

Anti-Tick Activity of Leaves of *Azadirachta indica*, *Dalbergia sisso* and *Morus alba* against *Rhipicephalus microplus* (Acari: Ixodidae)

¹Mohsin Nawaz, ¹Sajid Mahmood Sajid, ¹Zulfiqar Ahmed, ¹Muhammad Waqas, ¹Tanveer Ahmed, ¹Abid Hussain, ¹Abrar Mohi-ud-Din, ¹Asim Shamim, ¹Muhammad Zubair and ²Imran Khalid

¹Faculty of Veterinary and Animal Sciences, The University of Poonch, Rawalakot, Azad Kashmir, Pakistan

²Veterinary Officer, Civil Veterinary Dispansory, Tehsil Bhowana, District Chiniot, Pakistan

Abstract: The purpose of present study was to evaluate anti-tick activity of *Azadirachta indica*, *Dalbergia sisso* and *Morus alba*. Water extract prepared from leaves of *A. indica*, *D. sisso* and *M. alba* was used to evaluate anti-tick activity of plants against 12-14 days old larvae of *Rhipicephalus microplus*. Acaricidal activity of plants and ivermectin (control group) was examined after 24h and 6 days of treatment using Syringe test. LC₅₀, LC₉₀ and LC₉₉ values were calculated for extract and ivermectin. LC₅₀ (CL 95%) of extract collected after 24 h was 2185% (1466-4061%), totally different ($P < 0.05$) when compared with the LC₅₀ of extract after 6 days 45% (30-62%). This significant difference between LC₅₀ values at 24 h and 6 days indicate that extract was more toxic after 6 days of treatment. Likewise, significant difference ($P < 0.05$) was observed between LC₉₀ and LC₉₉ of extract and ivermectin. Time dependent response of water extract of plants was observed. The results confirmed traditional use of *Azadirachta indica*, *Dalbergia sisso* and *Morus alba* against ticks and could be used for further development as herbal acaricide.

Key words: Syringe Test

INTRODUCTION

Ticks are dispersed widely in the world [1] and are responsible for severe economic losses in animals and these economic losses are principally due to the diseases which they spread [2]. Production losses due to ticks have been estimated as \$7000 million annually by McCosker [3]. Similarly, Biswas [4] reported that ticks resulted in 20-30% financial losses associated with low quality of hides and skin. Ticks are known to transmit various bacteria, protozoans, viruses and rickettsiae. As ticks are blood suckers, therefore, heavy infestation leads to stunted growth as well as weakness of animals [5, 6]. Ticks are also the most significant group of vectors of many pathogens. They are liable for the transmission of many pathogens affecting not only domestic animals but also humans [7].

In order to control ticks, chemical acaricides have been widely used in the world but some problems including resistance developed by ticks [8] and environmental pollution [9] are also associated with the

use of such drugs. These issue necessities the promotion of alternate tick control sources. For this purpose, plants have been traditionally used worldwide to control ticks. Medicinal plants symbolize the ancient and most prevalent form of medication [10]. *Azadirachta indica* as well as *Gynandropsis gynandra* have shown significant anti-tick activity in Africa [11, 12]. Volatile oil derived from leaves of lemon grass and citronella grass was tested to evaluate its activity against larvae and adults of *Rhipicephalus microplus* [13]. In an experiment by Malonza *et al.* [14], *Gynandropsis gynandra* was found to have repellent as well as acaricidal activities against larvae, nymphs and adult stages of *Amblyomma variegatum* and *Rhipicephalus appendiculatus* ticks. Kaaya *et al.* [15] reported high mortalities of *R. appendiculatus* (nymphs and adults) and *A. variegatum* (nymphs) when treated with water extract of *Margaritaria discoidea*. Mortality of ticks (*R. microplus*) treated with crude extract of *Tamarindus indicus* was examined after 24h, 48h and 7 days [16]. Pereira and Famadas [17] evaluated the efficacy of the roots of *Dahlstedtia*

pentaphylla against *R. microplus* infestations and observed an efficacy rate of 76.10% of the extract. Chamomile flowers (*Matricaria chamomile*) were studied for their acaricidal potential against engorged females of *Rhipicephalus annulatus*. Mortality of ticks was examined after 5 days of treatment [18]. Adult immersion and the larval immersion tests were used to evaluate the antitick activity of *Hesperozygis ringens* against larvae and engorged female ticks of *R. microplus* [19]. Similarly, methanolic extracts prepared from leaves and stem of *Petiveria alliacea*, *Havardia albicans* and *Caesalpinia gaueri* were used against larvae of *R. microplus* and found very effective [20]. The particular goal of this study was to develop formulations for the control of ticks, based on easily available plants.

