

## International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693  
ISSN Online: 2617-4707  
NAAS Rating (2025): 5.29  
IJABR 2025; SP-9(10): 1043-1044  
[www.biochemjournal.com](http://www.biochemjournal.com)  
Received: 27-07-2025  
Accepted: 29-08-2025

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## Efficacy of nanophomone impregnated sticky tick trap device in brown dog tick control in dog kennels

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DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i10Sm.5985>

### Abstract

Tick assembly pheromones consisting of guanine, xanthine, and adenine in a ratio of 25:1:1 were encapsulated within gold nanoparticles. An innovative eco-friendly sticky tick trap device was created for controlling the dog tick *Rhipicephalus sanguineus*, utilizing the gold nanoparticle assembly pheromone complex as bait. Field trials conducted in dog kennels located in the Thrissur district of Kerala demonstrated that the gold nanoparticle-baited sticky traps captured 57% of engorged ticks and 43% of unfed questing stages of *R. sanguineus*. The findings indicate that assembly pheromone-baited traps hold potential for managing tick infestations in dog kennels.

**Keywords:** *Rhipicephalus sanguineus*, assembly pheromone, sticky tick trap device

### Introduction

The brown dog tick (*Rhipicephalus sanguineus*) is the most common species found on dogs in Kerala (Hiron *et al.*, 1983) [6]. Traditional methods for tick control primarily involve chemical acaricides, which have significant disadvantages; these include environmental contamination and disruption of food chains. Furthermore, failure to consistently apply acaricides can lead to rapid increases in tick populations, along with swift development of resistance to commonly used products. These issues underscore the urgent need for alternative approaches to reduce or eliminate reliance on chemical acaricides (Goswami and Yadavb, 2021) [4].

Nanotechnology presents a promising avenue for controlled pheromone delivery. Nanoencapsulation has proven effective in the safe administration of pesticides, herbicides, and pheromones within agriculture (Daniel and Kehera, 2016) [7]. This method facilitates slow release of active ingredients while enhancing bioavailability of volatile pheromones through nanoformulations. Pheromones exhibit increased reactivity at the nanoscale compared to their bulk forms, making smaller quantities sufficient for greater insect attraction. Therefore, this study aimed to utilize gold nanoparticles for encapsulating assembly pheromones to attract and eliminate stages of the brown dog tick within a stray dog shelter (Das *et al.*, 2019) [3].

### Materials and Methods

#### Encapsulation Procedure

A 2% chitosan solution was prepared by dissolving 0.8 g chitosan in 40 mL distilled water containing 1% acetic acid, continuously stirred for approximately 18 hours. Stock solutions of assembly pheromones were made (1 mg per mL distilled water), with adenine fully dissolving in hot water while guanine and xanthine dissolved completely in cold water. The assembly pheromone mixture (25:1:1) was added to 500  $\mu$ L chitosan matrix and stirred continuously for about an hour. Subsequently, 200  $\mu$ L prepared gold nanoparticles were added to this matrix and stirred overnight until well homogenized (Sivan *et al.*, 2016) [10].

#### Sticky Tick Trap Device Design

The trap was constructed from an acrylic sheet measuring 15 cm x 10 cm as its base. A transparent stiff sheet was attached using cellophane tape at each corner, onto which double-sided sticky tape was affixed. This tape was cut into strips measuring 1 cm wide; ten strips were placed on each trap surface.

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A total volume of 200  $\mu$ L assembly pheromone nanogold complex was absorbed into filter paper discs; thus, forty discs received an infusion of five  $\mu$ L each. Control devices were assembled identically but used plain gold nanoparticles without any pheromone addition before labeling them for field trials.

### Field Evaluation

Field testing involved placing baited traps inside kennels housing stray puppies and adult dogs across Thrissur district in Kerala. Traps were positioned on window sills at approximately three feet off the ground so that dogs could not access them directly. Six replicates per polymer type alongside corresponding controls were deployed over seven days, after which dead ticks stuck to traps were counted.

### Statistical Analysis

Results are expressed as mean  $\pm$  standard deviation ( $n = 3$ ). ANOVA (Analysis of Variance) and chi-square tests determined significant differences among groups with  $p < 0.05$  considered statistically significant.

### Results

After one week from placement within kennel shelters, six replicates indicated that traps baited with assembly pheromone nanogold complex attracted both questing and engorged phases of *R. sanguineus* ticks significantly more than controls where only one or two nymphal stages were captured on devices lacking formulation enhancements. The mortality rates recorded included:

**Table 1:** Percent tick mortality in sticky trap device

S. No.	Tick Stages	Mortality %
1.	Engorged Larvae	27
2.	Engorged Nymphs	15
3.	Engorged Female	16
4.	Unfed Larvae	30
5.	Unfed Nymphs	05
6.	Unfed Adult	07

Statistical analysis using chi-square tests showed no significant attraction differences between engorged versus questing stages; all life stages displayed similar levels of response towards the nanogold encapsulated assembly pheromone lure.

### Discussion

Four types of sex-specific pheromones have been identified within ticks; notably, assembly/arrestment pheromones serve to decrease distances between individuals naturally leading them toward clustering behavior around hosts (Franklin and Sterling, 1990) [12]. These compounds are prevalent across both hard and soft ticks—facilitating mating success while improving host detection rates through collective behavior observed particularly among argasid species including multiple *Ixodes* species (Katoch, 2018) [8]. While generally volatile or subject to rapid degradation due to instability concerns surrounding traditional use cases involving other types like adhesion-based formulations suggest modifications may be required—assembly variations exhibit considerable stability necessitating integration into consistent release mechanisms that prolong efficacy against environmental factors targeting *R. sanguineus* populations (Levine, 2012) [15].

Existing studies such as those patented by Christen *et al.*, (2002) [1] highlight prior use cases incorporating similar compounds alongside permethrin targeting developmental phases effectively within ecological niches occupied by other vector systems like *Ixodes scapularis*. Natural polymers such as calcium alginate have demonstrated promise alongside synthetic variants enabling successful encapsulation techniques facilitating environmental management addressing challenges posed by pests while reducing dependencies on toxic chemicals during application cycles (Kennedy *et al.* 2013) [9].

This investigation successfully utilized nanoscale vehicles employing sustainable practices yielding noteworthy results concerning pest control strategies without reliance upon conventional biocides indicative towards fostering greener methodologies applicable under Integrated Pest Management frameworks benefiting animal husbandry sectors overall.

### Conclusion

For the first time ever reported literature indicates successful application involving nanoparticles utilized specifically designed captures pertaining directly targeting assembly type-derived signals originating from *R. sanguineus*. Gold nanoparticle formulations exhibited high effectiveness attracting varying life stage entities present throughout samples collected during routine monitoring practices revealing promising potential toward environmentally conscious alternatives capable replacing traditional methods lacking efficacy henceforth opening avenues promoting adoption green technologies across agricultural interfaces alike enhancing quality standards relating livestock welfare initiatives safeguarding ecosystem integrity.

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