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Effect of 1-octen-3-ol, the ruminant kairomone on ixodid tick larvae

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ABSTRACT

In vitro evaluations were made to evaluate the efficacy of 1-octen-3-ol on five ixodid larval stages using Petridish bioassay. The species of the ruminant ticks which are common in Indian sub continent including *Rhipicephalus (Boophilus) microplus, Haemaphysalis bispinosa, Rhipicephalus haemaphysaloides*, *Hyalomma marginatum* and dog tick *Rhipicephalus sanguineus* were made use in the current study. Amongst the ruminant tick larvae tested with 1-octen-3-ol, maximum attraction of 72 per cent and 71 per cent was recorded for *H. marginatum* and *R. microplus* respectively while *H. bispinosa* and *R. haemaphysaloides* showed 52 and 50 per cent attraction respectively. 1-octen-3-ol being a compound found characteristically in bovine odour failed to evoke any response (questing / attraction) in the brown dog tick.

Key words: 1-octen-3-ol, ruminant kairomones, ixodid tick larvae, India, Tamil Nadu

Introduction

Ticks have attracted a great deal of scientific attention primarily because of their considerable medical and veterinary importance. The impact of ticks and tick-borne diseases on the livelihood of resource poor farming communities have been ranked high in India (PERRY et al., 2002). These problems are closely associated with domestic animals and pets in the tropical and subtropical regions of the world. The direct and indirect effects of ticks are either through their role as vectors of disease or by their feeding activities. In Tamil Nadu, *Rhipicephalus sanguineus* (brown dog tick) is the most common ixodid tick parasitizing dogs, while *Haemaphysalis bispinosa, Rhipicephalus haemaphysaloides*

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and *Hyalomma marginatum* are seen infesting small and large ruminants in addition to *Rhipicephalus microplus (Boophilus microplus*) infestation on large ruminants. *Rhipicephalus sanguineus* transmits ehrlichiosis, babesiosis and hepatozoonosis. Ruminant ticks are the vectors for theileriosis, babesiosis and anaplasmosis. In India, the economic losses due to tick and tick-borne diseases are estimated to be US$ 498.7 million per annum (GHOSH et al., 2007) with a global annual loss of $109 billion (JABBAR et al., 2007).

Currently the mainstay of tick control measure relies mainly on the use of chemical acaricides. However the use of acaricides is often accompanied by its well-known drawbacks. So there is an urgent need for an effective alternate tick control method in near future. Semiochemicals were used with considerable success in countries other than India. In Tamil Nadu no research work has been done to exploit kairomones for tick control. 1-octen-3-ol is a known semiochemical reported in tsetse flies and mosquitoes (HALL et al., 1984). McMAHON et al. (2001) first reported 1-octen-3-ol as being attractive to ticks. Ticks recognise and differentiate between their hosts through characteristic host odours. Reports of NORVAL et al. (1987) suggests the activation of *Amblyomma habraeum* in the field by odours of cattle and sheep. Amongst the semiochemicals 1-octen-3-ol which is characteristically found in bovine odours evoked a significantly stronger response from *R. microplus* (OSTERKAMP et al., 1999). Moreover these volatiles are present in various life stages of ixodid ticks like *Amblyomma variegatum* and *A. habraeum.* Behavioural responses were recorded to ruminant odours like 1-octen-3-ol and several short chain aldehydes (HALL et al., 1984; STEULLET and GUERIN, 1994). Among these the parsimonious use of 1-octen-3-ol was explained as a cue for aggregation with conspecifics and for host finding when in search for a blood meal (McMAHON et al., 2001). OSTERKAMP et al. (1999) observed that 1-octen-3-ol and 2- nitro phenol was highly effective kairomones for the larvae of *R. microplus*. Moreover they found that cattle ticks always preferred bovine odours while *Ixodes ricinus* was equally stimulated by all mammalian odours clearly indicating the ability of ticks to identify their hosts via olfactory cues. 1-octen-3-ol enhanced the questing behaviour of *R. microplus*, at a source dose of 3 μg (McMAHON et al. 2001). 1-octen-3-ol could elicit an attractive response when used along with Attraction-Aggregation-Attachment Pheromone (AAAP) but the combination of butyric acid and AAAP failed to elicit attraction (NCHU et al., 2009). Trials using different concentrations of 1-octen-3-ol revealed that 16 ng of 1-octen-3-ol was capable of eliciting relative attraction of 50 per cent of *A. variegatum* adult ticks. This paper explores the effect of ruminant kairomone 1- octen-3-ol on the five larval ixodid ticks of Indian sub continent.

