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Efficacy of *Nicotiana tabacum* as a biocontrol agent against cattle ticks

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Abstract

Tick and tick borne diseases are one of the biggest public health and veterinary problems in the world. Control of ticks using chemical acaricides have developed resistance to wide range of acaricides and also producing environmental pollution. Nowadays more emphasis is given on use of environment free biocontrol agents such as ethno veterinary herbal medicines. Use of *Nicotiana tabacum* (Tobacco) was a traditional control remedy for control of ectoparasites in India. *In vitro* acaricidal efficacy of crude aqueous leaf extract of *Nicotiana tabacum* on immature and adult stages of *Rhipicephalus (Boophilus) microplus* was carried out at concentrations of 15, 30, 45 and 60% with 12 hour time interval. The extract was found to have a significant effect on the mortality of nymphal and adult stages of ticks. The 100% mortality of Nymphal and adult stages of ticks was observed at 36 hours post treatment with 60% concentration. 100% mortality of all the Nymphal and adult stages of ticks was observed with the concentration 15, 30, 45 and 60% at 48 hours post treatment. The mortality rates increased with the extract concentrations. It is concluded that *Nicotiana tabacum* is effective biocontrol agent for control of ticks.

Keywords: *Nicotiana tabacum*, Biocontrol, acaricidal efficacy, *Rhipicephalus (Boophilus) microplus*

Introduction

Parasitic diseases is a global problem and considered as a major obstacle in the health and product performance of animals. The disease may be mild or severe, the infected animals shows reduced growth rate, incapable to reach its full growth potential, ultimately results in economic losses for the producers. (Das and Laha, 2017)^[4]. Amongst ectoparasites, about 80% of the cattle population is affected by the ticks in the world. Ticks causes the deleterious effects on the health of animals which includes tick bites, sucking of blood, tick worry, tick toxicosis, tick paralysis, transmission of diseases hence affects the health of animals and ultimately responsible for economic losses.

Control of ticks using chemical acaricides has controversial effects due to problems of acaricide resistance, residues of chemical acaricides in food and the environment and the non-suitable population of tick resistant cattle make the current situation unsatisfactory. Use of Chemical acaricides for control of ticks was considered as one of the best methods, but ticks have developed resistance to wide range of acaricides to all currently-used organophosphate-carbamates, synthetic pyrethroids and amidines. (Martins *et al.*, 1995)^[8]. To overcome all these issues there is a call for the development of an alternate and absolute control method, such as biological control. Control of ticks using herbal medicines are ecofriendly, safe, cheap, easy to use and are beneficial to overcome the problems of acaricidal resistance and environmental pollution. *Nicotiana tabacum* (Tobacco) is commonly used in rural areas in India and found to be more effective against all the stages of ticks. Choudhary *et al.*, (2004)^[2] observed the paralysis and absence of pedal reflex in *Rhipicephalus haemaphysaloides* ticks after application of aqueous leaf extract of *Nicotiana tabacum*. *In vivo* acaricidal efficacy of *Nicotiana tabacum* leaf extract studied by Neira *et al.*, (2009)^[10], reported the mortality of adult *Rhipicephalus* spp. ticks in dogs. The leaf extract of *Nicotiana tabacum* against *Rhipicephalus* spp. of ticks was effective to cause paralysis and mortality in adult ticks (Moharana; 2014)^[9]. Lan *et al.*, (2016)^[6] reported that the NaOH (5%) extract of leaves of *Nicotiana rustica* was effective against brown dog ticks. The present study was conducted to evaluate the acaricidal efficacy of *Nicotiana tabacum* leaves against cattle ticks.

