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 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. 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 nematicidal activity against plant-parasitic nematode X. index [4].

Saponins from *Medicago spp.* are complex mixture of high molecular weight triterpene glycoside with medicagenic acid, hydagenin, 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 (*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 (Teymur 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 (*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 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 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

*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*

Received September 30, 2013; Accepted November 23, 2013; Published November 30, 2013

Citation: Ibrahim MAR, Srour HAM (2013) Saponins Suppress Nematode Cholesterol Biosynthesis and Inhibit Root Knot Nematode Development in Tomato Seedlings. Nat Prod Chem Res 2: 123 doi:10.4172/ 2329-6836.1000123

Copyright: © 2013 Ibrahim MAR, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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 ± 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 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
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. Nematicidal 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 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

1. Malinow MR, McLaughlin P, Papworth L, Stafford C, Kohler GO, et al. (1977) Effect of alfalfa saponins on intestinal cholesterol absorption in rats. Am J Clin Nutr 30: 2061-2067.
2. D’Addabbo T, Curto G, Greco P, Di Silvestro D, Ciro MI, et al. (2005) Preliminary trials with extracts of Quillaja saponaria Molina for the control of root-knot nematodes. Nematologia Mediterranea 33: 29-34.
3. Pelah D, Abramovich Z, Markus A, Wiesman Z (2002) The use of commercial saponin from Quillaja saponaria bark as a natural larvicide agent against Aedes aegypti and Culex pipiens. J Enthemopathol 81: 407-409.
4. Argentieri MP, Addabbo TD, Tava A, Agostinelli A, Jurzyzta M, et al. (2008) Evaluation of nematicidal properties of saponins from Medicago spp. European Journal of Plant Pathology 120: 189-197.
5. Tava A, Mella M, Avato P, Argentieri MP, Bialy Z, et al. (2005) Triterpenoid glycosides from leaves of Medicago arborea L. J Agric Food Chem 53: 9954-9965.
6. Tava A, Avato P (2006) Chemistry and biological activity of triterpene saponins from Medicago species. Natural Product Communications 1: 1159-1180.
7. Bialy Z, Jurzyzsta M, Mella M, Tava A (2004) Triterpene saponins from aerial parts of Medicago arbatica L. J Agric Food Chem 52: 1095-1099.
8. Avato P, Bocci R, Tava A, Vitali C, Rosato A, et al. (2006) Antimicrobial activity of saponins from Medicago spp.: structure-activity relationship. Phytother Res 20: 454-457.
9. Meher HC, Welia S, Sethi CL (1988) Effect of steroidal saponins on the mobility of juveniles of Meloidogyne incognita. Indian Journal of Nematology 18: 244-247.
10. Omar SA, Abdel-Massih MI, Mohamed BE (1994) Use of saponin to control the root-knot nematode Meloidogyne javanica in tomato plants. Bulletin of Faculty of Agriculture of Cairo 45: 933-940.
11. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC (1974) Enzymatic determination of total serum cholesterol. Clin Chem 20: 470-475.
12. Snedecor GW, Cochran WG (1967) Statistical methods (6th edition) Iowa State University Press p 327.
13. San Martin R, Magnunacelaya JC (2005) Control of plant-parasitic nematodes with extracts of Quillaja saponaria. Nematology 7: 577-585.
14. Chitwood DJ (2002) Phytochemical based strategies for nematode control. Annu Rev Phytopathol 40: 221-249.
15. Francis G, Kerem Z, Makkar HP, Becker K (2002) The biological action of saponins in animal systems: a review. Br J Nutr 88: 587-605.
16. Merris M, Wadsworth WG, Khamrai U, Bittman R, Chitwood DJ, et al. (2003) Sterol effects and sites of sterol accumulation in Caenorhabditis elegans: developmental requirement for 4alpha-methyl sterols. J Lipid Res 44: 172-181.
17. Heng L, Koningsveld van GA, Gruppen H, Boekel van MAJS, Vincken JP, et al. (2004) Protein-flavour interactions in relation to development of novel protein foods. Trends in Food Science & Technology 15: 217-224.
18. Ikedo S, Shimoyada M, Watanabe K (1996) Interaction between bovine serum albumin and saponin as studied by heat stability and protease digestion. J Agric Food Chem 44: 279-285.
19. Pollter SM, Jimenez-Flores R, Pollack J, Lone TA, Berber-Jimenez MD (1993) Protein- saponin interaction and its influence on blood lipids. J Agric Food Chem 41: 1287-1291.
20. Page AP, Winter AD (2003) Enzymes involved in the biogenesis of the nematode cuticle. Adv Parasitol 53: 85-148.
21. Bialy Z, Jurzyzta M, Mella M, Tava A (2006) Triterpene saponins from the roots of Medicago hybridra. J Agric Food Chem 54: 2520-2526.
22. Houghton P, Patel N, Jurzyzta M, Biey Z, Cheung C (2006) Endermatophyte activity of medicago extracts and contained saponins and their structure-activity relationships. Phytother Res 20: 1061-1066.
23. Julier B, Guy P, Castillo-Acuna C, Caubel G, Ecalle C, et al. (1996) Genetic variation for disease and nematode resistances and forage quality in perennial diploid and tetraploid lucerne populations (Medicago sativa L.). Euphytica 91: 241-250.

