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Research Article

Effectiveness of Silver Nano-particles of Extracts of *Urtica urens* (Urticaceae) Against Root-knot Nematode *Meloidogyne incognita*

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Abstract

Natural products are an important resource for finding new pesticides. Additionally, preparation of elemental nano-particles of natural products was proven to be promising as anti-microbial agents. Present study aimed to examine the petroleum ether, ethyl acetate and ethanol extracts of *Urtica urens* and their silver-nano formulations as nematicides against root-knot nematode *Meloidogyne incognita*. Leaf extracts of *U. urens* were prepared through successive extraction and the Ag-nanoparticles of extracts and the reference nematicide (rugby®) were synthesized. Results revealed that Ag-nano formulations of extracts were effective in the management of *M. incognita*. There was an 11-fold of activity between Ag-rugby and the least toxic extract (ethyl acetate) against eggs. The LC_{50} values showed significant effects of Ag-rugby, rugby® and Ag-petroleum ether against the larvae compared with petroleum ether, ethanol and ethyl acetate extracts. Petroleum ether extract and its Ag nano-particles might be considered as environmental-safe and effective nematicides alternatives against the invasive *M. incognita*.

Key words: Nematicides alternatives, Ag-nanoparticles, successive soxhlet extraction, toxicity index, scanning electron microscope

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INTRODUCTION

Nematode infestation causes significant economic loss worldwide that was estimated by about $80 billion (Handoo, 1998). About 4,100 species of plant-parasitic nematodes have been identified and most of these species are restricted to specific geographical areas but *Meloidogyne* spp. is distributed globally (Nicol *et al*., 2011). *Meloidogyne incognita* infects a wide range of host plants causing the formation of root galls. The nematode initiates development of multinucleate giant cells, which serve as permanent feeding sites within the root and provide nutrition for the developing worm to complete its life cycle. Root-knot nematodes are considered one of the most serious soil-borne pests of many crops in Egypt (Balbaa, 2010) and worldwide (Anderson and Mulvey, 1979; Roberts *et al*., 2005; Sikora *et al*., 2007).

Management of root-knot nematodes are difficult because of its wide host range and ability to survive in various environmental conditions. Control strategies include the use of biological control predators and parasites, cover crops (*Tagetes* spp., sorghum-sudangrass and sunn hemp plants) through allelochemicals, fertilization, crop rotation, organic amendments, resistant cultivars, sanitation, solarization and nematicides (McSorley, 2002; Wang *et al*., 2007). Nematicides are the most commonly used management method, but they are costly and contaminate the environment (Adegbite *et al*., 2005) so alternatives are required.

Researchers investigated the use of plant natural products such as essential oils and other plant metabolic derivatives as nematicides. For example, saponins from *Asparagus adscendens, Albizzia chinensis* and *Acacia concinna* were active against *Meloidogyne incognita* (Meher *et al*., 1988). Thiarubrine A (a naturally occurring disulphide polyine) of *Aspilia mossambicensis* was very effective to control *Meloidogyne javanica* (Rodriguez, 1988). The combinations of thymol and benzaldehyde exhibited broad-spectrum nematicidal effect against root-knot and cyst nematode (Soler-Serratosa *et al*., 1996). The alkaloid 1,2-dehydropyrrolizidine was found in many plants (Asteraceae, Boraginaceae, Fabaceae and Apocynaceae) and had effective nematicidal activity against the Northern root-knot nematode *Meloidogyne hapla* (Thoden *et al*., 2009). Burning (dwarf) and stinging nettle (*Urtica* spp.) are rich source of secondary metabolites including 5-hydroxytryptamine, (-)-3,4-divanillyltetrahydrofuran lignin, acetylcholine, acetic acid, butyric acid, formic acid, histamine, leukotrienes and the steroids; stigmasterol, stimast-4-en-3-one and campesterol (Emmelin and Feldberg, 1949; Hirano *et al*., 1994; Wagner *et al*., 1994; Hryb *et al*., 1995; Schottner *et al*., 1997; Jimoh *et al*., 2010).

