Veterinary Parasitology 235 (2017) 57–63

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Research paper

A novel assembly pheromone trap for tick control in dog kennels

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ARTICLE INFO

Article history:
Received 1 August 2016
Received in revised form 5 December 2016
Accepted 9 January 2017

Keywords:
Rhipicephalus sanguineus
Assembly pheromone
Petridish bioassay
Sticky tick trap device

ABSTRACT

A novel ecofriendly sticky tick trap device for the control of dog tick *Rhipicephalus sanguineus* using gold nanoparticle assembly pheromone complex as a bait was developed. Assembly pheromones comprising of guanine, xanthine and adenine in the ratio of 25:1:1 was encapsulated in gold nanoparticle. The response of the different stages of unfed *R. sanguineus* ticks was evaluated using petridish bioassay. Statistical analysis was done using chi-square test. Petridish bioassay with unfed stages of *R. sanguineus* revealed that 100% of the larvae, nymph and adults were attracted to assembly pheromone nanogold complex within 24 h. Of the 952 ticks trapped, ticks of different stages trapped in total by the baited sticky trap device, 543 (57%) were engorged and 409 (43%) were unfed ticks. The study revealed that assembly pheromone baited traps has the potential to control tick infestations in dog kennels.

1. Introduction

The brown dog tick, *Rhipicephalus sanguineus* is the prevalent species in dogs in Tamil Nadu, India (Koshy et al., 1983). It plays a major role in the transmission of *Ehrlichia canis*, *Babesia canis* and *Hepatozoan canis* (Vairamuthu et al., 2014). The mainstay of tick control measure is the use of chemical acaricides. This has serious drawbacks such as chemical pollution of the food chain and environment (Dipeolu and Ndungu, 1991). Moreover, any failure to continue with strict application of acaricides results in rapid propagation of tick populations (Imamura et al., 2007). In addition, there is rapid development of resistance to the acaricides commonly used for control (Kah et al., 2012). These drawbacks point to an urgent need for novel tick control measure to reduce or replace acaricidal use.

Nanotechnology holds the promise for controlled delivery of pheromones (Bhattacharyya et al., 2010) Nanoencapsulation has been used in efficient and safe administration of pesticides, herbicides and pheromones in the agricultural sector (Kuzma and Verhage, 2006). It aids in slow release of active ingredient and the bioavailability of volatile pheromones increases through use of nanoformulations (Kah et al., 2012). The pheromones also show high reactivity at nanoscale when compared to their bulk counterparts thereby ensuring that less quantity of nanopheromone is sufficient for enhanced insect attraction (Debnath et al., 2011). Hence, the present study attempted to use gold nanoparticle for encapsulation of assembly pheromone to lure and kill stages of the brown dog tick in a dog shelter for strays.

2. Materials and methods

2.1. Collection of ticks

The brown dog tick, *Rhipicephalus sanguineus* was collected from the dogs presented at Small animal clinics of Madras Veterinary College Teaching Hospital and those housed in Blue Cross of India, Velachery, Chennai, India. The brown dog ticks were identified by observing the morphological features such as brevi-rostrum, hexagonal basis capitulum and bifid first coxa using a stereo zoom microscope (Koch, 1982). The engorged female ticks were collected and were placed in dry vials and covered with porous cloth. They were maintained in the laboratory for oviposition by placing the vial in a tray filled with moist sand to maintain a relative humidity (RH) of ≥80%. They were left undisturbed until they oviposited and the larvae hatched out. Engorged larvae and engorged nymphal stages were maintained in the same way until they moulted to unfed nymph and adult respectively. The unfed larvae, nymphs and adults were utilized for the in vitro trials.

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http://dx.doi.org/10.1016/j.vetpar.2017.01.005
0304-4017/

2.2. Synthesis of gold nanoparticles

Gold nanoparticles were synthesized by reducing the 1 mM gold chloride (Sigma-Aldrich) solution with 0.5% w/v aqueous pectin solution (PS) by under microwave irradiation. Pectin at the rate of 0.5 g was dissolved in 100 mL of distilled water and varying volumes of PS (0.5%, 1% and 2%,) was added to 0.2 mL of 1 mM of gold chloride solution and stirred well at room temperature. This was followed by microwave irradiation 700 W and heating for 8–10 min. The reaction mixture turned red wine colour indicating the formation of pectin reduced gold nanoparticles (Pec-GNPs). UV–vis spectroscopic analysis was performed to confirm the formation of GNPs. Pec-GNPs were purified by removing the excess unreacted pectin by repeated washing with centrifuge operated at 18,000 rpm for 20 min and the pellet was redispersed in deionised water (Raja et al., 2016)

