

Saponins Suppress Nematode Cholesterol Biosynthesis and Inhibit Root Knot Nematode Development in Tomato Seedlings

Mervat AR Ibrahim and Hany AM Srour*

Biochemistry Department, Faculty of Agriculture, Ain Shams University, 68 Hadayek shoubra, PO 11241, Cairo, Egypt

Abstract

Root knot nematodes *Meloidogyne incognita* are responsible for heavy economic losses to many agricultural crops and considered the most difficult crop pest to control. Recently attention on environmental and food safety in addition to human health has led to increase the limitations on using chemical nematocides and searching for environmental safe natural nematocides. Saponins extracted from *Medicago sativa* L., alfalfa was used to control the infection of tomato seedlings with root knot nematode *Meloidogyne incognita*. The results indicated that saponin treatments led to significant reduction in the numbers of larva of root knot nematodes in tomato roots and in soil. The highest nematode inhibition was observed in the treatment of 100% of saponin crude extracts. The observed reduction of the number of nematode larva in tomato roots and in soil were found to be correlated with the decline of cholesterol level in root knot nematode eggs which is due to saponins from *Medicago sativa*, in a concentration dependent manner. Also saponin treatments showed a general improvement in plant growth and performance.

Keywords: Saponins; *Medicago sativa*; Root knot nematode; *Meloidogyne javanica*; Cholesterol

Introduction

Saponins are secondary metabolites widely presented in many plant species. Most saponins are hemolytic and display many biological activities, such as anti-inflammatory and hypocholesterimic effects. Saponins from alfalfa showed a hypocholesterimic effect in rats through the ability of natural saponins to reduce cholesterol uptake from rat's intestine [1]. The hypocholesterimic mechanism of saponins included their ability to form insoluble complex with sterols. Many commercial products containing saponins are used in pharmaceutical, cosmetic and food industry. Triterpene saponins from *Quillaja saponaria* are used to control insect and nematode development [2,3]. Also, saponins from *Medicago arborea*, *Medicago Arabica* and *Medicago sativa* all possess nematocidal activity against plant-parasitic nematode *X. index* [4].

Saponins from *Medicago spp.* are complex mixture of high molecular weight triterpene glycoside with medicagenic acid, hydragenin, zahnic acid, bayogenin and soyasapogenol A and B as the dominant aglycones [5-7]. The chemical structure of the main aglcones of saponins from *Medicago sativa* is presented in Figure 1, reported by Argentieri et al. [4]. All saponins from *Medicago spp.* have been reported as human leukemia inhibitors against some plant pathogens and gram positive pathogenic bacteria [6,8].

Plant parasitic nematode are c for heavy economic loss in many agricultural crops Root knot nematode *Meloidogyne javanica* is the most harmful nematode species to many crops such as tomato (*Lycopersicon esculentum* L.). Root knot nematode attacks tomato roots and reduces plant growth and causes plant death. Also nematode participates in viral infection whereas it acts as virus vector and cause a severe damage of tomato crop. Cholesterol is a structural component of nematode membrane. Nematode requires too little amount of cholesterol to control molting and signaling for other functions (Teymuraz et al. (2003)) Nematode management depends upon chemical nematocides which have high harmful effects on human health and environment, So the environmental safe natural products will be highly required. Few data about the nematocidal activity of saponins are available [2,4,9,10], and there is no information about the nematocidal mechanism of saponins from *Medicago spp.*

The present study aims to evaluate the nematocidal activity of saponins crude extract from alfalfa against root knot nematode. In addition, the correlation between nematocidal activity of *Medicago sativa* saponins and their inhibitory effect on cholesterol synthesis in nematode was highlighted.

Materials and Methods

Preparation of saponin crude extracts

Shoots of *Medicago sativa* were used for saponins extraction. *Medicago sativa* were grown in Giza, Egypt. Samples of plant leaves were collected and dried at 40°C. Saponins were extracted by the method described by Tava et al. and Bialy et al. [5,7]. Saponins extract (100% concentration) was diluted to 75%, 50% and 25%.

