiques et potentiel bio-pesticide. Thèse de Doctorat en Sciences Agronomiques. Ecole Supérieure d'Horticulture et d'Elevage de Chott Mariem, Tunisia, 157 pp.
15. Chaieb, I., Ben Halima-Kamel, M., and Ben Hamouda M.H. 2001. Effects of diet addition of *Cestrum parquii* (*Solanaecae*) extracts on some Lepidoptera pests: *Pieris brassicae* (Pieridae) et *Spodoptera littoralis* Boisduval (*Noctuidae*). Med. Fac. Lanbouww. Uni. Gent. 66: 479-480.
16. Chaieb, I., Ben Halima-Kamel, M., and Ben Hamouda M.H. 2004. Modifications cuticulaire et protéinique de *Spodoptera littoralis* Boisduval *(Lepidoptera)* sous l'action d'une alimentation additionnée d'extrait sec de *Cestrum parquii* l'Hérit *(Solanaceae).* Ann. INRAT 77: 119-135.
17. Chaieb, I., Ben Halima-Kamel, M., and Ben Hamouda, M.H. 2006. Insect growth regulator activity of *Cestrum parqui* saponins: an interaction with cholesterol metabolism. Commun. Agric. Appl. Biol. Sci. 71: 489-496.
18. Chaieb, I., Ben Hamouda, A., Trabelsi, M., Ben Halima, M., and Ben Hamouda, M.H. 2009. Toxicity investigation of *Cestrum parqui* saponins to *Culex pipiens* larvae. Pest Tech. 3: 73-75.
19. Chaieb, I., Trabelsi, M., Ben Halima-Kamel, M., and Ben Hamouda, M.H. 2007. Histological effects of *Cestrum parqui* saponins on *Schistocerca gregaria* and S*podoptera littoralis*. J. Biol. Sci. 7: 95-101.
20. Cheeke, P.R. 1971. Nutritional and physiological implications of saponins: A review. Can. J. Ani. Sci. 51: 621-632.

21. Chuan-Chun, Z., Shu-Jie, H., Yang, S., Ping-Sheng, L., and Xiao-Tian, L. 2003. The synthesis of gracillin and dioscin: two typical representatives of spirostanol glycosides. Carbohy. Res. 338: 721-727.
22. De Geyter, E., Lambert, E., Geelen, D., and Smagghe, G. 2007. Novel Advances with plant saponins as natural insecticides to control pest insects. Pest Tech. 1: 96-105.
23. De Geyter, E., Geelen, D., and Smagghe, G. 2007. First results on the insecticidal action of saponins. Commun. Agric. Appl. Biol. Sci. 72: 645-648.
24. Duke, S.O. 1990. Natural pesticides from plants. Pages 511-517. In: Advances in new crops. J. Janick, and J.E. Simon, éd. Timber press, Portland, Oregon, USA, 540 pp.
25. Febvay, G., Bourgeois, P., and Kermarrec, A. 1985. Antifeedants for attine ant, *Acromymex octospinosus* (Reich) (*Hymenoptera-Formicidae*), in several ignam spices (*Discoreaceae*) cultivated in Antilla. Agronomie 5: 439-444.
26. Gestetener, B., Assa, Y., Henis, Y., Tencer, Y., Rotman, M., Birk, Y., and Bondi, A. 1972. Interaction of lucerne saponin with sterols. Biochem. Biophys. Acta 270: 181-187.
27. Haridas, V., Arntzen, C.J., and Gutterman, J.U. 2001. Avicins, a family of triterpenoid saponins from *Acacia victoriae* (Bentham), inhibit activation of nuclear factor-kB by inhibiting both its nuclear localization and ability to bind DNA. Proc. Nat. Acad. Sci. USA 98: 11557-11562.
28. Harmatha, J. 2000. Chemo-ecological role of spirostanol saponins in the interaction between plants and insects. Pages 129-141. In: Saponin in food, feedstuffs and medicinal plants. W. Olezek and A. Marston, éd, Kluwer Academic Publisher, Netherlands, 304 pp.
29. Harmatha, J., Mauchamp, B., Arnault, C., and Salama, K. 1987. Identification of spirostane type saponin in the flowers of leek with inhibitory effect on growth of leek-mouth larvae. Biochem. System. Ecol. 15: 113-116.
30. Hu, M., Konoki, K., and Tachibana, K. 1996. Cholesterol independent membrane disruption caused by triterpenoid saponins. Biochem. Biophys. Acta 1299: 252-258.
31. Jain, D.C. and Tripathi, A.K. 1999. Insect feeding deterrent activity of some saponin glycosides. Phytother. Res. 5: 139-141.
32. Keukens, E.A., De Vrije, T., Van den Boom, C., De Waard, P., Plasman, H.H., Thiel, F., Chupin,V., Jongen, W.M., and De Kruijff, B. 1995. Molecular basis of glycoalkaloid induced membrane disruption. Biochem. Biophys. Acta 1240: 216-228.
33. Kreuger, B. and Potter, D.A. 1994. Changes in saponin and tannins in ripening holly fruits and effects of fruit consumption on non adapted insect herbivore. Am. Midl. Nat. 132: 183-191.
34. Milgate, J. and Roberts, C.K. 1995. The nutritional and the biological significance of saponin. Nutr. Res. 15: 1223-1249.
35. Mitra, S. and Dungan, S.R. 2000. Micellar properties of *Quillaja* saponin. 2. Effect of solubilized cholesterol on solution properties. Coll. Surf. Biointer. 17: 117-133
36. Nozzolillo, C., Arnason, J.T., Campos, F., Donskov, N., and Jurzysta, M. 1997. Alfalfa leaf sapnins and insect resistance. J. Chem. Ecol. 23: 995-1002.
37. Oleszek, W.A., Hoagland, R., and Zablotowicz, E. 1999. Ecological significance of plant saponins. Pages 451-465. In: Principles and practices in plant ecology allelochemical interactions. K.M.M. Dakshini and C.L. Foy, Eds. Chemical Rubber Company Press, 608 pp.
38. Papadopoulou, K., Melton R.E., Leggett M., Daniels M.J., and Osbourn, A.E. 1999. Compromised disease resistance in saponin-deficient plants. Proc. Nat. Acad. Sci. USA 96: 12923-12928.
39. Paré, P.W. and Tumlinson, J.H. 1996. Plant volatile signals in response to herbivore feeding. Fla. Entomol. 79: 93-103.
40. Pelah, D., Abramovich, Z., Markus, A., and Wiesman, Z. 2002. The use of commercial saponin from *Quillaja saponaria* bark as a natural larvicidal agent against *Aedes aegypti* and *Culex pipiens*. J. Ethnopharmacol 81: 407-409.
41. Plasman, V., Braekman, J.C., Daloze, D., Luhmer, M., Windsor, D., and Pasteels, J.M. 2000. Triterpene saponins in the defensive secretion of a chrysomelid beetle, *Platyphora ligata*. J. Nat. Prod. 63: 646-649.
42. Potter, D.A. and Kimmerer, T.W. 1989. Inhibition of herbivory on young holly leaves evidence for defensive role of saponins. Oecologia 78: 322-329.
43. Pracros, P. 1988. Mesure de l'activité des saponines de la luzerne par les larves du ver de la farine: *Tenebrio molitor* L. (Coléoptère, *Tenebrionidae*). I - Comparaison avec les divers tests biologiques. Agronomie 8: 257-263.
44. Rahbe, Y., Febavy, G., and Kermarrec, A. 1988. Foraging activity of attine ant *Acromyrex octospinosus* (reich) (*Hymnoptera: Fomicidae*) on resistant susceptible yam varieties. Bul Entomol. Res. 78: 339-349.
45. Serizawa, H., Schinoda, T., and Kawai, A. 2001. Occurrence of a feeding deterrent in *Barbarea vulgaris* (*Brassicales*: *Brassicaceae*), a crucifer unacceptable to the diamondback moth, *plutella xylostella*. Appl. Entomol. Zool. 36: 465-470.