Materials and Methods

Preparation of extract

The crude aqueous leaf extract was prepared by maceration and digestion method. The dried leaves of *Nicotiana tabacum* were purchased from local market and were identified from the Department of Botany, Botanical garden, Vayusena Nagar, Nagpur. Leaves were washed thrice to remove dust and dirt and were dried under shade. About 100 grams of dried leaves were grinded into to fine powder using mixer grinder. The dried leaf powder was taken in conical flask to which 400 ml of sterile distilled water was added. The flask was then gently heated in water bath at the temperature of 60°C for 5 minutes. The flask was then kept at room temperature for three days to release the active gradients with frequent agitation and mixing. The extract was then strained using muslin cloth. The extract was maintained in deep freezes for cold evaporation for 10 days.

Collection of ticks

The nymphal and adult stages of ticks were collected from body of animals with hand picking. The adult ticks were collected irrespective of male and female ticks. The ticks were morphologically identified as *Rhipicephalus (Boophilus) microplus* using the morphological keys as described by Alan Walker (1994)^[1].

Results and Discussions

Table 1: Acaricidal activity of *Nicotiana tabacum* against *Rhipicephalus (Boophilus) microplus*

Time in Hrs.	Status of ticks	Concentrations of <i>Nicotiana tabacum</i> leaf extract				Amitraz	Control
		15%	30%	45%	60%	2%	D. W.
0 Hrs.	Nymph (Exposed)	14	14	14	14	14	14
	% mortality	0	0	0	0	0	0
	Adults(Exposed)	12	12	12	12	12	12
	% mortality	0	0	0	0	0	0
12 Hrs.	Nymph (Live)	12	12	6	6	8	14
	% mortality	14.28	14.28	42.85	42.85	57.14	0
	Adults (Live)	11	10	8	8	8	12
	% mortality	8.33	16.66	33.33	33.33	33.33	0
24 Hrs.	Nymph (Live)	6	4	3	3	2	14
	% mortality	57.14	71.42	78.57	78.57	85.71	0
	Adults(Live)	9	8	8	6	4	12
	% mortality	25	33.33	33.33	50	66.66	0
36 Hrs.	Nymph (Live)	3	2	2	0	0	14
	% mortality	78.57	85.71	85.71	100	100	0
	Adults(Live)	4	3	2	0	0	12
	% mortality	66.66	75	83.33	100	100	0
48 Hrs.	Nymph (Live)	0	0	0	0	0	14
	% mortality	100	100	100	100	100	0
	Adults(Live)	0	0	0	0	0	12
	% mortality	100	100	100	100	100	0

Nymphal and Adult stages of *Rhipicephalus (Boophilus) microplus* were exposed to 15, 30, 45 and 60% concentration of aqueous extract of *Nicotiana tabacum*. The efficacy was compared with Amitraz solution. The observations were taken by observing the number of ticks died at one hourly interval and percent mortality was calculated.

Mortality of Nymphal ticks

Percent mortality of Nymphal stages of *Rhipicephalus (Boophilus) microplus* after 12 hour of treatment showed 14.28, 14.28, 42.85 and 42.85% mortality at 15, 30, 45 and 60% concentrations respectively. Mortality of ticks treated with Amitraz was 57.14% whereas 0% mortality was

In vitro Acaricidal efficacy

The ticks were washed thrice with distilled water to avoid the contamination if any. The concentrations of 15%, 30%, 45% and 60% of crude aqueous extract were prepared by dissolving the extract in distilled water. The ticks were treated by using the adult emersion method with slight modification. The filter papers impregnated with 1ml of plant extract of various concentrations were kept in petri plates. The ticks were suspended in treatment solution for one minute and then kept in petri plates impregnated with treated filter paper. Total six groups (N=14) of Nymphal ticks and adult ticks (N=12) were prepared to observe the efficacy against ticks. (Table: 1). First four groups of the ticks were treated with 15, 30, 45 and 60% concentration of extract. Fifth group was treated with commercially available Amitraz solution at the dose rate of 2%. Sixth group of ticks was control group treated with distilled water. The efficacy of *Nicotiana tabacum* was compared with commercially available Amitraz solution at the dose rate of 2%. Also the control group treated with distilled water was maintained to compare the efficacy. The tick mortality was observed at one hourly time interval by pricking the ticks with entomological needle and also by observing the movement of ticks under dissecting microscope.

observed in control group treated with Distilled water.