Materials and methods

Ticks. Ticks were located by visual appraisal and by running the hand across the body of the animal. Brown dog ticks, *R. sanguineus* were collected from the animals presented in the Small Animal Clinics of Madras Veterinary College, Chennai. Ruminant ticks namely *R. microplus, R. haemaphysaloides, H. bispinosa* and *H. marginatum* were collected from the cases presented in the Large Animal Clinics of Madras Veterinary College and Livestock Research Station, Kattupakkam. The above species of ticks were used in the current study. Engorged female ixodid ticks were collected from dogs and ruminants. They were placed in dry vials covered with porous cloth. These ticks were maintained in the laboratory for oviposition. The hatched out larvae of all species were maintained at a RH (Relative Humidity) of 93 per cent using saturated solution of potassium chloride. The hungry larval stages were maintained alive by keeping them at 4 ^{o}C in a BOD (Biological Oxygen Demand) which ensured dormancy of larvae. Larval ticks with intact first pair of legs were only selected for use in the bioassays since the Haller's organ is located on the dorsal surface of the tarsus of the forelegs (SONENSHINE, 2006).

Filter paper. Whatman® qualitative filter paper grade 3 having a diameter of 11 cm (Whatman International Ltd., Maidstone, England) was used to impregnate both the assembly pheromone and acaricide (SONENSHINE, 2003). Filter paper discs of size 2x2 cms were used in petridish bioassay. The discs were handled with a gloved hand. Sterile forceps were used to take the filter paper discs. This ensured that the filter paper discs did not come into contact with human skin lipids which were found attractive to ticks (YODER et al., 1998).

Kairomone. Synthetic analogue of 1-octen-3-ol obtained from Sigma Alderich, Germany were used as the kairomone source. In the present study 16 ng of 1-octen-3-ol was used and it was diluted in Dichloromethane (DCM) procured from Sigma Alderich, Germany.

Petri dish bioassay was conducted with 100 larvae of each five tick species to evaluate the effect of 1-octen-3-ol. Dichloromethane in 4 µL quantities served as controls.

Petri dish bioassay. A method followed by YODER and STEVENS (2000) was adopted in this study with modifications. Glass petri dishes of 9 cm diameter were used for the assay. Filter paper discs of size 2 × 2 cms were pasted at one quadrant of the petri dish. Larvae were placed 10 at a time in the bioassay arena, opposite to the filter paper discs. Tests were replicated until N = 100 for the larvae. Petri dish was covered with another petri dish of same diameter and sealed with laboratory grade parafilm in order to prevent the escape of ticks as well as to avoid responses by the tick to carbon dioxide emitted by the investigator which could lead to misinterpretation of results. Care was taken while handling the ticks in order to avoid damage of the first pair of legs.

Controls were maintained in separate petri dishes. All tests were conducted at room temperature. Unconditioned ticks (ticks which were unexposed to the semiochemicals), fresh chemicals, filter paper discs and clean petri dishes were used for each test. Number of ticks that were attracted onto the disc or in the same quadrant of source was counted. Observations were made up to 1 hour.

Statistical analysis. Statistical analysis of the data obtained was done with chi- square test.

Results

In vitro evaluation of the effect of kairomone 1-octen-3-ol on the larval stages of five ixodid tick species was conducted. The questing was very rapid with ruminant ticks and the attracted larvae visited the kairomone treated filter paper discs frequently. Amongst the ruminant tick larvae tested with 1-octen-3-ol, maximum attraction of 72 per cent and 71 per cent was recorded for *H. marginatum* and *R. microplus* respectively while *H. bispinosa* and *R. haemaphysaloides* showed 52 and 50 per cent attraction respectively. 1-octen-3-ol being a compound found characteristically in bovine odour failed to evoke any response (questing / attraction) in the dog tick. The larvae did not show any attraction but exhibited avoidance behaviour. No change in behaviour was observed in control. The response of the larvae to 1-octen-3-ol is depicted in table. Statistical analysis revealed significant difference (chi - square value: 137.01**) in attraction to 1-octen-3-ol between the five ixodid tick species.

