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Comparative acaricidal properties of different solvents and surfactants on *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae)

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ABSTRACT

Use of herbal acaricides is considered one of the options for integrated tick management. Many natural compounds have low water solubility and use of solvents/surfactants is obligatory to test the efficacy of the herbal extracts in experimental conditions. In an attempt to identify best suitable solvents, experiments were conducted on laboratory reared homogenous colonies of adults of *R.(B.) microplus*. Adult immersion test was conducted by exposing the adults on solvents (methanol, ethanol, acetone, n-butanol, di-methyl sulphoxide) and surfactant (tween 20). The effect of solvents and surfactant was monitored in terms of causing mortality and inhibition of oviposition (IO).

Out of 4 solvents used, n-butanol was found most toxic to tick system with a mean mortality of 20.0±8.2% at 10% concentration. Though no mortality was seen in 25% ethanol treated ticks but percentage inhibition of oviposition (%IO) of 10.3 was recorded. In contrast, upto 50% concentration acetone and methanol were found to be safe as a solvent since it had least toxic effect on tick mortality. Amongst the two detergents tested up to 6% concentration tween-20 found safe while DMSO can be used even at higher concentration up to 10% without any toxic effect. The results revealed that of the 4 commonly used solvents tested, acetone was the least toxic while n-butanol was highly toxic to ticks both in terms of causing mortality and IO. The surfactant DMSO was found safe at 10% level against adults of *R. (B.) microplus*.

Key words: Mortality, Oviposition, *Rhipicephalus (Boophilus) microplus,* Safety, Solvent

Rhipicephalus (Boophilus) microplus parasitization on animals causes blood loss, reduction in milk production and severe deterioration in the quality of leather in livestock (Ducornez *et al.* 2005). The use of synthetic acaricides for the control of tick populations is beset with limitations like environmental pollution, residues in milk and meat, development of acaricide resistance in ticks and the demise of non-targeted species (Boeke *et al.* 2004, Martin *et al.* 2006). To address these problems, research on plant based acaricides is increasing to establish plants as potential sources of anti-tick agents (Franscisco *et al.* 2003, Fernandes *et al.* 2005, Magano *et al.* 2008), because plant based acaricides has the biodegradability properties. As plant extracts has low water solubility, various organic solvents are used in extraction of active components of plants/herbs. However, organic solvent may cause toxic effects on their own and can interact with pesticides to alter their toxicity (Stratton and Corke 1981).

Comparative study on toxicity of organic solvents was reported on fish, aquatic invertebrates and on insects (Stratton 1989, Tadros *et al.* 1994, Gorb *et al.* 2000). Although the effect of different solvents on *R. (B.) microplus* of Brazilian strain was studied (Chagas *et al.* 2003, Goncalves *et al.* 2007), it is imperative to test the effect of these solvents on laboratory reared acaricide susceptible homogenous colony of *R. (B.) microplus*. Hence, the present experiment was conducted to identify the suitable solvents to be used in preparation of antitick herbal formulations.

MATERIALS AND METHODS

Test materials: The solvents and detergents i.e. methanol (99.8%), ethanol (99.9%), acetone (99.5%), n-butanol (99.5%), di-methyl sulphoxide (DMSO) (99.8%), tween-20 were of analytical grade. The concentration of the solvents and detergents are given in % volume. In all the experiments

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distilled water was used as control.

Maintenance of disease-free homogenous colony of R. (B.) microplus (Izatnagar isolate): The homogeneous colony of *R. microplus*, maintained at IVRI as per Khan *et al.* (1982) was used. The isolate is kept in the absence of acaricides pressure for multiple generations and was used as the susceptible reference strain. *Babesia bigemina* free crossbred calves were used for feeding of larvae and adults of *R. microplus.* Crossbred male calves (6) were maintained in tick proof shed under standard feeding and managemental conditions. After 2–3 feeding cycles, the calves were kept free from tick feeding for 1 month. The biological parameters were noted to confirm the homogeneity in the colony. These laboratory reared ticks were used to test *in vitro* efficacy of different solvents and detergents. Animal experimentations were conducted in accordance with the approved guidelines laid down by the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), a monitoring Indian statutory body.