Leaf extracts of *U. urens* showed nematicidal properties against the citrus nematode *Tylenchulus semipenetrans* (Mohammad *et al*., 1981) and it was among several weeds that were effectively controlled *M. incognita* (Ornat and Sorribas, 2008). Stinging nettle (*Urtica dioica*) significantly reduced numbers of several species of nematodes (Nasiri *et al*., 2014). Increasing the biological activity and bio-compatibility of plant extracts through the preparation of new formulation might be useful. Synthesis of nanoparticles using plants extracts was reported to increase the anti-pathogenic activity.

Nanoparticles (up to 100 nm) offer improved properties compared to the larger particles because of small particle size, which help in distribution and coverage of plants (Wiesman and Chapagain, 2006). Biosynthetic of metal (Ag, Au, Cu and Cd) nano-formulation of plant extracts has received an increasing attention because of their potential application in pest control. Of particular interest, Ag nano-particles revealed antibacterial (Sathishkumar *et al*., 2009), antivirals (Rogers *et al*., 2008) and antifungal activities (Panacek *et al*., 2009). Therefore, present study investigated the effectiveness of Ag-nano formulations of polar and non-polar extracts of *Urtica urens* against the root-knot nematode (*M. incognita*).

MATERIALS AND METHODS

Rugby® 20% SC (Cadusafos: organophosphorodithioate; an insecticide and a nematicide) was used in present study as a reference nematicide and was purchased from local suppliers. Solvents, silver nitrate (ACS reagent, >99%, 209139, Sigma-Aldrich) and other chemicals were of analytical grade and purchased from reputed local suppliers.

Plant collection and sample preparation: Plants of burning nettle (*U. urens*) were collected from tomato and eggplant fields at Al-Nubaryia area, Albeheira Governorate, Egypt. Plants were identified and washed under running tap water and dried using tissue paper. Leaves were freeze-dried at -60 to -70°C for 2 days (Christ Freeze-Dryer, Gamma 1-16 LSC, Osterode, Germany). Freeze-dried samples were ground to a fine powder and stored in at -20°C freezer until extraction.

Plant extracts preparation: Approximately 25 g of freeze-dried leave samples were extracted successively in soxhlet apparatus with 200 mL of each of petroleum ether (PE), ethyl acetate (EA) and absolute ethanol (E) at 60-80°C for 6 h. Before, each successive extraction, the powder was

carefully spread on a sheet of paper to dry at room temperature. Extracts were dried over anhydrous sodium sulfate and the solvent was completely evaporated under reduced pressure using a rotary evaporator (Unipan vacuum rotary evaporator type 350 P, Poland). Dried crude extracts were kept in tightly closed brown bottles at -20°C until use.

Synthesis of silver nanoparticles: About 10 mL silver nitrate (5 mM) were added slowly to 10 mL of the plant crude extract (2500 mg L^{-1}) and 5 mL of 10 mM ascorbic acid (as reducing agent) in brown glass bottles (ca. 90 mL). Then the mixture was incubated at room temperature in dark place for 24 h. Mixture color was changed into brown indicating the formation of silver nanoparticles (Fig. 1) (Cruz *et al*., 2010; Annamalai *et al*., 2011). The Ag-nanoparticles of rugby® was prepared following a modified method of Tien *et al*. (2008). The reduction reaction of $AgNO_3$ to the nematicide was started by adding 10 mL of $AgNO_3$ (5 mM) slowly to 10 mL of rugby® in amber screw cap tubes at room temperature. Then tubes were incubated at 40°C for 12 h. The reddish-brown color solution was synthesized and was used as the Ag-rugby® formulation.

