

## ***In vitro* EFFICACY OF AQUEOUS EXTRACTS OF *Stylosanthes scabra* AGAINST IXODID TICKS**

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One of the most common arthropod pest and vector of animal diseases is ticks. Ticks and tick-borne diseases are major inhibitory factors on livestock production all over the world including tropical countries like India. Traditionally, the control of arthropod parasites of livestock has depended upon the use of chemical pesticides. The control of certain ticks using aqueous extract of *Stylosanthes scabra* was attempted in this study since its secretions were reported to possess acaricidal property (Sutherest *et al.*, 1982).

### **Materials and Methods**

#### **A. Cultivation of *Stylosanthes scabra***

Seeds of *Stylosanthes scabra* were sown and grown in 20 cm (top) diameter pots. 1.5 g of urea and 0.7 g of superphosphate were added at the time of sowing and thereafter every 4 months and the pots were watered daily. The plants thus grown were utilised in the present experiments. Six and seven months old plants of *S. scabra* were selected for *in vitro* trials, since they showed highest acaricidal property (Khudrathulla and Jagannath, 1998).

#### **B. Collection and maintenance of different stages of ticks**

Engorged female ticks of *Boophilus microplus*, *Hemaphysalis intermedia* and *Rhipicephalus sanguineus* were collected from naturally infested cattle, sheep and dogs respectively. The specific identification of ticks were based on the keys adopted by Arthur (1960) for *Boophilus*, *Trapidoet al.* (1964) for

*Haemaphysalis* and Sharif (1928) for *Rhipicephalus* species. Individual ticks were placed in different petri dishes for egg laying. Eggs laid were transferred to glass tubes tied with muslin cloth and rubber band and kept for hatching. Larvae and nymphs raised on rabbits by the ear bag method were preserved in a desiccator containing saturated potassium chloride solution at room temperature of 25°C and relative humidity of 80%.

#### **Aqueous extraction of *Stylosanthes scabra* leaves**

Aqueous extraction of leaves of different concentrations was prepared by triturating 0.5, 1.0, 1.5, 2, 3, 4, 5 and 6g of fresh leaves separately with 100 ml of distilled water. The extract was then sieved to remove fibrous material. The different concentrations of the filtrate thus obtained was stored separately for later use.

*In vitro* trials were conducted by tea bag method (Gladney *et al.*, 1972). Teabags containing known number of larvae/nymphs were dipped in different concentrations for four minutes. Their mortality were recorded after 24 hours of the trial. The control group was dipped in distilled water.

### **Results**

The percentage mortality of larval ticks is presented in Table 1 and 2.

Significant differences (P < 0.01) were seen in the mortality of *B. microplus* larvae in aqueous extract when exposed to different concentrations (Anova Table 1).

Anova Table 1 Efficacy of aqueous extract of *S. scabra* plant against larval and nymphal stages

Source	df	MSS		F	
		Larvae	Nymphs	Larvae	Nymphs
<b><i>B. microplus</i></b>					
Extract concentration	7	199.78	255.06	57.24**	57.71**
Age of the plant	1	3.66	0.57	0.88 NS	0.13 NS
Error	7	3.49	4.42		
<b><i>H. intermedia</i></b>					
Extract concentration	7	119.13	69.57	11.28**	23.50**
Age of the plant	1	10.57	72.26	1.00 NS	24.41**
Error	7	10.56	2.96		
<b><i>R. sanguineus</i></b>					
Extract concentration	7	981.13	303.56	47.88**	133.14**
Age of the plant	1	68.07	45.57	3.32 NS	19.99**
Error	7	20.49	2.28		

\*\* (P ≤ 0.01) NS - Not significant

A cent per cent mortality of larvae was observed in 3.0 per cent concentration and above followed by  $99.50 \pm 0.50$  per cent in 2.0 per cent (Table 1)

Table 1 Percentage mean larval mortality of aqueous extract of *S. scabra* leaves