MATERIALS AND METHODS

Extraction of Plant Material: Fresh leaves (10 kg) of *Azadirachta indica*, *Dalbergia sisso* and *Morus alba* were collected from Faisalabad (Pakistan). After collection, plants were identified by a botanist from the Department of Botany, University of Agriculture, Faisalabad. First the leaves were chopped and then soaked in sufficient quantity of distilled water in plastic buckets (up to 10 lit/bucket) and were vigorously shaken after every 24 hour. After a period of 30 days, contents of buckets were heated at 25-30 °C till 10% of the total volume was achieved. Material was then sieved and extract was stored at 4 °C for further use [21].

In vitro Acaricidal Activity: Adult female ticks of *Rhipicephalus microplus* were collected from naturally infested animals and were identified in the Department of Parasitology, University of Agriculture, Faisalabad. After confirmation of the specie, these ticks were washed with tap water and placed in incubator at 27 °C and 90% relative humidity (RH) for egg laying. Acaricidal activity of water extract of plants was determined using syringe test [22].

Syringe Test: A 3 ml syringe was used. Nozzle end of syringe was cut open and plunger was pulled back to the 2 ml mark. Approximately 200 eggs (approx. 10 gm of eggs) were placed in the syringe and open end of the syringe was sealed with double layer of nylon gauze. These prepared syringes were incubated at 27 °C and 90% Relative Humidity (RH) for hatching. Syringes containing 12-14 day old larvae were used for evaluation of acaricidal activity.

Syringes containing larvae were divided into 3 groups. One of the groups received extract while the second group was treated with ivermectin. Third group didn't receive any treatment (negative control). Six two-fold serial dilutions of the extract (100-3.125%) were prepared for treatment. Two ml of the solution was drawn inside the syringe containing 12-14 day old larvae which was then shaken for 30 seconds to treat the larvae. After 30 seconds test solution was discarded by pushing the plunger, till it touches the gauze. A facial tissue paper was placed on the gauze to completely remove the test solution and plunger was pulled back to 2 ml mark. All the treated syringes were placed in fume hood for one hour, cut end facing upwards. After one hour all the treated syringes were incubated at 27 °C and 90% RH. Two sets of tests were performed to evaluate the acaricidal activity of herbal formulation against all three groups. One set was used to check the acaricidal activity after 24h and other to check the activity after 6 days of treatment (time mortality experiment).

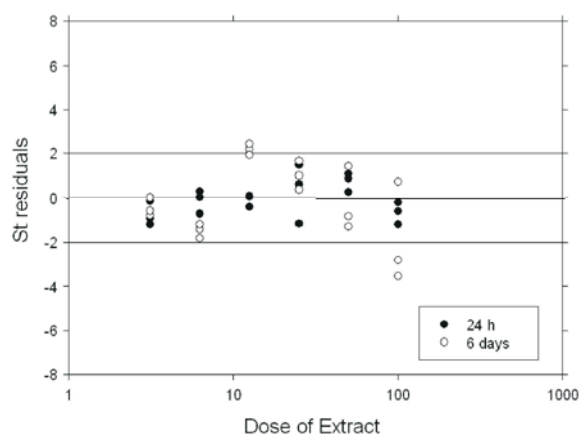
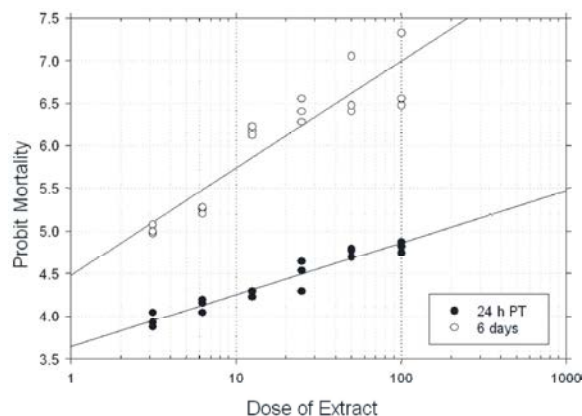
Data Analysis: Live and dead larvae of ticks for each concentration of extract and ivermectin were counted at the end of time to calculate the percent mortality. Data was subjected to Probit analysis using PoloPlus [23]. Probit transformation of percentage mortality and natural Logarithm transformation of dose was analyzed. Standardized residual plots were generated to show how results fit the log-probit model. Standardized residuals were calculated by taking the difference of the observed value and the expected value and dividing the result by their standard errors. These results were plotted against the lethal concentration estimate for the expected values. Good fit was considered as residuals scattered randomly within a horizontal band around zero and mostly between -2 and 2.

