

Effects of solvents and surfactants against *Haemaphysalis bispinosa*

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Abstract. As per the report published by FAO (2004), the tick population in India has developed resistance against all the available acaricides. Hence, newer methods of control including potential herbal agents are required to reduce the problems caused by the ticks. Most of the herbal extracts or their fractions are dissolved in polar or non-polar solvents or detergents before tested for acaricidal activity and these diluents should be of little acaricidal activity. In the present study, adult immersion test (AIT) was carried out on adult engorged female *Haemaphysalis bispinosa* ticks to detect the acaricidal activity of different solvents *viz.*, n-butanol, glycerol, acetone, ethanol, methanol and surfactants (at 1 per cent dilution) like dimethyl sulphoxide (DMSO), tween 20 and triton X- 100. The study revealed that methanol was the least toxic solvent while tween 20 (1 per cent) was the least toxic detergent against *H. bispinosa*.

INTRODUCTION

Ticks and the diseases they transmit are widely distributed throughout the world, particularly in tropical and subtropical regions. It has been estimated that 80 per cent of world cattle population is exposed to tick infestation causing a global annual loss of US\$ 7000 million (FAO, 1984). In India, the cost of tick and tick borne disease (TTBD) control in animals has been estimated to be US \$ 498.7 million per annum (Minjauw & Mc Leod, 2003). At present, TTBD control is mainly performed by the widespread use of chemical acaricides like organophosphates, carbamates, pyrethroids, BHC-cyclodienes, amidines, macrocyclic lactones and benzoyl phenyl ureas (Ghosh *et al.*, 2007). Since the first report of the development of resistance of *Boophilus microplus* to arsenic in

Australia in 1937, acaricidal resistance was frequently reported from various countries against drugs like DDT, cyclodienes and toxaphenes, organophosphorous-carbamate group, formamidines, pyrethroids and recently introduced macrocyclic lactones (George *et al.*, 2008).

Some of the future strategies laid down for the sustainable tick control involves development of vaccines, newer generation chemical acaricides, herbal acaricides and transgenic animals (Ghosh *et al.*, 2007). Hence the effective tick control around the world requires a range of newer compounds with different modes of action, good safety characteristics for the animals and soft environmental toxicity profile (Graf *et al.*, 2004). Herbal insecticides show properties like rapid degradation, less persistence in environment, reduced risks to non-target

organisms and may not leave excessive residues (Dipeolu & Ndungu, 1991). Thus identification and validation of herbal acaricidal components is highly essential.

The most common species under the genus *Haemaphysalis*, the most species rich genus under the family Ixodidae, in the oriental region is *Haemaphysalis bispinosa*. They were reported on domestic cattle, buffalo, horse, goat, sheep, wild mammals, rodents and several bird species from many states of India (Geevarghese *et al.*, 1997; Prakasan & Ramani, 2007). This species acts as vector of *Babesia motasi*, *Babesia ovis* in sheep and goat, *Babesia equi* in horses and donkeys and *Babesia canis* and *Babesia gibsoni* in dogs (Taylor *et al.*, 2007).

The herbal extracts or their fractions must be dissolved in a polar or non-polar solvent or detergent which should have minimum acaricidal property. Gonçalves *et al.* (2007) tested the acaricidal effect of four solvents and two detergents against *Rhipicephalus (Boophilus) microplus*. But there is paucity of information on the effect of solvents and detergents on multi-host ticks especially, *H. bispinosa*. Hence, the present investigation focuses on effect of common solvents and detergents against *H. bispinosa*.

MATERIALS AND METHODS

Solvents and detergents

The effect of six commonly employed solvents and two detergents used in the extraction and *in vitro* testing were tested on adult female *H. bispinosa*. The solvents (in the increasing order of polarity) employed for the current study were, n-butanol, acetone, glycerol, ethanol and methanol. The surfactants used were di-methyl sulphoxide (DMSO), tween 20 and triton X-100.