Extract concentration (per cent)	<i>B. microplus</i> Mean ± S.E.	<i>H. intermedia</i> Mean ± S.E.	<i>R. sanguineus</i> Mean ± S.E.
0.5	73.50 <sup>a</sup> ± 1.50	34.00 <sup>a</sup> ± 5.00	41.50 <sup>a</sup> ± 1.50
1.0	84.00 <sup>b</sup> ± 3.00	39.00 <sup>ab</sup> ± 2.00	48.50 <sup>ab</sup> ± 4.50
1.5	91.50 <sup>c</sup> ± 1.50	47.00 <sup>bc</sup> ± 2.00	53.00 <sup>ab</sup> ± 6.00
2.0	99.50 <sup>d</sup> ± 0.50	54.00 <sup>c</sup> ± 1.00	60.00 <sup>bc</sup> ± 3.00
3.0	100.00 <sup>d</sup> ± 0.00	49.50 <sup>c</sup> ± 1.50	75.00 <sup>cd</sup> ± 3.00
4.0	100.00 <sup>d</sup> ± 0.00	49.00 <sup>c</sup> ± 1.00	85.00 <sup>de</sup> ± 3.00
5.0	100.00 <sup>d</sup> ± 0.00	54.00 <sup>c</sup> ± 1.00	95.50 <sup>ef</sup> ± 4.50
6.0	100.00 <sup>d</sup> ± 0.00	56.00 <sup>c</sup> ± 2.00	100.00 <sup>f</sup> ± 0.00

whereas lowest mortality ( $73.50 \pm 1.50$ ) was observed in 0.5 per cent.

The mortality of larvae of *H. intermedia* was  $56.00 \pm 2.00$  per cent in 6.0 per cent followed by  $54.00 \pm 1.00$  in 5.0 per cent (Table 1). The lowest ( $34.00 \pm 5.00$ ) mortality was observed in 0.5 per cent concentration. Significant differences were not seen in the mortality of larvae when different age plant extracts were tested (Anova Table 1).

A cent per cent mortality of larvae of *R. sanguineus* was observed at 6.0 per cent followed by  $95.50 \pm 4.50$  in 5.0 per cent (Table 1). The lowest ( $41.50 \pm 1.50$ ) was observed in 0.5 per cent. The effect of age of plant on larval mortality of the 3 species of ticks was not significantly different.

A mortality of  $41.00 \pm 2.00$  was observed in 6.0 per cent followed by  $41.00 \pm 1.00$  in 5.0 in nymphs of *B. microplus* (Table 2).

**Table 2** Percentage mean nymphal mortality of aqueous extract of *S. scabra* leaves

Extract concentration (per cent)	<i>B. microplus</i> Mean $\pm$ S.E.	<i>H. intermedia</i> Mean $\pm$ S.E.	<i>R. sanguineus</i> Mean $\pm$ S.E.
0.5	10.00 <sup>a</sup> $\pm$ 2.00	12.50 <sup>a</sup> $\pm$ 0.50	18.00 <sup>a</sup> $\pm$ 2.00
1.0	15.50 <sup>ab</sup> $\pm$ 1.50	17.00 <sup>ab</sup> $\pm$ 2.00	21.50 <sup>ab</sup> $\pm$ 2.50
1.5	20.50 <sup>bc</sup> $\pm$ 0.50	16.50 <sup>ab</sup> $\pm$ 2.50	27.00 <sup>b</sup> $\pm$ 2.00
2.0	22.00 <sup>bc</sup> $\pm$ 1.00	18.50 <sup>ab</sup> $\pm$ 3.50	27.00 <sup>b</sup> $\pm$ 3.00
3.0	26.00 <sup>cd</sup> $\pm$ 1.00	21.50 <sup>bcd</sup> $\pm$ 1.50	30.00 <sup>b</sup> $\pm$ 1.50
4.0	31.50 <sup>d</sup> $\pm$ 1.50	25.50 <sup>cde</sup> $\pm$ 2.50	39.50 <sup>c</sup> $\pm$ 0.50
5.0	41.00 <sup>e</sup> $\pm$ 1.00	27.00 <sup>de</sup> $\pm$ 2.50	45.50 <sup>d</sup> $\pm$ 1.00
6.0	41.00 <sup>e</sup> $\pm$ 2.00	29.50 <sup>e</sup> $\pm$ 2.50	54.00 <sup>e</sup> $\pm$ 2.00

Means bearing any one common superscript in column do not differ with each other

The lowest ( $10.00 \pm 2.00$ ) mortality was observed at 0.5 per cent. A high mortality of nymphs of *H. intermedia* ( $29.50 \pm 2.50$ ) was observed in 6.0 per cent followed by  $27.00 \pm 3.00$  in 5.0 per cent (Table 2) and the lowest

( $12.50 \pm 0.50$ ) in 0.5 per cent. A mortality of  $23.13 \pm 2.31$  was observed in the extract of leaves of 6<sup>th</sup> month old plants and  $18.88 \pm 1.88$  in 7<sup>th</sup> month old plants (Table 3).