UV/vis spectra and scanning electron microscopy analysis: The synthesized Ag-nanoparticles were verified using the distinctive absorption pattern from 300-700 nm (Jenway UV/vis spectrophotometer, Model 6305, Bibby Scientific Limited, Staffordshire, UK). The morphology and size of the nano-particles were measured and photographed by the Scanning Electron Microscope (SEM) (Model JSM-5300, JEOL, USA) after coating the sample using the ion sputtering device (Fine Coat, Model JFC-1100E, JEOL, USA). In the SEM, an electron beam was focused into affine probe and raster scanned over a small rectangular slide. As the beam interacts with the sample, it generates signals (i.e., secondary electrons, internal currents and/or photon emission), which can be appropriately detected.

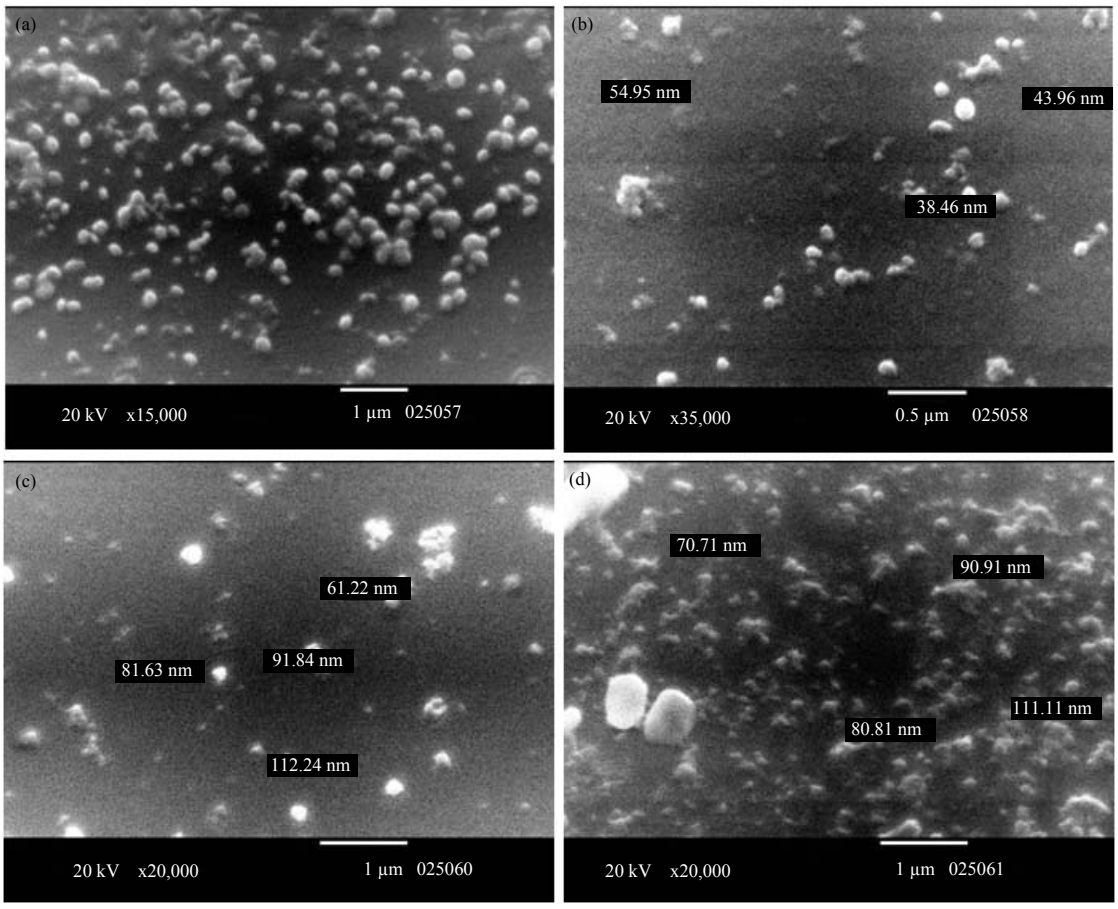

Fig. 1(a-d): Scanning electron microscopy images of (a) Ag-nanoparticles of rugby, extracts of *U. urens*, (b) Ethyl acetate, (c) Ethanol and (d) petroleum ether