After 24 hours of treatment the percent mortality was recorded as 57.14, 71.42, 78.57 and 78.57% at the concentration of 15, 30, 45 and 60% respectively. Mortality of ticks treated with Amitraz was 85.71% whereas 0% mortality was observed in control group treated with Distilled water.

Mortality percentage of ticks after 36 hours was noted as 66.66, 75, 83.33 and 100% at 15, 30, 45 and 60% concentration respectively. Mortality of ticks treated with Amitraz was 100% after 36 hours post treatment whereas 0% mortality was observed in control group treated with Distilled water.

After 48 hours of post treatment 100% nymphal mortality was

observed in all the groups at 15, 30, 45 and 60% concentration. No mortality of ticks was observed in control group.

Mortality of Adult ticks

Percent mortality of Nymphal stages of *Rhipicephalus (Boophilus) microplus* after 12 hour of treatment showed 8.33, 16.66, 33.33 and 33.33% mortality at 15, 30, 45 and 60% concentrations respectively. Mortality of ticks treated with Amitraz was 33.33% whereas 0% mortality was observed in control group treated with Distilled water.

After 24 hours of treatment the percent mortality was recorded as 25, 33.33, 33.33 and 50% at the concentration of 15, 30, 45 and 60% respectively. Mortality of ticks treated with Amitraz was 66.66% whereas 0% mortality was observed in control group treated with Distilled water.

Mortality percentage of ticks after 36 hours was noted as 78.57, 85.71, 85.71 and 100% at 15, 30, 45 and 60% concentration respectively. Mortality of ticks treated with Amitraz was 100% after 36 hours post treatment whereas 0% mortality was observed in control group treated with Distilled water.

After 48 hours of post treatment 100% nymphal mortality was observed in all the groups at 15, 30, 45 and 60% concentration. No mortality of ticks was observed in control group. It was noticed that, mortality percentage was related with increased dose of treatment. As the dose of treatment increases the mortality percentage also increases.

In vitro acaricidal efficacy mixture of *N. tabacum* leaves and a mineral salt, was evaluated by Dipeolu and Ndungu (1991)^[3], observed that the larvae and nymphs of *R. appendiculatus* were found dead within 24 hours whereas adult ticks were dead within 2-3 days. In present study 100% mortality of both the mature and immature stages of ticks was observed within 48 hours post treatment. *In vitro* acaricidal efficacy of the aqueous leaf extract of *Nicotiana tabacum* was evaluated on brown dog tick, *Rhipicephalus sanguineus* was evaluated Jeyathilakan *et al.* (2019)^[5] using the concentration of 25%, 50% and 100% in comparison with the standard acaricide deltamethrin and water. The extract at 50% and 100% concentration caused 100% mortality in adult ticks on 10th day of exposure whereas standard drug deltamethrin caused immediate mortality. The mortality of *Boophilus microplus* ticks was observed as 45.8% when treated with 8% ethanol extract of *Nicotiana tabacum* at 24 hours of exposure. (Magadam *et al.*, 2009)^[7] Zaman *et al.*, (2012)^[12], reported the anti-tick efficacy of combined aqueous herbal extracts of *Azadirachta indica* leaves, *Nicotiana tabacum* leaves, *Calotropis procera* flowers and *Trachyspermum ammi*, observed reduced tick intensity on the infested calves at 45% concentration. Lan *et al.*, (2016)^[6] observed acaricidal effect of 5% NaOH extract of leaves of *Nicotiana rustica* (1-20% extract) on brown dog ticks with LT50 and LT100 values of 35 and 55 min, respectively. Tobacco leaf extracts of different alkaloid contents exhibited tick repellency. Waber *et al.*, (2019)^[11], during his experiment reported that, tobacco leaf extracts of different alkaloid contents exhibited tick repellency. The tobacco leaf extracts with high nicotine levels were lethal for ticks. The alkaloid anatabine present in tobacco exhibited the highest tick repellency.

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