In vitro *bioassay:* Ticks were treated separately with 5, 10, 50 and 100% concentration of ethanol, methanol, n-butanol and acetone while 1,5 and 10% concentrations were used for tween-20 and for DMSO by 2 min adult immersion test (AIT) (Benavides *et al.* 2001). After treatment ticks were transferred to the tissue paper for drying and were kept in covered petri dishes padded with Whatman filter paper no. 1. For each concentration 5 replication and in each replication 5 ticks were maintained. Simultaneously, control ticks were treated in distilled water and 5 replications were maintained. The treated ticks were kept at 28±2°C and relative humidity of 85±5%. The mortality was carefully observed daily by motility under light and pedal reflexion of ticks up to 14 days. The inhibition of oviposition was evaluated after weighing the eggs using the following formula (Stendel 1980).

Statistical analysis: Data were statistically analyzed by software, Graph Pad Prism 4 and significance of data was calculated using one-way ANOVA (Finney 1962).

RESULTS AND DISCUSSION

Data on the effects of different concentration of solvents and surfactant on engorged *R. (B.). microplus* are presented in Tables 1–6. Out of four solvents used at 5–100% concentrations, the effect of n-butanol was severe and a mean mortality of 20.0±8.2% was recorded even at 10% concentration. The mortality level reached at 35.0±5.0 and 50.0±5.7% at 25 and 50% concentrations, respectively, and the mortality data were statistically significant at P<0.001 level when compared with control ticks where no mortality was recorded. At 100% concentration, both n-butanol and acetone were highly toxic to *R.(B.) microplus* as both the chemicals adversely effected on tick survivality and a mortality of 100±0.0 (P<0.001) and 70±8.4% (P<0.001), respectively, within 14 days post-treatment (dpt) (Tables 1,2).

Table 1. Effect of n-butanol on mortality and biological activity of *R. (B.) microplus*

Conc. (%)	Tick weight (mg)±SE	Final mortality (%)±SE	Egg mass (mg)±SE	RI±SE	IO (%)
5	132.0±4.5	0.0±0.0	65.5±2.7	0.496±0.01	–
10	132.7±4.7	20.0±8.2	57.3±3.3	0.424±0.05	5.4
25	127.4±2.6	35.0±5.0[c]	44.3±11.6	0.383±0.06	14.5
50	123.0±11.1	50.0±5.7[c]	45.3±4.3	0.343±0.06	23.4
100	112.7±5.1	100.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	100.0
Control	121.6±5.5	0.0±0.0	54.5±1.9	0.448±0.02	–

[c]Significant at P< 0.001 level

Table 2. Effect of acetone on mortality and biological activity of *R. (B.) microplus*

Conc. (%)	Tick weight (mg)±SE	Final mortality (%)±SE	Egg mass (mg)±SE	RI±SE	IO (%)
5	114.0±10.0	0.0±0.0	51.1±2.6	0.452±0.02	–
10	169.4±2.8	0.0±0.0	84.9±4.3	0.501±0.02	–
25	105.8±0.8	0.0±0.0	49.5±2.4	0.467±0.03	–
50	121.2±10.1	0.0±0.0	58.8±1.5	0.496±0.04	–
100	124.4±10.6	70.0±8.4[c]	40.5±1.6	0.348±0.02	22.3
Control	121.6±5.5	0.0±0.0	54.5±1.9	0.448±0.02	–

[c]Significant at P< 0.001 level

Table 3**.** Effect of ethanol on mortality and biological activity of *R.(B.) microplus*

Conc. (%)	Tick weight (mg)±SE	Final mortality (%)±SE	Egg mass (mg)±SE	RI±SE	IO (%)
5	128.7±2.2	0.0±0.0	55.1±3.6	0.418±0.06	6.7
10	149.9±7.2	0.0±0.0	62.4±3.6	0.412±0.07	8.0
25	126.1±1.1	0.0±0.0	51.6±2.6	0.402±0.06	10.3
50	118.4±3.5	15.0±5.0	47.3±3.7	0.399±0.06	11.0
100	124.8±0.0	30.0±5.8[b]	47.2±2.4	0.383±0.04	14.5
Control	121.6±5.5	0.0±0.0	54.5±1.9	0.448±0.02	–

[b]Significant at P< 0.01

However, up to 50% concentration, acetone was safe as a solvent since it had no toxic effect on tick mortality and% inhibition of oviposition.