Root-knot nematode (*M. incognita*) culture: Root-knot nematode (*M. incognita*) was collected from eggplants at Al-Nubaryia area and used to establish a nematode culture in the greenhouse on eggplant plants. Eggplants were planted in plastic pots (30 cm diameter, one plant per pot) that filled with autoclave-sterilized peat moss. Plants were grown under greenhouse conditions (24±2°C and 70±5% RH), watered once every 3-4 days. Fertilization was done every 6-8 days with commercial fertilizer that has N (19%), P (19%), K (19%), S (5.94 %), Fe (1000 mg L^{-1}), Zn (500 mg L^{-1}) and Mn (500 mg L^{-1}) (Farmers VASCON, Moubarak Industries City, Quesna, Menoufia, Egypt) at 1 kg/1000 L of water. The *M. incognita* egg and larval suspensions were prepared from infected roots with obvious galls (about 60 days of inoculation).

Nematicidal activity against eggs and larvae of *M. incognita*: The efficiency of the *U. urens* extracts and their nano-formulations to eggs and larvae of *M. incognita* were tested by applying 1 mL of series of concentrations 125, 250, 500 and 1000 mg L^{-1} of each treatment to 1 mL of the eggs or larvae suspension. Egg suspension (100 eggs mL^{-1}) and 50 larvae (2nd stage) mL^{-1} of *M. incognita* were prepared using a special microscopic slide for counting the nematodes and used for each replicate. Glass tubes were incubated at 27±2°C for 2 and 7 days for larvae and eggs, respectively. Eggs hatchability and larval mortality were evaluated. Assays were repeated twice and each treatment was replicated 5 times. Average results of the two assay times were compared with the recommended nematicide and water as control. The median lethal concentration (LC_{50}) and toxicity index values of each treatment were calculated using Log dose Probit (LdP) line software (Ehabsoft, Cairo, Egypt).

RESULTS

Synthesis of Ag-nano formulation: Data presented in Fig. 1 showed that the reduction reaction of rugby® and *U. urens* extracts with silver nitrate was successful in the synthesis of circular nano-sized particles. The ESM photographs showed that the size of Ag nano-particles of rugby® (Ag-rugby, Fig. 1a) was homogeneous. The *U. urens* extracts were reduced by silver nitrate; petroleum ether extract (Ag-PE, Fig. 1d) showed more homogeneous and smaller size compared to ethyl acetate (60-112 nm) (Ag-EA, Fig. 1c) and ethanol extract (80-111 nm) (Ag-E, Fig. 1b). Nano-formulation of Ag and petroleum ether extract was the most homogenous particle size compared with rugby®, ethyl and extracts of ethyl acetate and ethanol of *U. urens*.

Biological activity against eggs stage of *M. incognita*: Efficacy of natural extracts of *U. urens* in the management of *M. incognita* compared to rugby® (recommended nematicide) and their Ag-nano formulations (Ag-rugby, Ag-PE, Ag-EA and Ag-E) were assayed under laboratory condition (Table 1). Ag-rugby suppressed the hatchability of eggs with an LC_{50} value of 5.62 mg L^{-1} compared to 7.21 mg L^{-1} for rugby® itself. The Ag-PE had the most suppressive effect to eggs hatchability compared to other extracts but was less than Ag-rugby and rugby®. Petroleum ether extract was more toxic (LC_{50}; 28.77 mg L^{-1}) than ethanol, Ag-E, Ag-EA and ethyl acetate extracts with LC_{50} values of 40.92, 42.39, 45.71, 47.92 and 61.96 mg L^{-1}, respectively.