All the reagents used in this study were of analytical grade and purchased from Merck (India) and were used undiluted except DMSO, tween 20, and triton X-100 which were used at 1 per cent concentration.

Ticks

Fully engorged adult *H. bispinosa* female ticks were collected from infested calves washed with distilled water and dried using clean soft tissue paper.

Experimental protocol

Adult immersion test (AIT) was performed as per the protocol described by Drummond *et al.* (1973). Four replicates of six ticks each were used for testing of single solvent or detergent. Six ticks were immersed in the solution (10 ml) at room temperature for two minutes in a 50 ml beaker with gentle agitation. Distilled water was used as control. Ticks were recovered from the solutions, dried and placed in a plastic specimen tube (25 X 50 mm). They were incubated at 28°C and 80 per cent relative humidity in a BOD incubator.

Per cent adult mortality, inhibition of fecundity, hatching

Adult tick mortality was observed up to 19th day after immersion. After oviposition, the eggs laid by the female ticks were collected and weighed. The index of egg laying (IE) and percentage inhibition of fecundity (IF) were calculated (FAO, 2004; Gonçalves *et al.*, 2007) as follows:

Index of egg laying (IE) = weight of eggs laid (g)/weight of females (g)

Percentage inhibition of fecundity (IF) = [(IE control group - IE treated) X 100] / IE control group.

Hatching percentage of eggs was calculated visually.

Statistical analysis

All the data were expressed as the mean \pm SEM. Groups were compared using one-way ANOVA for repeated measurements using SPSS software. Duncan's test was used for post-hoc analysis. A value of $P < 0.05$ was considered significant.

RESULTS

The ticks dipped in distilled water showed mortality from 19th day onwards. Hence, mortality of ticks, up to 19 days post-treatment was taken as due to acaricidal effect of solvent or detergent. The results of adult immersion test using various solvents and detergents are shown in Table 1. Among the tested solvents, acetone caused 100 per cent mortality to the adult ticks within 24 hours of immersion. N-butanol and glycerol produced more than 80 and 66 per cent mortality respectively within 19 days of treatment. Both solvents produced more than 50 per cent inhibition of fecundity also. The toxic effect of ethanol and methanol were almost similar but the latter exhibited moderately lower percent inhibition of fecundity. Ethanol inhibited hatching of laid ova by the treated ticks. Compared to other solvents, methanol was observed as safe for use in dissolving the herbal extracts for testing the acaricidal properties.

At one per cent dilution, both DMSO and tween 20 did not produce any mortality of adult ticks within 19 days of treatment. Inhibition of fecundity was more than 24 per cent for all the three detergents. DMSO caused 90 per cent blocking of hatching of laid eggs. Out of the three detergents tested, tween 20 was identified as the best detergent for dissolving herbal extract.

DISCUSSION

Currently there is paucity of information on the effect of solvents and detergents on the multi-host ticks. Hence for the first time the acaricidal effect of common solvents and detergents used for herbal extraction and dilution were analyzed.

The immediate mortality of all ticks treated with acetone resulted in 100 per cent inhibition of fecundity and complete blocking of eclosion whereas lesser effect was observed with ticks treated with n-butanol. The outermost waxy layer of epicuticle of ixodid ticks is composed mainly of cholesterol and its derivatives (Sobhy *et al.*, 1994). As cholesterol is freely soluble in acetone, the wax lipid is destroyed, resulting in hundred per cent mortality due to dehydration (Cherry *et al.*, 1969; Gonçalves *et al.*, 2007). N-butanol is also non-polar solvent and thus can solubilize lipids.

Glycerol is less polar but causes dehydration (Booth & Mc Donald, 1982). It is highly penetrable and viscous, and thus resulted in mortality. Ethanol and methanol cause dehydration and thereby results in mortality. Inhibition of fecundity was lower in the case of methanol compared to ethanol. Methanol being highly polar and less penetrable of all the solvents tested in this study, showed the lower adult acaricidal effects.

