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

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Abstract

Root knot nematodes *Meloidogyne incognita* are responsible for heavy economic losses to many agricultural crops and considered the most difficult 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 hypcholesterolimic effects. Saponins from alfalfa showed a hypcholesterolimic effect in rats through the ability of natural saponins to reduce cholesterol uptake from rat’s intestine [1]. The hypcholesterolimic 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. Tripterene saponins from *Quillaja saponaria* are used to control insect and nematode development [2,3]. Also, saponins from *Medicago arborea, Medicago Arbica* and *Medicago sativa* all possess nematicidal activity against plant-parasitic nematode X. *index* [4].

Saponins from *Medicago spp.* are complex mixture of high molecular weight triterpene glycoside with medigenic acid, hydragenic, zahnic acid, bayogénin and soyaspogenol A and B as the dominant aglycrones [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 bacterias [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 (Lycopersicum 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 (Teymuras 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 nematicidal activity of saponins are available [2,4,9,10], and there is no information about the nematicidal mechanism of saponins from *Medicago spp*.

The present study aims to evaluate the nematicidal activity of saponins crude extract from alfalfa against root knot nematode. In addition, the correlation between nematicidal 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 nematicidal activity

*M. incognita* were green house-propagated on tomato seedlings (Lycopersicum 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 crude 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 was 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
distilled water and lipids were extracted with chloroform: methanol 2:1. The mixture was centrifuged at 10000 g then total cholesterol was determined in supernatant according to the method described by Allan et al. [11].

Statistical analysis
Data presented are Means ± 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 ≤ 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 1: All values are expressed as mean ± 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*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Population of root knot nematode</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Larva / Kg Soil</td>
<td>µg/g</td>
</tr>
<tr>
<td>Control</td>
<td>1358 ± 133</td>
<td>336 ± 11*</td>
</tr>
<tr>
<td>25 % v/v saponins extract</td>
<td>465 ± 37*</td>
<td>276 ± 16*</td>
</tr>
<tr>
<td>50% v/v saponins extract</td>
<td>393 ± 26.5*</td>
<td>261 ± 13*</td>
</tr>
<tr>
<td>75 % v/v saponins extract</td>
<td>272.6 ± 22.6µ</td>
<td>178 ± 12*</td>
</tr>
<tr>
<td>100% v/v saponins extract</td>
<td>221 ± 21.6µ</td>
<td>170 ± 9*</td>
</tr>
</tbody>
</table>

Table 2: All values are expressed as mean ± 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*.

![Chemical structure of the main saponins from Medicago spp.](image)
Medicago spp of saponins extracts from Medicago spp was reported by D'Addabbo et al. [2] where they found that aqueous extracts 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 glycones 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 Qu saponaria and soybean saponins [17-19] have shown that the interaction between the saponins and proteins is quite complex and also involves the glycone 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 sapogenins and sapogenins 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 sapogenin 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.

References


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