24. Jurzysta M (1982) M. Polski Urzad Patentowy. Patent No. 114171.

25. Jurzysta M, Price K, Ridout C, Fenwick R (1989) The structure of four Interpenoid saponins isolated from the seed of Trifolium incarnatum. Acta Societas Botanicorum Poloniae 58: 575-582.

26. McSorley R (1987) Extraction of nematodes and sampling methods. Principles and practises of nematode control in crops. Brown RH, Kerry BR (Editors), (pp. 13–47), Academic Press, USA.

27. Morein B, Hu KF, Abusugra I (2004) Current status and potential application of ISCOMs in veterinary medicine. Adv Drug Deliv Rev 56: 1367-1382.

28. Oleszek W, Jurzysta M, Ploszynski M, Colquhoun IJ, Price KR, et al. (1992) Zanthic acid tridesmoside and other dominant saponins from Alfalfa (Medicago sativa L.) aerial parts. Journal of Agricultural and Food Chemistry 40: 191-196.

29. Pedersen MW, Barnes DK, Sorensen EL, Griffin DG, Nielsen MW, et al. (1976) Effects of low and high saponin selection in alfalfa on agronomic and pest resistance traits and the interrelationship of these traits. Crop Science 16: 193-199.

30. Raski DJ (1996) Dagger and needle nematodes. Compendium of grape diseases Pearson RC, (pp. 56–59), APS Press, USA.

31. Rönnberg B, Fekadu M, Morein B (1995) Adjuvant activity of non-toxic Quillaja saponaria Molina components for use in ISCOM matrix. Vaccine 13: 1375-1382.

32. San Martin R (2004) Use of Quillaja saponins to control nematodes. Development of a commercial product: QL AGRI (p. 6). Pulawy, Poland: Abstract Book – International Conference on Saponins.

33. Tanaka O, Tamura Y, Masuda H, Mizutani K (1996) Application of saponins in foods and cosmetics: saponins of Mohave yucca and Sapindus mukurossi. Adv Exp Med Biol 405: 1-11.

34. Tava A, Oleszek W, Jurzysta M, Berardo N, Odoardi M (1993) Alfalfa saponins and sapogenins: Isolation and quantification in two different cultivars. Phytochemical Analysis 4: 269–274.

35. Kurczhalia TV, Ward S (2003) Why do worms need cholesterol? Nat Cell Biol 5: 684-688.

36. Thorne G, Allen MW (1950) Paratylennchus hamamatus. sp. and Xiphinema index n. sp. two nematodes associated with fig roots, with a note on Paratylennchus anceps Cobb. Proceedings of the Helminthological Society of Washington 17: 27-35.

37. UNEP (2000) The montreal protocol on substances that deplete the ozone layer.

38. T