2.3. Procedure for encapsulation of assembly pheromone

Chitosan (2%) solution was prepared by dissolving 0.8 g of chitosan in 40 mL of distilled water with 1% acetic acid and kept at constant stirring for about 18 h continuously. All the pheromones and chemicals were purchased from Sigma-Aldrich, Bangalore, India, unless specified otherwise. The composition of assembly pheromone used was guanine 95 mg, xanthine 3.8 mg and adenine 3.8 mg (Sonenshine, 2004 and Ranju, 2011). Assembly pheromone stock solutions were prepared (1 mg pheromone in 1 mL distilled water). Moreover, adenine dissolved completely in hot water whereas guanine and xanthine dissolved completely in cold water. The assembly pheromone (25:1:1) was dispersed in the chitosan matrix (500 μL) and was continuously stirred for about

1 h. Further, add 200 μL of prepared gold nanoparticle to the assembly pheromone chitosan solution and was continuously stirred overnight to get the well homogenized solution.

2.4. In vitro assessment

2.4.1. Petridish bioassay

A modified method of Yodder and Stevens (2000), adopted by Ranju (2011) and Dhivya (2013) was employed in the current study. The filter paper disc (1.5 × 1.5 cm) impregnated with 200 μL assembly pheromone gold nanoparticles was placed in one quadrant of the petridish. Similarly, control trials were done using filter paper discs impregnated with 200 μL assembly pheromone control as well as gold nanoparticle polymer control. Unfed larvae, nymph and adult stages were released in the opposite quadrant. The petridish was covered with another petridish and sealed with laboratory grade parafilm inorder to avoid response of the ticks to carbon dioxide emitted by the investigator which may lead to erroneous results. Ticks were handled only with camel hair brushes (No. 9). Nitrile gloves were worn to prevent the direct contamination of tick sensory organs by human skin volatiles. For each trial, only ticks that were active were used. This ensured that all ticks tested were in a similar state and provided an accurate estimate of the attractiveness of the assembly pheromone complex. Utmost care was taken in handling ticks so that the first pair of legs was not damaged. All tests were conducted at room temperature and results were read after 10 min, 30 min, 2 h and 24 h. Fresh, unexposed ticks were used for each trial. The trial was applied to 500 larvae, 50 nymphs and 100 adult ticks.

2.5. Ultra violet (UV) spectrophotometry

The encapsulation of assembly pheromones by nanoparticles was confirmed by UV Spectrophotometry (Shimadzu UV 1800Ver. 2.43).

2.6. Transmission electron microscope (TEM) analysis

HRTEM images were recorded using FEI Tecnai G2 F20 S-Twin Transmission electron microscope.

2.7. Field trials

2.7.1. Design of sticky tick trap device

The tick trap device was designed using an acrylic sheet of size 15cm × 10 cm which forms the base of the trap. A transparent stiff sheet was stuck on to the acrylic sheet using cellotape at the corners. A double sided sticky tape was stuck to this sheet. The double sided sticky sheet was cut into strips of 1 cm breadth and 10 such strips were placed on each sticky trap. A total of 40 filter paper discs were impregnated with each 5 μL of assembly pheromone nanogold solution. Control tick trap devices were prepared in the same manner as described above with plain gold nanoparticle without addition of the assembly pheromone. The lures were labelled and then the field trials were carried out.

2.7.2. Field evaluation of the assembly pheromone baited traps

Field trials were carried out by placing the pheromone trap devices in the kennels housing stray pups and adults at The Blue Cross of India, Velachery, Chennai. These devices were placed on the window sill about 3 ft from the ground in such a manner that dogs did not have access to the device. Six replicates for each polymer type and its corresponding control were placed simultaneously in different parts of the kennels for a period of 7 days. The number and stages of ticks that were found dead and stuck to the trap were counted and recorded after 7 days. The per cent mortality of different stages was calculated.

2.8. Statistical analysis

Results are presented as mean ± S.D. (n = 3). ANOVA (Analysis of variance) and chi-square test was done to determine the significant differences among the groups. The observed differences were statistically significant when $p < 0.05$.

3. Results

3.1. Nanoparticles

3.1.1. TEM analysis

The surface morphology revealed a smooth surface. The nanoparticle was more or less spherical and the particle size was 15–25 nm (Fig. 1)

3.1.2. UV spectrophotometry

UV spectrophotometry revealed individual peaks for assembly pheromone (400 nm) and gold nanoparticle (534 nm), thereby confirming the encapsulation of the assembly pheromone by gold nanoparticle (Fig. 2)

3.1.3. Assembly pheromone gold nanoparticle solution

3.1.3.1. In vitro assessment—petridish bioassay.

3.1.3.1.1. Response of larva. Five hundred unfed *R. sanguineus* larvae were exposed to assembly pheromone encapsulated in gold nanoparticle in petridish bioassay. The response of the larvae to the assembly pheromone—nanogold solution was evaluated at 10 min,