Research in Veterinary Science 93 (2012) 303–309

Contents lists available at SciVerse ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Acaricidal activity of aqueous extract and synthesized silver nanoparticles from *Manilkara zapota* against *Rhipicephalus* (*Boophilus*) *microplus*

G. Rajakumar, A. Abdul Rahuman *

Unit of Nanotechnology and Bioactive Natural Products, Post Graduate and Research Department of Zoology, C. Abdul Hakeem College, Melvisharam 632 509, Vellore District, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 31 January 2011
Accepted 1 August 2011

Keywords:
Rhipicephalus (*Boophilus*) *microplus*
Aqueous extract
Manilkara zapota
Silver nanoparticles
SEM-EDX
XRD

ABSTRACT

Traditional parasite control is primarily based on the use of chemical acaricides, which unfortunately have many negative side effects. The aim of the present study was to evaluate the effect of plant synthesized silver nanoparticles (AgNPs) using aqueous leaf extract of *Manilkara zapota* to control *Rhipicephalus* (*Boophilus*) *microplus*. The synthesized AgNPs were characterized by UV–vis spectrum, scanning electron microscopy (SEM), Fourier transform infrared and X-ray diffraction. The UV–vis spectrum of the aqueous medium containing silver nanostructures showed a peak at 421 nm corresponding to the surface plasmon resonance band of AgNPs. SEM supports the biosynthesis and characterization of AgNPs with spherical and oval in shape and size of 70–140 nm. Acaricidal activity of aqueous leaf extract of *M. zapota* and synthesized AgNPs were carried out against *R.* (*B.*) *microplus* and the results showed the LC_{50} values of 16.72 and 3.44 mg/L; r^2 = 0.856 and 0.783), respectively.

1. Introduction

Rhipicephalus (*Boophilus*) *microplus* is one of the most widely distributed tick species and constitutes a major problem for the cattle industry in tropical and subtropical regions of the world. The tick is responsible for severe losses caused by tick worry, blood loss, hide damage, injection of toxins, and diseases transmitted by the parasite (Sabatini et al., 2001; Ducornez et al., 2005). Almost of all dairy and meat animals in India are suffering from tick infestation and cause significant economic loss (Ghosh et al., 2006). In a recent estimate, the control cost of ticks and tick borne diseases in dairy sector has been estimated in the tune of $498.7 million per annum (Minjauw and Mc Leod, 2003). *R. microplus* is a one-host tick that usually infests cattle and is responsible for economic losses that range over hundreds of millions of dollars per year (Guerrero et al., 2006).

Tick control, in general, is based on the use of chemical acaricides. Unfortunately, continual use of this method has many negative side effects, including the possibility of causing the development of chemical resistance in some ticks population, and food and environmental contamination if these products are improperly used. Public concerns about the environmental impact and safety of chemical applications are driving research into alternative, sustainable methods for tick control, including biological control (Chandler et al., 2000). Natural bioactive compounds are a promising alternative for tick control (Ribeiro et al., 2007; Fernandes and Freitas, 2007). They might offer additional advantages such as low toxicity to mammals and more environmentally friendly (Batish et al., 2008; Rosado-Aguilar et al., 2010).

Manilkara zapota L. (*Sapotaceae*) is evergreen, glabrous tree with a milky juice. Aqueous extracts of *M. zapota* was prepared and evaluated the anti-quorum-sensing inhibitory activity of extract was tested against *N*-acyl-homoserine lactone-mediated phenotypic expressions of *Chromobacterium violaceum* and virulence factor expression in *Pseudomonas aeruginosa* PAO1 (Musthafa et al., 2010) and the same extract was tested for antibacterial activity against 10 Gram positive, 12 Gram negative bacteria and one fungal strain (Nair and Chanda, 2008). *In vitro* antibacterial activity assay against 14 important human pathogenic bacteria was done in aqueous extract of *M. zapota* and showed highest inhibitory activity against *Staphylococcus aureus*, *Citrobacter* sp., and *Proteus mirabilis* (Satish et al., 2008). The maximum efficacy was observed in the aqueous extract of *Musa paradisiaca* with LC_{50} values of 28.96, 31.02 mg/L and synthesized AgNPs with LC_{50} values of 1.87 and 2.02 mg/L against the *Haemaphysalis bispinosa*, *Hippobosca maculata*, respectively (Jayaseelan et al., 2011b). Acaricidal activity of the active constituent derived from *Pyrus ussuriensis* fruits against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* was examined and the LD_{50} value of the ethyl acetate fraction obtained from the aqueous extract of *P. ussuriensis* fruits was 9.51 and 8.59 μg/cm^3, respectively (Lee, 2007). The aqueous leaf extract of *Mimosa pudica* and synthesized AgNPs were tested

* Corresponding author. Tel.: +91 94423 10155/04172 269009; fax: +91 04172 269487.
E-mail address: abdulrahuman6@hotmail.com (A. Abdul Rahuman).

0034-5288/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2011.08.001

for acaricidal activity against *R.* (*B.*) *microplus*, which showed increased activity from the synthesized AgNPs (Marimuthu et al., 2010).

The use of plants for synthesize of nanoparticles are rapid (a single-step method for biosynthesis process), low cost, eco-friendly and safe for human therapeutic use (Huang et al., 2007; Kumar and Yadav, 2009). Furthermore the aim of the present study was to evaluate the acaricidal activity of plant synthesized AgNPs using aqueous leaf extract of *Manilkara zapota* to control *R.* (*B.*) *microplus*.