Evaluation of nematocidal activity

M. incognita were green house-propagated on tomato seedlings (*Lycopersicon esculentum*) grown in a plastic pots 15 cm. After 10 days of infection tomato seedlings were divided into five groups, one of them acts as untreated control and the others were treated with 100 ml of 100%, 75%, 50% and 25% saponin crud extract. After 15 days of treatments tomato roots were harvested and cleaned thoroughly with low pressure water stream to remove adhering soil, roots were incubated for 3 days at 28°C. Then nematode larva war filtrated through a screen of 20 meshes. Nematode population was determined by microscopic investigation.

Determination of cholesterol

Nematode larva and Eggs were collected in a small volume of

*Corresponding author: Hany AM Srour, Biochemistry Department, Faculty of Agriculture, Ain Shams University, 68 Hadayek shoubra, PO 11241 Cairo, Egypt, Tel: +2010-660-9868; E-mail: info@radcoegypt.com

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distilled water and lipids were extracted with chloroform: methanol 2:1. The mixture was centrifugated at 10000 g then total cholesterol was determined in supernatant according to the method described by Allain *et al.* [11].

Statistical analysis

Data presented are Means \pm Standard Deviation of five replicates. The recorded data were statistically analyzed using the one way analysis of variance as described by Snedecor and Cochran [12]. The means were compared by least significant difference test at $P \leq 0.05$.

Results and Discussions

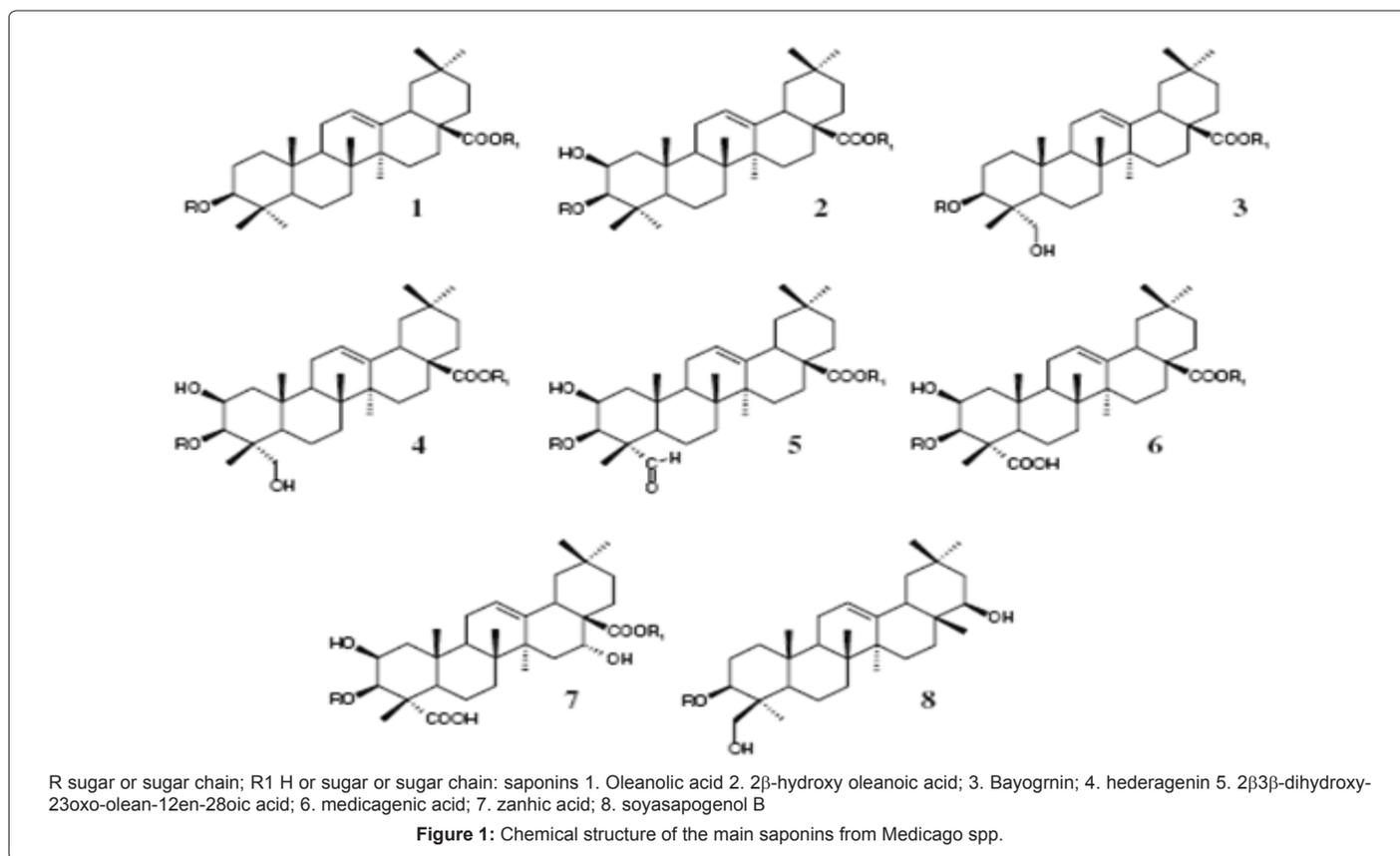
The nematocidal activity of different concentrations of saponins extract from *Medicago sativa* was measured by determination of nematode populations in soil and tomato roots after 10 days of treatment. Table 1 indicates that all tested saponins concentrations are able to inhibit the development of *M. incognita*. The concentration of 100% of crude extract of alfalfa saponins showed the highest inhibitory effect on root knot nematode. The population of root knot nematode in

