

Assessment of the Repellent Efficacy of Allomones against the Larval Stages of Brown Dog Tick

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ABSTRACT

Ticks and tick-borne diseases are a major threat to livestock and companion animals. Hematophagous ticks transmit a variety of pathogens along with the direct effects such as tick worry and skin damages. Acaricides has been the mainstay in fighting off ticks until the emergence of resistance which forced researchers to look out for alternate tick control strategies. Allomones (non-host semiochemicals-NHC's) are semiochemicals which benefit the host and deter the pests. The NHC's in certain breeds of dogs have been elucidated and their function has been decoded. The current study was attempted in formulating a sustained release of these allomones using vapour patches and assessing their efficacy by *in-vitro* assay. It was found that allomones tested had a strong repellent effect on *Rhipicephalus sanguineus* comparable to that of the commercial repellent DEET.

Key words: Allomones, Brown dog tick, Repellent, *Rhipicephalus sanguineus*, Semiochemicals

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INTRODUCTION

Ticks are hematophagous ectoparasites ranked second to mosquitoes based on their vector potentiality (Sonenshine, 1991). The brown dog tick, *Rhipicephalus sanguineus* sensu lato is cosmopolitan in distribution and commonly infests domestic dogs, although there are reports of this species parasitizing birds, horses, cattle, sheep, goats, and even human beings (Dantas-Torres *et al.*, 2006). Tick bite allergy, irritation, alopecia, blood loss, associated weight loss, eczema, and even flaccid paralysis are few of the direct effects of *R. sanguineus* s.l. (Dantas-Torres, 2008). The indirect effect of *R. sanguineus* s.l. is because of its potentiality to maintain, transmit and even amplify pathogens. The brown dog tick is known to be the vector for *Babesia canis*, *B. vogeli*, *B. gibsoni* and *Ehrlichia canis* (Reck *et al.*, 2018). These adverse effects of ticks on their host warrant effective strategies to control them.

The indiscriminate use of synthetic acaricides has resulted in the emergence of resistant tick population to these active agents (Oliveira *et al.*, 2017). The development of resistance to most of the commonly available acaricides has paved way for research on alternate tick control strategies. One such alternate strategy which has yielded fruitful result is the utilization of semiochemicals for tick control. Semiochemicals are biologically active, information bearing compounds that are associated with inter- and intra-species communication which modify the behavioural pattern when perceived (Sonenshine, 2004). Semiochemicals in ticks fall under three major categories namely, allomones, kairomones and pheromones. Allomones and kairomones aid in inter-species communication. Allomones (also known as non-host semiochemicals-NHC's) are chemical signals which benefit the emitter and they are mostly defensive compounds that repel the predator.

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Louly *et al.* (2009) found that the Beagle breed had less infestation of *Rhipicephalus sanguineus* ticks compared to the English Cocker Spaniels. It was elucidated that the volatile organic compounds in the skin emissions of the Beagle breed repelled the ticks thus making them resistant to infestation (Louly *et al.*, 2010). Borges *et al.* (2015) deciphered the chemical composition of allomones which is a mixture of aldehydes and ketones. Allomonal compounds have been detected in previous studies from the skin secretions of various animals

such as waterbuck which is deterrent to the tsetse flies (Bett *et al.*, 2015), while Ferreira *et al.* (2019) have found similar odour compounds from donkeys that are deterrent to *Amblyomma sculptum* ticks. The current study was thus designed to assess the repellent efficacy of allomones against the larval stages of *Rhipicephalus sanguineus* s.l. by *in-vitro* assay using a vapour patch as sustained release formulation.

MATERIALS AND METHODS

Collection and Maintenance of Ticks

Engorged females of *Rhipicephalus sanguineus* s.l. ticks identified based on morphology were collected from infested animals that were presented for treatment at the Dermatology unit of Madras Veterinary College Teaching Hospital and from community dogs available in the MVC, campus, Chennai, Tamil Nadu, India. The engorged female ticks were maintained in the laboratory in order to allow them to oviposit and the larvae to hatch out of the egg. The larvae of 7-14 days old were utilized for the trials.

Allomones and DEET

Allomones used were synthetic analogues of 2-hexanone and benzaldehyde, procured from Sigma-Aldrich, Germany. They were used in the ratio of 2:1 of Benzaldehyde and 2-hexanone (Oliviera *et al.*, 2017). The compounds were diluted in Hexane (Loba Chemie, India) and the concentrations of the compounds were 0.200 mg cm⁻². DEET procured from Supelco, USA diluted in ethanol (Northman, India) was used as repellent standard for comparing the repellent efficacy (Carroll *et al.*, 2004).

Vapour Patches for Sustained Release of Allomones

Fabric based vapour patches of 150 gm measuring 350 mm procured from Crawford wisdom, New Delhi were utilized to prepare sustained release formulation of the allomone similar to Gowrishankar *et al.* (2021), who used them to prepare sustained release formulations of pheromones. A total volume of 400 µL of the allomone was impregnated onto the vapour patch, air dried for 15 min and utilized for the trials. The vapour patches were impregnated with allomones just before the start of the trial and used afresh for every trial.