Table 1. Effects of different solvents against *H. bispinosa*

Sl. No	Solvent / detergent	Mean ticks weight per replicate \pm SEM (g)	Mean % adult mortality within 19 days \pm SEM	Mean eggs mass per replicate \pm SEM (g)	Index of fecundity of \pm SEM	Percentage Inhibition Fecundity (%)	Hatching % (Visual)
1.	n-Butanol	0.628 \pm 0.0162 ^{ab}	83.33 \pm 9.624 ^c	0.123 \pm 0.0471 ^b	0.194 \pm 0.0729 ^b	61.19	100
2.	Acetone	0.585 \pm 0.0771 ^{ab}	100 \pm 0 ^d	0 \pm 0 ^a	0 \pm 0 ^a	100	10
3.	Ethanol	0.666 \pm 0.0547 ^{ab}	4.165 \pm 4.165 ^a	0.239 \pm 0.0072 ^{cd}	0.358 \pm 0.0064 ^{cd}	28.15	10
4.	Methanol	0.703 \pm 0.0133 ^{ab}	4.165 \pm 4.165 ^a	0.273 \pm 0.0347 ^{cd}	0.386 \pm 0.0453 ^{cd}	22.56	100
5.	Glycerol	0.528 \pm 0.0524 ^a	66.66 \pm 6.804 ^b	0.130 \pm 0.0269 ^b	0.248 \pm 0.0447 ^{bc}	50.25	100
6.	Dimethyl sulphoxide (1%)	0.580 \pm 0.0215 ^{ab}	0 \pm 0 ^a	0.219 \pm 0.0086 ^{cd}	0.379 \pm 0.0036 ^{cd}	24.08	10
7.	Tween 20 (1%)	0.591 \pm 0.1011 ^{ab}	0 \pm 0 ^a	0.191 \pm 0.0058 ^{bc}	0.346 \pm 0.0461 ^c	30.66	100
8.	Triton (1%)	0.649 \pm 0.0225 ^{ab}	12.50 \pm 7.978 ^a	0.246 \pm 0.0209 ^{cd}	0.372 \pm 0.0217 ^{cd}	25.29	100
9.	Control (H ₂ O)	0.584 \pm 0.0221 ^{ab}	0 \pm 0 ^a	0.296 \pm 0.0519 ^d	0.499 \pm 0.0748 ^d	0	100

Values are Mean \pm SEM, n = 4 in each group, values in a column with different superscripts differ, (P<0.05)

Detergents are amphipathic (amphiphilic) compounds with both lipophilic and lipophobic sites within one molecule. Triton X 100 and tween 20 are non-ionic polyoxyethylene detergents. DMSO [(H₃C)₂S=O] is partly soluble in both aqueous and organic media (Stammati *et al.*, 1996). DMSO was reported to interact with the metabolism and membrane of cells, resulting in severe cell damage (Penninckx *et al.*, 1983; Brayton 1986). The acaricidal effects observed in the present study could be attributed to the lipophilicity of these compounds which can remove the epicuticular waxy layer of the ticks. Since they cause increase in the lipid fluidity, they can also reduce the resistance of waxy layer to the diffusion of drugs thereby delivering the active ingredients present in the herbal extract onto the integument.

Gonçalves *et al.* (2007) evaluated the effect of various solvents / detergents *viz.*, acetone, methanol, ethanol, DMSO, triton X-100 and tween 80 on *R. (B.) microplus* based on inhibition of fecundity and observed that ethanol, and one per cent dilution of DMSO, triton X-100 or tween 80 were suitable for bioassays. The present study revealed that methanol and tween 20 (1 per cent) were the best solvent and detergent respectively for use of bioassays involving *H. bispinosa*.

Also, it can be concluded that the combined acaricidal effects contributed by active ingredients of the extract along with the solvent or detergent will be helpful in controlling the ticks when the extract is used as topical formulation.

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