the soil treated with saponin crude extract was significantly less than that of untreated control. Table 1 clearly demonstrates that saponins extract from *Medicago sativa* showed a high potential as nematocidal natural products.

Table 2 indicates the level of total cholesterol in eggs and juvenile of *Meloidogyne incognita* treated with different concentrations of saponins extract from *Medicago sativa*. Data clearly indicated that saponins treatments led to a significant reduction in the cholesterol level in eggs and larva. The results also indicate that 100% and 75% saponins crude extract exhibited the lowest cholesterol level in root knot nematode. Also 50% and 25% of saponins crude extracts led to a significant reduction in cholesterol level compared to untreated control.

Discussion

Literature on the nematocidal activity of saponins is limited and data are mainly related to the assay of *Q. saponaria* extracts. Omar *et al.* [10], reported that saponins solutions reduced the total population, number of egg masses and viable juveniles of the root knot nematode *Meloidogyne javanica*. Moreover, another study on a formulation from



Treatment	Population of root knot nematode	
	Larva / Kg Soil	Larva / g tomato roots
Control	1358 \pm 133 ^a	212.8 \pm 6.1 ^a
25 % v/v saponins extract	465 \pm 37 ^b	114 \pm 9.7 ^b
50% v/v saponins extract	393 \pm 26.5 ^c	83.4 \pm 6.46 ^c
75 % v/v saponins extract	272.6 \pm 22.6 ^d	37.6 \pm 3.65 ^d
100% v/v saponins extract	221 \pm 21.6 ^e	27.4 \pm 3.97 ^e

Table 1: All values are expressed as mean \pm SD (n=5). Different letters refer to significant difference. Population of root knot nematode *Meloidogyne incognita* in soil and tomato roots after 10 days of treatment with different concentration of saponin crude extracts from *Medicago sativa*.