Biological activity against 2nd larval stage of *M. incognita*: Results in Table 2 showed the nematicidal activity of natural extracts of *U. urens* and their Ag-nano formulations compared with rugby® and its Ag-nano-sized formulation. The toxicity order was Ag-rugby, rugby®, Ag-PE, Ag-E, Ag-EA, petroleum ether extract, ethyl acetate extract and ethanol extract with LC_{50} values of 6.72, 7.81, 40.92, 42.39, 61.96, 169.23, 175.36 and 188.81 mg L^{-1}, respectively. The Ag-rugby, rugby® and Ag-PE were more toxic to 2nd larval stage compared to petroleum ether, ethanol and ethyl acetate

Table 1: Median lethal concentration (LC_{50}) and toxicity index values of the ethanol, ethyl acetate and petroleum ether extracts of *U. urens* and their nano-formulations compared to the nematicide rugby® and its Ag-nano particles against eggs of *M. incognita*

	LC_{50} (mg L^{-1})				
Treatments	LC_{50}	Lower limit	Upper limit	Slope	Toxicity index[1]
Ag-rugby	5.62	0.06	16.90	1.374	100.0
Rugby®	7.21	0.52	17.78	1.362	78.00
Ag-petroleum ether	28.77	2.49	67.02	0.892	19.55
Petroleum ether	40.92	2.63	93.14	0.718	13.74
Ethanol	42.39	1.38	101.31	0.637	13.27
Ag-ethanol	45.71	11.18	84.08	1.080	12.30
Ag-ethyl acetate	47.92	10.45	89.96	0.982	11.74
Ethyl acetate	61.96	11.69	115.78	0.819	9.08

[1]Comparison among all tested materials according to the most toxic one (Sun, 1950)

Table 2: Median lethal concentration (LC_{50}) and toxicity index values of the ethanol, ethyl acetate and petroleum ether extracts of *U. urens* and their nano-formulations compared to the nematicide rugby® and its Ag-nano formulation against 2nd larval stage of *M. incognita*

	LC_{50} (mg L^{-1})				
Treatments	LC_{50}	Lower limit	Upper limit	Slope	Toxicity index[1]
Ag-rugby	6.72	0.35	17.33	1.386	100.0
Rugby®	7.81	0.43	19.42	1.340	86.01
Ag-petroleum ether	40.92	2.63	93.14	0.718	16.42
Ag-ethanol	42.39	1.38	101.31	0.637	15.85
Ag-ethyl acetate	61.96	11.69	115.77	0.819	10.85
Petroleum ether	169.23	100.55	230.78	1.052	3.97
Ethyl acetate	175.36	94.66	247.20	0.917	3.83
Ethanol	188.81	112.47	258.32	0.986	3.56

[1]Comparison among all tested materials according to the most toxic one (Sun, 1950)

extracts. Limits of confidence of LC_{50} values showed significant effects between Ag-rugby, rugby® and Ag-PE and petroleum ether, ethanol and ethyl acetate extracts.

DISCUSSION

Nanoparticles (up to 100 nm) offer improved properties and activities compared to the original material (Wiesman and Chapagain, 2006). To the best of our knowledge, few researchers tested the nematicidal activity of extracts of burning nettle against nematode; Mohammad *et al*. (1981) reported that leave extracts of *U. urens* effectively control the *Tylenchulus semipenetrans* nematode, Decker (1982) listed *U. urens* (stinging nettle) among plants that control root-knot nematode and Nasiri *et al*. (2014) found that exudates of *U. dioica* effectively controlled the Pratylenchus, Aphelenchoides and Helicotylenchus nematodes.

Present study was the first study to report about testing the efficacy of Ag-nano formulations of extracts of *U. urens* against the worldwide nematode *M. incognita*. Results reported herein revealed an 11-fold of activity between Ag-rugby and the least toxic extract (ethyl acetate) against eggs. However, limits of confidence of the median lethal concentrations showed overlap among tested extracts, nano-extracts, rugby® and Ag-rugby, which highlights similar suppressive effects of eggs hatchability. There was small difference in the activity between extracts of *U. urens* and their corresponding nano-formulations, where only a 1.3-fold of activity was reported. No difference was noticed between the ethanol extract and its Ag-formulation.