Table 1. Effect of the kairomone, 1-octen-3-ol on the ixodid tick larvae

Tick	No. of larvae attracted to the kairomone			No. of larvae not attracted to the kairomone	
	On disc	In the same quadrant	Total	With orientation	Without orientation
Rhipicephalus sanguineus	Nil	Nil	Nil	Nil	100 (100)
Hyalomma marginatum	23 (31.94)	49 (68.06)	72 (72	11 (11)	17 (17)
Haemaphysalis bispinosa	13 (25)	39 (75)	52 (52)	4 (4)	44 (44)
Rhipicephalus haemaphysaloides	6 (12)	44 (88)	50 (50)	13 (13)	37 (37)
Rhipicephalus microplus	30 (42.25)	41 (57.75)	71 (71)	6 (6)	23 (23)
Chi-square value and significance			137.01**		

**Highly significant (P≤ 0.01), Figures in parenthesis indicate percentage

Discussion

The current study used 16 ng of the ruminant kairomone, 1-octen-3-ol to attract the questing larval stages of the five ixodid tick species which is equivalent to about 1/20th of the amount present in bovine breath. With this concentration a percentage of attraction in the range of 50-72 was obtained among the ruminant ticks. McMAHON et al. (2001) used 10 ng of 1-octen-3-ol and demonstrated that this compound functions s a kairomone among ruminant ticks. NCHU et al. (2009) observed a relative attraction of 50 per cent of the *A. variegatum* ticks with 16 ng of 1-octen-3-ol. Amongst the ruminant tick larvae tested with 1-octen-3-ol maximum attraction of 72 per cent and 71 per cent was recorded for *H. marginatum* and *R. microplus* respectively while *H. bispinosa* and *R. haemaphysaloides* showed 52 and 50 per cent attraction respectively. Since 1- octen- 3-ol simulate bovine odour it is natural for ruminant tick to be attracted to this kairomones. The higher sensitivity with which *R. microplus* larvae responded to 1-octen-3-ol may be contributing to the cattle specificity of the tick. OSTERKAMP et al. (1999) demonstrated that this compound enhanced questing behaviour of *R. microplus.*

1-octen-3-ol being a compound found characteristically in bovine odour failed to evoke any response (questing / attraction) in the dog tick. Such differences in tick sensitivity to different host odour profiles explain how ticks recognize their preferred host and proceed to feed on them (OSTERKAMP et al., 1999). Often kairomonal compounds are most effective when combined with visual cues, body heat and even sounds made by the vertebrate host.

In conclusion amongst the ruminant tick larvae tested with 1-octen-3-ol, maximum attraction of 72 per cent and 71 per cent was recorded for *H. marginatum* and *R. microplus* respectively while *H. bispinosa* and *R. haemaphysaloides* showed 52 and 50 per cent attraction respectively. Ticks seem to be equipped with similar array of receptor type for olfaction and the difference in response between different tick species might be achieved only by the quantitative differences in the contribution of the individual receptor signal to the response. 1-octen-3-ol being a compound found characteristically in bovine odour failed to evoke any response (questing / attraction) in the brown dog tick. No characteristic behavioural response was observed with dichloromethane which served as the control. 1-octen-3-ol attracts the hungry larval stages of ruminant ticks. A combination of different tick pheromones and 1-octen-3-ol can be used to attract the various stages of ruminant ticks. Standardisation of *in vivo* trials will help to introduce this host cue as one of the most effective tick control method.

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SAŽETAK

U radu je istražena *in vitro* učinkovitost 1-okten-3-ola, sastojka karakterističnog mirisa goveda, na ličinke pet različitih vrsta krpelja preživača koji se javljaju u Indiji: *Rhipicephalus (Boophilus) microplus, Haemaphysalis bispinosa, Rhipicephalus haemaphysaloides*, *Hyalomma marginatum*, *Rhipicephalus sanguineus*. Među pretraženim krpeljima privlačnost u iznosu od 72% bila je dokazana za krpelja *H. marginatum* te 71% za krpelja *R. microplus*. Manja privlačnost zabilježena je za *H. bispinosa* i *R. haemaphysaloides* (52 i 50%). Isti sastojak uopće ne privlači krpelja *R. sanguineus*.

Ključne riječi: 1-okten-3-ol, kairomoni preživača, ličinke, *Ixodidae*, Indija, Tamil Nadu