Treatment	Cholesterol	
	$\mu\text{g/g}$	% of control
Control	336 \pm 11 ^a	100 \pm 3.3 ^a
25 % v/v saponins extract	276 \pm 16 ^b	82 \pm 4.8 ^b
50% v/v saponins extract	261 \pm 13 ^b	77.8 \pm 3.9 ^b
75 % v/v saponins extract	178 \pm 12 ^c	52.9 \pm 3.5 ^c
100 % v/v saponins extract	170 \pm 9 ^c	50.5 \pm 2.5 ^c

Table 2: All values are expressed as mean \pm SD (n=5). Different letters refer to significant difference. Cholesterol level in root knot nematode *Meloidogyne incognita* after 10 days of treatment with different concentration of saponin crude extracts from *Medicago sativa*.

Q. Saponaria containing 6% polyphenols and 25% saponins indicated that association of polyphenols and saponins resulted in the best nematode control at low dosage of plant extract [13]. Similar results were reported by D'Addabbo et al. [2] where they found that aqueous extracts of *Q. saponaria* significantly reduced the density of *M. incognita* in soil and increased tomato and melon crop yield. Nematocidal activity of saponins extracts from *Medicago spp* was reported by Argentieri et al. [4]. They found that saponins from *M. arborea*, *M. Arabica*, and *M. sativa* to different concentrations all possess nematocidal activity against the plant parasitic nematode *X. index*. The exposure of juveniles of *M. incognita* to eight different steroid and triterpenoid saponins led to significant reduction in the motility of the juveniles [14].

In agreement with the previous data our results clearly demonstrate the nematocidal activity of saponin crude extracts from *Medicago sativa* as shown in Table 1. The observed nematocidal activity in alfalfa crude extract could be explained by the fact that these crude extract contain a polyphenolic compounds which have a synergetic effect between saponins and polyphenols [13]. Also, Argentieri et al. [4] explain the nematocidal activity of all saponins from *Medicago spp* by interaction between saponin and collagen protein from the cuticle of nematode. Also, the biological activity of saponins is explained by their specific interaction with cell membranes [6] causing changes in cell membrane permeability. In addition, the saponin nematocidal activity might involve the formation of saponin cholesterol insoluble complex [15].

Data in Table 2 demonstrated the ability of saponins extracts from *Medicago sativa* to reduce the level of cholesterol in nematode eggs and larva. The biological functions for cholesterol in nematode can be summarized as follows: (1) as structural component of cell membranes, (2) as precursor of the molting hormone ecdysone [16], (3) as the moiety required for activation by covalent attachment to morphogen protein hedgehog (Proter et al 1996).

Biological effects of saponins are normally ascribed to their specific interaction with cell membranes [6] causing changes in cell permeability. The implication in the process of cholesterol-saponin insoluble complexes is, however, still controversial [15]. Moreover, it has been shown that the side sugar chains on the aglycones might contribute to saponin effects on cell membranes; that is, monodesmosides have generally a stronger haemolytic activity than bidesmosides [6]. In addition to the activity of saponins on biological systems, it has been demonstrated that they are also able to interact with proteins [17-19]. Moreover, specific studies with *Q. saponaria* and soybean saponins [17-19] have shown that the interaction between the saponins and proteins is quite complex and also involves the aglycone moiety. A critical structure for nematode viability is the protective cuticle, an extracellular matrix that forms their exoskeleton. This structure is primarily composed of collagen proteins assembled into higher order complexes [20-25]. It appears reasonable to speculate whether saponin interaction with collagen proteins from the cuticle might also be responsible for the observed nematotoxic effects [4,8,11]. Furthermore, the rate of nematocidal activity induced by both pro saponins and saponins also suggests the possible implication of the saponin aglycone [26-32]. Natural saponins used in these in vitro tests were from different *Medicago spp.* and were chosen for their different saponin and saponin profiles [32-38].

Conclusion

Thus, the nematocidal activity of saponins from *Medicago sativa* could be attributed to their ability to inhibit cholesterol accumulation in egg and/or larva. Our results suggested different mechanisms for the nematocidal activity of saponins involved inhibition of cholesterol

biosynthesis, binding to plant sterol and formation of insoluble complexes which cannot metabolize to cholesterol and/or inhibition of sterol conversion enzymes which convert plant sterol such as argosterol to animal sterol such as cholesterol.

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