Ethanol 5–25% concentrations did not show any mortality while IO (%) of 6.7–10.3 was recorded (Table 3). The IO (%) of 10.3 as recorded in 25% is considerable, hence ethanol can be safely used at below 25% level. In contrast, methanol can be effectively used as solvent in different herbal preparations against ticks up to 50% level as it showed no adverse effect on mortality and IO% (Table 4). A statistically significant (P<0.01) mortality of 30±5.8% and IO% of 14.5 were recorded in ticks treated with 100% ethanol. On the other hand, absolute methanol showed only 15±5.0%

Table 4. Effect of methanol on mortality and biological activity of *R.(B.) microplus*

Conc. (%)	Tick weight (mg)±SE	Final mortality (%)±SE	Egg mass (mg)±SE	RI±SE	IO (%)
5	129.2±4.8	0.0±0.0	56.4±2.6	0.438±0.05	2.2
10	131.4±10.0	0.0±0.0	53.5±2.9	0.416±0.04	6.5
25	119.0±3.4	0.0±0.0	46.3±2.5	0.42±0.06	6.7
50	122.0±7.5	0.0±0.0	50.7±2.5	0.419±0.03	6.5
100	114.0±4.1	15.0±5.0	43±3.8[a]	0.373±0.07	16.7
Control	121.6±5.5	0.0±0.0	54.5±1.9	0.448±0.02	–

[a]Significant at P< 0.05

Table 5. Effect of tween-20 on mortality and biological activity of *R.(B.) microplus*

Conc. (%)	Tick weight (mg)±SE	Final mortality (%)±SE	Egg mass (mg)±SE	RI±SE	IO (%)
1	155.8±1.7	0.0±0.0	74.3±3.9	0.498±0.02	–
2	131.4±1.4	0.0±0.0	65.6±5.8	0.485±0.11	–
4	133.9±1.1	0.0±0.0	63.7±3.8	0.475±0.03	–
6	127.7±2.3	0.0±0.0	63.7±2.1	0.498±0.01	–
8	129.8±3.2	25.0±5.0[b]	53.2±3.7	0.409±0.03	8.7
10	132.5±5.8	65.0±9.6[c]	39.4±4.5[a]	0.277±0.01[c]	38.2
Control	121.6±5.5	0.0±0.0	54.5±1.9	0.448±0.02	–

[b]Significant at P< 0.01, [c]Significant at P< 0.001 level

Table 6. Effect of DMSO on mortality and biological activity of *R.(B.) microplus*

Conc. (%)	Tick weight (mg)±SE	Final mortality (%)±SE	Egg mass (mg)±SE	RI±SE	IO (%)
1	163.8±4.1	0.0±0.0	80.0±3.5	0.479±0.02	–
5	125.9±3.5	0.0±0.0	57.4±3.0	0.457±0.01	–
10	135.1±4.5	0.0±0.0	56.9±3.6	0.454±0.01	–
20	131.6±4.0	10.0±5.8	61.8±1.64	0.470±0.01	–
Control	121.6±5.5	0.0±0.0	54.5±1.9	0.448±0.02	–

mortality and 16.7% IO. Results revealed that except n-butanol and ethanol, the other 2 solvents can be used up to 25% concentration for extraction of active components from plant materials and for screening of anti-tick activities.

It is interesting to note that although the mean mortality of ticks treated with 5–100% concentration of methanol was 0 to 15±5.0%, the IO % was 16.7% in 100% methanol treated ticks. While in ethanol, the maximum IO % of 14.5 was recorded in 100% ethanol treated ticks (Tables 4, 3). Of the 4 solvents tested, methanol was found least toxic and n-butanol was highly toxic both at the level of mortality and at IO (%).