RESULTS AND DISCUSSION

LC₅₀, LC₉₀ and LC₉₉ of extract and ivermectin were recorded. Standardized residuals indicate better fit of results in log probit model as the residuals lie within a range of 2 and -2 (Fig. 1). LC₅₀ (CL 95%) calculated after 24 h was 2185% (1466-406%), statistically different ($P < 0.05$) when compared with the LC₅₀ of extract after 6 days 45.996% (30.327-62.056%). This statistically significant difference in lethal concentration after 24 h and 6 days indicate that the onset of activity of extract was slow (Fig. 2). Similarly, significant difference ($P < 0.05$) was observed between LC₉₀ and LC₉₉ of extract and ivermectin after 24 h and 6 days post treatment. LC₉₀, LC₉₉ and

Table 1: LC₅₀, LC₉₀, LC₉₉ and chi-square values of plants extract and ivermectin

Name	Exp. time	Slope (SL)	X ²	(95% CL)	LC ₅₀ % v/v LC ₉₀ (95% CL)	% v/v LC ₉₉ % v/v (95% CL)
Extract	24h (%)	1.026 (0.129)	10.775	2185 (1466-4061)	38740 (15286-177472)	403743 (101060-3946440)
	6 days	1.457 (0.092)	50.987	45 (30-62)	348 (255-542)	1819 (1025-4494)
Ivomec (PPM)	24h	0.827 (0.054)	118.38	1.563 (0.55-3.46)	55 (19-406)	1020 (182-38628)
	6 days	0.502 (0.054)	27.401	0.000 (0.00-0.001)	0.110 (0.06-0.24)	13 (3.29-159)

Fig. 1: Standardized residuals of data from *Boophilus microplus* submitted to syringe test with aqueous extract of *Azadirachta indica*, *Dalbergia sisso* and *Morus alba*Fig. 2: Probit Mortality \times log concentration plot from *Rhipicephalus microplus* submitted to syringe test with herbal formulation containing aqueous extracts of *Azadirachta indica*, *Dalbergia sisso* and *Morus alba*

chi-square values of extract and ivermectin are presented in Table 1. In negative control group, zero mortality was observed after 24 h as well as 6 days of experiment.

Results of this study showed similarities with the results of previous studies. Kaposhi [24], Williams [25] and Kandil [26] reported severe effect of *Azadirachta*

indica, *Sea anemone* and *Simmondisa chinensis* extracts on the reproduction as well as mortalities of *Rhipicephalus microplus* and *Rhipicephalus annulatus*. 50% concentration of *Stemona collinsae* induced 100% (nymph) and 93.33% (adult) mortalities of *R. microplus* at 24 h post treatment [27]. Similarly, all stages of *Rhipicephalus appendiculatus* were repelled and killed by oil extracted from leaves of *Ocimum suave* [28]. Kaaya *et al.* [15] treated nymphs (*R. appendiculatus*) and adult ticks (*R. appendiculatus* and *Amblyomma variegatum*) with 6.25% concentrated hexane extract of *Nicotiana tabacum* and observed 100% mortalities of ticks. Herbal preparation containing extract of *A. indica* resulted in 100, 80 and 70% efficacy against all stages (larvae, nymph and adult) of *R. microplus*, *Rhipicephalus haemaphysaloides* and *Hyalomma anatolicum* [29]. Kaaya and Saxena [30] observed that neem oil inhibited 47-55% egg hatching while 90-100% feeding of ticks.

CONCLUSION

Plants used in this study showed some potential to be used against parasites. Aqueous extract was prepared to reflect the use of these plants in traditional medicine. Although the plants showed moderated activity but the study support the observation that further optimization as well as characterization of extract is needed.

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