**Table 3** Percentage mean nymphal mortality in aqueous extract of *S. scabra* leaves

Age of the plant (month)	<i>H. intermedia</i> Mean $\pm$ S.E.	<i>R. sanguineus</i> mean $\pm$ S.E.
6	23.13 <sup>a</sup> $\pm$ 2.31	31.31 <sup>a</sup> $\pm$ 4.52
7	18.88 <sup>b</sup> $\pm$ 1.88	34.50 <sup>b</sup> $\pm$ 4.20

Means bearing any one common superscript in column do not differ with each other

Mortality of  $54.00 \pm 2.00$  was observed in 6.0 per cent followed by  $45.50 \pm 1.00$  in 5.0 per cent in nymphal stages of *R. sanguineus* (Table 2) and lowest ( $18.00 \pm 2.00$ ) in 0.5 per cent concentration. The mortality of  $31.13 \pm 4.52$  per cent was observed when extract of leaves of 6 month old plant was used and  $34.50 \pm 4.20$  in the 7<sup>th</sup> month plant extract (Table 3).

## Discussion

There are various methods of controlling ticks as reviewed by Wharton and Norris (1980), Sutherst (1983), and Young *et al.* (1988). The control measures included methodologies ranging from brief repellency to more permanent control. Traditionally, the control of ticks has depended upon the use of chemical acaricides. Widespread use of acaricides for tick control has its own problems such as the development of resistance. In addition the presence of chemical residues in animal products and its effect on environment, warrants greater care and therefore, their usage should be minimized.

*Stylosanthes scabra* which is used as grass/legume mixture in cattle feed and it has an acaricidal property. *S. scabra* produces a sticky secretion which immediately immobilizes the larvae of ticks and are poisoned within 24 hours by an unidentified vapour from the secretion (Sutherst *et al.*, 1982). The secretion appears to have the potential to substantially reduce the populations of all species of ticks because of the acaricidal property and therefore, in this study the effect of its aqueous extract was studied.

Varying degrees of mortality of larval stages of *Boophilus microplus*, *B. decoloratus*, *Rhipicephalus appendiculatus* and *Amblyomma variegatum* on exposure to plants of *S. scabra* had been reported (Sutherst *et al.* 1982, 1988; Zimmerman *et al.*, 1984; Wilson and Sutherst, 1986; Wilson *et al.*, 1989). Similar results on

mortality of *B. microplus*, *R. sanguineus* and *Haemaphysalis intermedia* were reported (Khudrathulla and Jagannath., 1998).

In the present study aqueous extracts of *S. scabra* leaves has been tried on larval and nymphal stages of *B. microplus*, *H. intermedia* and *R. sanguineus* (Table 8) on 6<sup>th</sup> and 7<sup>th</sup> month old plants, since they showed high acaricidal property and no published reports are available on these aspects.

In aqueous extract hundred per cent mortality of *B. microplus* larvae was observed at 3.0 per cent and above and same in *R. sanguineus* at 6.0 per cent. Maximum mortality of  $56.00 \pm 2.00$  was seen in *H. intermedia* larval stages at 6.0 per cent concentration. In nymph the highest mortality of  $41.00 \pm 2.00$ ,  $29.50 \pm 2.50$  and  $54.00 \pm 2.00$  was seen at 6.0 per cent in *B. microplus*, *H. intermedia* and *R. sanguineus* respectively. Significant nymphal mortality was noticed at various age of the plant  $23.13 \pm 2.31$  per cent mortality was observed in six month plant extract and  $18.88 \pm 1.88$  in seven month in *H. intermedia*. In *R. sanguineus*  $31.13 \pm 4.52$  was seen in six month plant extract and  $34.50 \pm 4.20$  in seven month plant extract.

## Summary

*In vitro* acaricidal efficacy of aqueous extracts of 6 and 7 month old plants of *Stylosanthes scabra* against larval and nymphal stages of *Boophilus microplus*, *Haemaphysalis intermedia* and *Rhipicephalus sanguineus* was assessed.

Significant variation (P < 0.01) on the mortality of larvae and nymphs in various concentrations of aqueous extract was observed. One hundred percent mortality of *B. microplus* larvae was noticed in 3.0 per cent and above, whereas 6.0 per cent in *R. sanguineus*. However, highest percentage mortality of  $56 \pm 2$  was observed in *H. intermedia* at 6 per cent concentration.

Highest percentage of **nymphal** mortality was noticed in 6 per cent concentration viz.,  $54 \pm 2$  in *R. sanguineus*,  $41 \pm 2$  in *B. microplus* and  $29.5 \pm 2.5$  in *H. intermedia*. Mortality of nymphal stages of *H. intermedia* and *R. sanguineus* differed significantly ( $P < 0.01$ ) in extracts of 6 and 7<sup>th</sup> month plants.

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