Examination of the slope of the LDP-lines reported similarity in the nematicidal action among rugby® and the petroleum ether extract, which were different from that of the ethanol and ethyl ether extracts. Ag-petroleum ether extract was the most effective against both eggs and larval stages this might be due to the fact that Ag-PE had the most homogenous particle size. Moreover, present study showed similar effects of the petroleum ether extracts and their Ag nano-particles and rugby®, which highlights the potential use of such components in controlling such invasive pest with environmentally-friendly and less toxic plant extracts.

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REFERENCES

Adegbite, A.A., N.A. Amusa, G.O. Agbaje and L.B. Taiwo, 2005. Screening of cowpea varieties for resistance to *Meloidogyne incognita* under field conditions. Nematropica, 35: 155-159.

Anderson, R.V. and R.H. Mulvey, 1979. Errata for plant-parasitic nematodes in Canada. Part 1: An illustrated key to the genera. Monograph No. 20, Biosystematics Research Institute, Ottawa, Ontario, Agriculture Canada, pp: 152.

Annamalai, A., T.B. Sarah, A.J. Niji, D. Sudha and V.L. Christina, 2011. Biosynthesis and characterization of silver and gold nanoparticles using aqueous leaf extraction of *Phyllanthus amarus* Schum. And Thonn. World Applied Sci. J., 13: 1833-1840.

Balbaa, E.M.E., 2010. Biological and environmental studies on root-knot nematode affecting Leguminosae. M.Sc. Thesis, Faculty of Agriculture, Alexandria University, Egypt.

Cruz, D., P.L. Fale, A. Mourato, P.D. Vaz, M.L. Serralheiro and A.R.L. Lino, 2010. Preparation and physicochemical characterization of Ag nanoparticles biosynthesized by *Lippia citriodora* (Lemon Verbena). Colloids Surf. B: Biointerfaces, 81: 67-73.

Decker, H., 1982. Plant Nematodes and their Control. Pauls Press, New Delhi, India.

Emmelin, N. and W. Feldberg, 1949. Distribution of acetylcholine and histamine in nettle plants. New Phytol., 48: 143-148.

Handoo, Z.A., 1998. Plant-parasitic nematodes. United States Department of Agriculture, Agricultural Research Service, August, 1998. http://www.ars.usda. gov/Services/ docs. htm? docid=9628.

Hirano, T., M. Homma and K. Oka, 1994. Effects of stinging nettle root extracts and their steroidal components on the Na^+, K^+-ATPase of the benign prostatic hyperplasia. Planta Medica, 60: 30-33.

Hryb, D.J., M.S. Khan, N.A. Romas and W. Rosner, 1995. The effect of extracts of the roots of the stinging nettle (*Urtica dioica*) on the interaction of SHBG with its receptor on human prostatic membranes. Planta Medica, 61: 31-32.

Jimoh, F., A. Adedapo, A. Aliero and A. Afolayan, 2010. Polyphenolic and biological activities of leaves extracts of *Argemone subfusiformis* (Papaveraceae) and *Urtica urens* (Urticaceae). Revista Biologia Tropical, 58: 1517-1531.

McSorley, R., 2002. Losses to Nematodes. In: Encyclopedia of Pest Management, Pimentel, D. (Ed.). CRC Press, Boca Raton, ISBN: 9781439870587.

Meher, H.C., S. Walia and C.L. Sethi, 1988. Effect of steroidal and triterpenic saponins on the mobility of juveniles of *Meloidogyne incognita*. Indian J. Nematol., 18: 244-247.

Mohammad, H.Y., S.I. Husain and A.J. Al-Zarari, 1981. Effect of plant extracts of some poisonous plants of Iraq on mortality of citrus nematode, *Tylenchulus semipenetrans* Cobb. Acta Botanica Indica, 9: 198-200.

Nasiri, M., K. Azizi, H. Hamzehzarghani and R. Ghaderia, 2014. Studies on the nematicidal activity of stinging nettle (*Urtica dioica*) on plant parasitic nematodes. Arch. Phytopathol. Plant Protect., 47: 591-599.