2. Materials and methods

2.1. Rhipicephalus microplus collection

Engorged female ticks (20–50 individuals) were collected directly from infested animals, placed in identified cardboard boxes, and transported to the laboratory. In the laboratory, the engorged females were adhered by the dorsal surface in a glass plate (120 × 80 mm), with double sided adhesive tape. This glass plate was put inversely on a glass Petri dish (150 mm diameter), aiming at to collect the eggs laid. The engorged females were maintained in a biological oxygen demand incubator at 27 ± 1 °C, RH ⩽80% and a 12:12 h photoperiod. In order to obtain larvae of the same age cohort, egg batches were collected daily in separate hatchings tubes of polyethylene with screw caps. Larvae used for the bioassays were 14–21 days old (Fernandes and Freitas, 2007). The parasites were identified in the Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu.

2.2. Bioassays

The applied method in the present study to verify the acaricidal activity of AgNPs against the larvae of *R. microplus* was developed as per the method of FAO (2004), incorporating slight modifications to improve practicality and efficiency of tested materials (Fernandes et al., 2005). From the stock solution, 100 mg/L was prepared and a series of filter paper envelopes (Whatman filter paper No. 1, 12 × 18 cm) with micropores were treated with each concentration of extract of *M. zapota*. The synthesized particles were impregnated with 20 mg/L of which 3 ml solution of the stock was uniformly distributed with a pipette on internal surfaces. Five envelopes were impregnated with each tested solution. The control papers were impregnated with $AgNO_3$ and distilled water only. The opening of the envelopes (treated and inoculated with larval ticks) was folded (10 mm) and re-sealed with a metallic clip, with its identification mark (tested solution and concentration) on the outside. The packets are placed in the BOD incubator at a temperature of 28–30 °C and 80–90% RH for 24 h. The envelopes were opened 24 h after exposure and the number of live and mortality larvae were recorded (Fernandes and Freitas, 2007). Larvae used for the bioassays were 14–21 days old. The experimental media, in which 100% mortality of larvae occurs alone, were selected for dose–response bioassay (FAO (2004). The percentage mortality in all of the experimental batches of larvae was corrected by applying Abbott's formula:

$$\text{Corrected percent mortality} = \%\text{test mortality} - \%\text{control mortality}/100 - \%\text{control mortality} \times 100$$

2.3. Preparation of M. zapota leaf aqueous extract and silver nitrate solution

Aqueous extract was prepared by mixing 50 g of dried leaf powder with 500 mL of water (boiled and cooled distilled water) with constant stirring on a magnetic stirrer (Minjas and Sarda, 1986). The suspension of dried leaf powder in water was left for 3 h, filtered through Whatman No. 1 filter paper, and the filtrate was stored in amber colored air tight bottle at 10 °C and used within a week. 1 mM $AgNO_3$ powder was added in 100 ml distilled water and used within a day for the assay.

2.4. Synthesis of silver nanoparticles using M. zapota leaf extract

M. zapota leaves were washed thoroughly in tap water for 10 min in order to remove the dust particles and rinsed briefly in deionized water. The plant leaf broth solution was prepared by taking 10 g of washed and finely cut leaves in a 250 mL Erlenmeyer flask along with 100 mL of deionized water and then boiling the mixture at 60 °C for 5 min. After boiling, the solution was decanted, and 12 mL of this broth was added to 88 mL of 1 mM aqueous silver nitrate ($AgNO_3$) solution and the resulting solution became brown in color. This extract was filtered through nylon mesh (Spectrum), followed by Millipore hydrophilic filter (0.22 μm) and used for further experiments (Santhoshkumar et al., 2010). A control setup was also maintained without *M. zapota* extract and color intensity of the extracts was measured at 421 nm for different intervals (1, 2, 3, 4 and 5 h respectively).

2.5. Characterization of silver nanoparticles

Synthesis of AgNPs solution with leaves extract may be easily observed by UV–vis spectroscopy. The bioreduction of the Ag^+ ions in solutions was monitored by periodic sampling of aliquots (1 mL) of the aqueous component after 20 times dilution and measuring the UV–vis spectra of the solution. UV–vis spectra of these aliquots were monitored as a function of time of reaction on a Shimadzu 1601 spectrophotometer in 300–700-nm range operated at a resolution of 1 nm. Further, the reaction mixture was subjected to centrifugation at 60,000×*g* for 40 min; resulting pellet was dissolved in deionized water and filtered through Millipore filter (0.45 μm). An aliquot of this filtrate containing silver nanoparticles was used for X-ray diffraction (XRD), Fourier transform infrared (FTIR) and Scanning electron microscopy (SEM) and Energy-dispersive X-ray (EDX) analysis.

XRD measurements of the *M. zapota* leaf broth reduced Ag nanoparticles were carried out on films of the respective solutions drop-coated onto glass substrates on a Phillips PW 1830 instrument operating at a voltage of 40 kV and a current of 30 mA with CuKα1 radiation. For FTIR spectroscopy measurements, dry powders of the nanoparticles were obtained in the following manner. The AgNPs synthesized after 5 h of reaction of *M. zapota* leaf broth were centrifuged at 10,000 rpm for 15 min, following which the pellet was redispersed in sterile distilled water to get rid of any uncoordinated biological molecules. The process of centrifugation and redispersion in sterile distilled water was repeated three times to ensure better separation of free entities from the metal nanoparticles. The purified pellets were then dried and the powders subjected to FTIR spectroscopy measurement. Characterization involved FTIR analysis of the dried powder of AgNPs, by scanning it in the range 350–4000 cm^{-1} at a resolution of 4 cm^{-1}. These measurements was carried out on a Perkin–Elmer Spectrum One instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets and the pellets was mixed with KBr powder and pelletized after drying properly. The pellet was later subjected to FTIR spectroscopy measurement. For electron microscopic studies, 25 μl of sample was sputter coated on copper stub and the images of nanoparticles were studied using SEM-EDX (JEOL, Model JFC-1600).