46. Shinoda, T., Nagao, T., Nakayama, M., Serizawa, H., Koshioka, M., Okabe, H., and Kawai, A. 2002. Identification of a triterpenoid saponin from a crucifer, *Barbarea vulgaris*, as a feeding deterrent to the diamondback moth, *Plutella xylostella*. J. Chem. Ecol. 28: 587-599.

47. Simmonds, M.S.J. 2000. Molecular and chemo-systematics: do they have a role in agrochemical discovery? Crop Prot. 19: 603-608.

48. Soule, S., Guntner, C., Vazquez, A., Argandona, V., Moyna, P., and Ferreira, F. 2000. An aphid repellent glycoside from *Solanum laxum*. Phytochemistry 55: 217-222.

49. Szczepanik, M., Krystkowiak, K., Jurzysta, M., and Bialy, Z. 2001. Biological activity of saponins from alfalfa tops and roots against Colorado potato beetle larvae. Acta Agrobotanica 54: 235-245.

50. Tabassum, R., Nakvi S.H., Jahan, M., and Khan, M.Z. 1993. Toxicity and abnormalities produced by plant products (hydrocarbons and saponin) and dimethoate (Perfekthion) against fourth instar larvae of *Culex fatigans*. Proc. Pak. Cong. Zool. 13: 387-393.

51. Takagi, S., Otsuka, H., Akiama, T., and Sankawa, U. 1982. Digitonin cholesterol complex formation effect of varying the length of the side chain. Chem. Pharm. Bull. 30: 3485-3492.

52. Takechi, M., Doi, K., and Wakayama, W. 2003. Biological activities of synthetic saponins and cardiac gycosides. Phytother. Res. 17: 83-85.

53. Termonia, A., Pasteels, J.M., Windsor, D.M., and Milinkovitch, M.C. 2002. Dual chemical sequestration: a key mechanism in transitions among ecological specialization. Proc. R. Soc. Lond. 269: 1-6.

54. Vander Geest, L.S., Beerling, E.M., and Fransen, J. 2000. Can plants use entomopathogens as bodyguard? Ecol. Lett. 3: 228-235.

55. Weissenberg, M., Levy, A., Svoboda, J.A., and Ishaaya, I. 1998. The Effect of some *Solanum* steroidal alkaloids and glycoalkaloids on larvae of the red flour beetle, *Tribolium castaneum*, and the tobacco hornworm *Manduca sexta*. Phytochemistry 47: 203-209.

56. Wierenga, J.M. and Hollingworth, R.M. 1992. Inhibition of insect acetylcholinesterase by the potato glycoalkaloid alpha-chaconine. Natural Toxins 1: 96-99.