Nicol, J.M., S.J. Turner, D.L. Coyne, L. den Nijs, S. Hockland and Z.T. Maafi, 2011. Current Nematode Threats to World Agriculture. In: Genomics and Molecular Genetics of Plant-Nematode Interactions, Jones, J., G. Gheysen and C. Fenoll (Eds.). Chapter 2, Springer, Berlin, Germany, ISBN: 978-94-007-0433-6, pp: 21-43.

Ornat, C. and F.J. Sorribas, 2008. Integrated Management of Root-Knot Nematodes in Mediterranean Horticultural Crops. In: Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes, Ciancio, A. and K.G. Mukerji (Eds.). Chapter 14, Springer-Verlag, Netherlands, ISBN: 978-1-4020-6062-5, pp: 295-319.

Panacek, A., M. Kolar, R. Vecerova, R. Prucek and J. Soukupova *et al*., 2009. Antifungal activity of silver nanoparticles against *Candida* spp. Biomaterials, 30: 6333-6340.

Roberts, P.A., J.R. Matthews and J.D. Ehler, 2005. Root-knot nematode resistant cowpea cover crops in tomato production systems. Agron. J., 97: 1626-1635.

Rodriguez, E., 1988. Dithiopolyacetylenes as Potential Pesticides. In: Biologically Active Natural Products: Potential Use in Agriculture, Cutler, H.G. (Ed.). 2nd Edn., The Society, London, UK., ISBN: 9780841215566, pp: 432-437.

Rogers, J.V., C.V. Parkinson, Y.W. Choi, J.L. Speshock and S.M. Hussain, 2008. A preliminary assessment of silver nanoparticle inhibition of monkeypox virus plaque formation. Nanoscale Res. Lett., 3: 129-133.

Sathishkumar, M., K. Sneha, S.W. Won, C.W. Cho, S. Kim and Y.S. Yun, 2009. *Cinnamon zeylanicum* bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. Colloids Surf. B: Biointerfaces, 73: 332-338.

Schottner, M., D. Ganβer and G. Spiteller, 1997. Lignans from the roots of *Urtica dioica* and their metabolites bind to human sex hormone binding globulin (SHBG). Planta Medica, 63: 529-532.

Sikora, R.A., K. Schafer and A.A. Dababat, 2007. Modes of action associated with microbially induced in planta suppression of plant-parasitic nematodes. Australasian Plant Pathol., 36: 124-134.

Soler-Serratosa, A., N. Kokalis-Burelle, R. Rodriguez-Kabana, C.F. Weaver and P.S. King, 1996. Allelochemicals for control of plant-parasitic nematodes. 1. *In vivo* nematicidal efficacy of thymol and thymol/benzaldehyde combinations. Nematropica, 26: 57-71.

Sun, Y.P., 1950. Toxicity index-An improved method of comparing the relative toxicity of insecticides. J. Econ. Entomol., 43: 45-53.

Thoden, T.C., J. Hallmann and M. Boppre, 2009. Effects of plants containing pyrrolizidine alkaloids on the Northern root-knot nematode *Meloidogyne hapla*. Eur. J. Plant Pathol., 123: 27-36.

Tien, D.C., C.Y. Liao, J.C. Huang, K.H. Tseng and J.K. Lung *et al*., 2008. Novel technique for preparing a nano-silver water suspension by the ARC-discharge method. Rev. Adv. Mater. Sci., 18: 750-756.

Wagner, H., F. Willer, R. Samtleben and G. Boos, 1994. Search for the antiprostatic principle of stinging nettle (*Urtica dioica*) roots. Phytomedicine, 1: 213-224.

Wang, K.H., C.R. Hooks and A. Ploeg, 2007. Protecting crops from nematode pests: Using marigold as an alternative to chemical nematicides. Cooperative Extension Service, College of Tropical Agriculture and Human Resources, Plant Disease, July 2007, University of Hawai, pp: 1-6.

Wiesman, Z. and B.P. Chapagain, 2006. Larvicidal activity of saponin containing extracts and fractions of fruit mesocarp of *Balanites aegyptiaca*. Fitoterapia, 77: 420-424.