Treatment with 1–6% concentrations of tween-20 revealed no adverse effect on tick mortality and IO (%) while 8 and 10% tween-20 exhibited a significant mortality of 25±5.0 and 65±9.6%, respectively, with 8.7 and 38.2% of IO (Tables 5&6). Treatment with 1–2% DMSO against *R. (B.) microplus* showed that up to 10% level DMSO can be used as surfactant, however 20% DMSO was lethal to 10±5.8% treated ticks (Table 6).

The extraction of active phytochemical depends upon the polarity of the solvents used during bioassay experiment, as polar solvent will extract polar molecules and non polar solvents will extract non polar molecules. A survey of literature indicated that moderately polar solvents such as methanol and acetone (polarity index of 5.1), ethanol (pi 5.2), n-butanol (pi 4.0), mainly extract steroids, alkaloids etc. (Chowdhury and Chandra 2007, Ghosh *et al.* 2008). The strong polar solvents such as DMSO (polarity index 7.2) extract biochemicals with higher molecular weight such as proteins, glycans etc. (Madhumathy *et al.* 2007, Vineetha and Murugan 2009). For conducting bioassay tests against ticks using extracts of different plant products, polar solvents have been used extensively (Baki *et al.* 2002, Garcia *et al.* 2003, Ribeiro *et al.* 2008).

Many workers (Chungsamarnyart and Jiwajinda 1992, Chungsamarnyart and Jansawan 1996) used 95% ethanol to prepare working concentration of essential oils for testing against *R.(B.) microplus*. Similarly, methanol was used as solvent in bioassay of plant derived insecticides (Chungsamarnyart and Jansawan 1990, Matovu and Olila 2007). The lethal effect of acetone was tested against *Dermanyssus gallinae* and was found to be not toxic (Kim *et al.* 2007). Comparing the results obtained in the present experiments, it was observed that ethanol, methanol and acetone were non-toxic up to 5% concentration and suitable solvent for bioassay experiments. However, as reported earlier, higher concentration of acetone was lethal to *R.(B.) microplus* due to its effect on tick cuticle wax (Goncalves *et al.* 2007). Tick deposits no extra cuticular lipids during feeding, permitting maximum transpiratory water loss that probably helps to concentrate the blood meal. However, ticks deposit additional cuticular wax after apolysis that confers impermeability on the cuticle reducing integumental water loss, which helps tick to survive the host situation. Mean mortality of 70.0±12.9% was observed due to dehydration caused by 100% acetone. The mean mortality of 30.0±5.8, following treatment with absolute ethanol may also be attributed to a similar effect of these solvents in wax layer, since cholesterol is partially soluble in ethanol. The highest lethality of n-butanol is probably due to quick metabolization of butanol to CO_2 which causes depression of central nervous system of ticks leading to death (ECETOC JACC No 41, 2003). The adverse effect of butanol was also reported on pregnant mice in which 5% butanol causes significant decrease in maternal body weight gain accompanied by

reduced food and water consumption (Ema *et al.* 2005).

Besides, the above-mentioned solvents, DMSO and tween 20 have been used extensively for diluting extracts of plant products. The primary aim of use of detergent is to reduce surface tension and also to keep hydrophobic compounds in a soluble form through the formation of micelles. In most of the studies the detergents were used without any reported adverse effect on biological materials (Khan *et al.* 1982, Chungsamarnyart and Jansawan 1990, Matovu and Olila 2007). However, in the present experiment, a mean mortality of 65.0±9.6% and IO (%) of 38.2 were recorded when ticks treated with 10% concentration of tween-20 and thus not suitable for use in bioassay test on *R. (B.) microplus*. While DMSO was found suitable for making working concentration of herbal extracts in bioassay test. The result of the study indicated that for developing anti-tick formulation ethanol, methanol and acetone can be used at a specific concentration in the process of extraction while DMSO is the better option for making working concentration.

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