Petri Dish Bioassay

A modified method of Yoder and Stevens (2000) as adopted by Dhivya and Latha (2017) was utilized in the current study. For every trial, combination and replicate, fresh, unexposed larvae were used. The study was conducted at room temperature and responses were recorded at 10, 30, 60 min, 2, 4 and 24 h. The number of larvae per trial per combination was 500 and repeated until the cumulative count for each combination was 5000. Four different vapour patch combinations were tested, *viz.*, allomone-impregnated vapour patches, negative control (hexane- solvent), DEET (positive control), and ethanol (solvent control). The ticks

were considered attracted in if they were on the vapour patch or in the same quadrant and repelled if they were in the periphery as well as on the lid of other quadrants.

The response of ticks to DEET and allomone combination was assessed using chi-square test in SPSS software (IBM® technologies).

RESULTS AND DISCUSSION

The response of the larval stages to the allomones and the respective controls is presented in Table 1. The behavioural response of the larval stages included an initial questing, followed by kinetic activity towards the odour-source and then completely avoiding movement towards that quadrant wherein the vapour patches impregnated with allomones were placed. Similar behavioural responses were observed with DEET also. An additional attempt to escape from the petri dish also elicited with both DEET and allomones (Fig. 1). In the petri dish assay, it was observed that both DEET as well as allomones were equally repulsive. The percent repellence ranged between 63.12 and 88.66 with allomones, while with DEET it was 70.60 to 98.84 during the same period of observation. The repellent efficacy was statistically significant in case of DEET and allomone combination in comparison to the controls. The repellent efficacy of DEET and allomone showed no difference statistically after a period of 4 h of observation.

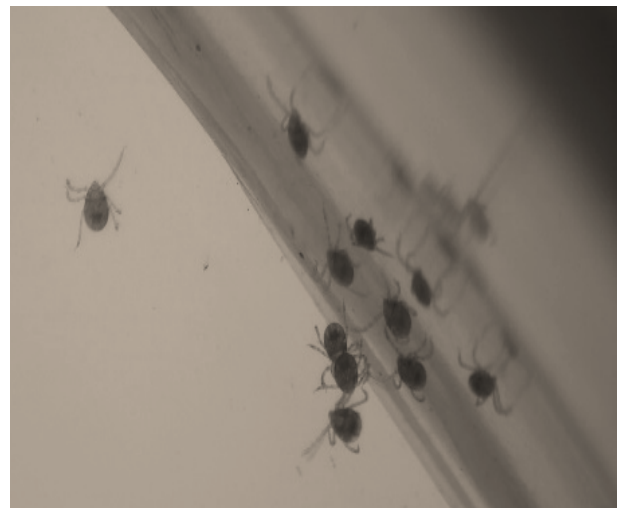


Fig. 1: Larvae in periphery and trying to escape in response to vapour patches with 2-hexanone + benzaldehyde

In the petri dish wherein vapour patches impregnated with ethanol and hexane (solvent controls) were tested no such behavioural responses were observed and the larvae moved throughout the circumference of the petri dish. It was observed that after 24 h of the assay, in all the four tested combinations the larvae were alive but repelled strongly only with allomones and DEET formulations.

In the current study, it has been evident that allomone impregnated vapour patches have comparable repellent effect similar to DEET as reported previously by Borges *et al.*

(2015) and Oliviera *et al.* (2017), who have used filter papers as allomone dispensers. Semiochemicals predominantly are volatile organic compounds (VOC's) and easily dissipate in field conditions. Sustained release formulations usually offer plausible solution to use them effectively. Therefore, this study attempted to utilize vapour patches for sustained release of allomones and the results concurred with the earlier reports.

Table 1: Response of larvae in petri dish bioassay (N=5000) expressed as % mean repellence

Observation time	Vapour patch with allomone (2:1 Benzaldehyde and 2-hexanone)	Vapour patch with DEET
10 min	63.12 (315.6±0.7)	70.60 (353±0.6)
30 min	68.64 ** (343.2±1.1)	76.18** (380.9±1.3)
60 min	75.00 ** (375±2.1)	84.12** (420.6±1.3)
2 hours	79.48** (397.4±1.5)	88.20** (444.1±1.2)
4 hours	86.56* (432.8±1.3)	90.64* (453.2±0.9)
24 hours	88.66 * (443.3±1.8)	98.84 * (494.2±0.8)

Figures in the parentheses represent mean ± SE

*Significant (p<.05) and **highly significant (p<.01)

In summary, this study has successfully attempted to assess the repellent properties of sustained release allomone formulation. It thus serves as a pilot study for further utilization of these compounds under field conditions as an effective target specific repellent for on-host control of brown dog tick, *Rhipicephalus sanguineus* s.l.

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