



Research Article

ACARICIDAL EFFECT OF CRUDE AQUEOUS EXTRACT OF NICOTIANA TABACUM ALONG WITH TURMERIC AND ANNONA LEAF EXTRACT ON CATTLE TICKS

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Article History: Received 2nd October 2023; Accepted 31st October 2023; Published 18th November 2023

ABSTRACT

The ectoparasites produces severe economic and production loss in farm animal's through tick bites, sucking of blood, tick worry, tick toxicosis, tick paralysis and transmission of haemoprotozoan diseases. Ticks developed resistance against chemical acaricides and also the residues of chemical acaricide producing environmental pollution. The herbal based acaricide used as biocontrol agent for protecting environment particularly hilly area. As per indigenous technical knowledge records, the *Nicotiana tabacum* was a traditional control remedy for control of ectoparasites in India. *In vitro* acaricidal efficacy of crude aqueous leaf extract of *Nicotiana tabacum* along with neem and turmeric followed by Annona leaves on immature and adult stages of *Rhipicephalus sp.* was carried out at different concentrations of with different time interval. These extract combo had a significant effect on the mortality of nymphal and adult stages of ticks. The 70%, 80% and 90% mortality of Nymphal and adult stages of ticks at 24-36 hours post treatment with 40%, 50% 70% concentration of herbal extract in *invitro* assay. The mortality rates of ticks increased with the increasing the extract concentrations both in *vivo* and *in vitro*. *Nicotiana tabacum* causes paralysis of ticks and leads to detachment from the body of animal and concluded that *Nicotiana tabacum* along with turmeric and annona extract is effective biocontrol agent for controlling ectoparasites without any environmental pollution and harmful side effect in animal health.

Keywords: Ectoparasites, Tick toxicosis, Haemoprotozoan diseases, *Nicotiana tabacum*.

INTRODUCTION

Parasitic diseases are a global health problem of livestock and poultry and produces major loss in the health and product performance of animals. The infestation may be mild or severe, the infected animals and bird's shows reduced growth rate, production loss, and immune suppression ultimately results in economic losses for the producers. (Das and Laha, 2017). The 80% of the cattle population is affected by the ticks infestation. 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 other bacterial and viral diseases which affects the health of animals and ultimately responsible for economic losses.

Recently the chemical acaricides were used for controlling ticks had controversial effects due to problems of acaricide resistance, residues of chemical acaricides in food and the environment which is affecting the animal as well as human. The 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) for avoiding these problems need an alternate control method such as biological control mandate for controlling tick infestation in animals. Lan *et al.*, (2016) 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*

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tabacum leaves along with other herbal extract against cattle ticks.

MATERIALS AND METHODS

Preparation of extract

The *Nicotiana tabacum* extract prepared by the method described by Lan *et al.*, (2016). The dried leaves of *Nicotiana tabacum* were purchased from local market and were identified and the crude aqueous leaf extract was prepared by maceration and digestion method. The tobacco 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 fresh leaves of annona plants leaves and neem leaves and turmeric were taken and grinded well added at the rate of 30%,40% and 60% level in tobacco leaf extract and mixed well kept for 2 hrs and the herbal mixture was used for both in animal (directly applied all over the body of animals) and *invitro* study

Collection of ticks

The nymphal and adult stages of ticks were collected from body of animals with hand picking method without damaging the mouth part. 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).

In vitro Acaricidal efficacy

The ticks were washed thrice with distilled water to avoid the contamination if any. The concentrations of 30%, 40% and 60% of crude aqueous extract of the herbal mixture were prepared by dissolving the extract in distilled water. The ticks were treated by using the adult emersion method. The filter papers impregnated with 1ml of plant extract of various concentrations and composition 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=20) of Nymphal ticks and adult ticks (N=20) were prepared to observe the efficacy against ticks (Table 1). First three groups of the ticks were treated with 30%, 40% and 60% concentration of tobacco extract alone. Fifth group was treated with 50% of tobacco leaf extract and 20% of annona leaf extract, 20% of neem leaf extract and 10% of tumeric Sixth group of ticks was control group treated with distilled water. The efficacy of *Nicotianata bacum* was compared with other herbal extract solution at the dose rate of 30, 40% and 60%. And 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

and observing the movement of ticks under dissecting microscope.

Nymphal and Adult stages of *Rhipicephalus* (*Boophilus*) *microplus* were exposed to 30%,40% and 60% concentration of aqueous extract of *Nicotiana tabacum*. The efficacy was compared with tobacco along other herbal extract solution. The observations were taken by observing the number of ticks died at one hourly interval and percent mortality was calculated (Table. 1)

RESULTS AND DISCUSSION

The freshly prepared herbal mixture applied over the animals and the direct application of herbal preparation (50% tobacco: 20% annona leaf extract: 20% neem extract: 10 % turmeric) showed detachment of adult ticks within 2 hrs of post application and death of adult ticks within 12 hrs of application due to starvation (figure1,2, 3&4). Percent mortality of Nymphal stages of *Rhipicephalus* (*Boophilus*) *microplus* after 12 hour of treatment showed 10%, 15%, 25% and 40% mortality rate in 30%,40% and 60% concentrations of aqueous extract of *Nicotiana tabacum* at 12 hrs. The mortality of ticks treated with 50% tobacco and 20% annona leaf extract and 20%neem and 10% turmeric extract was 60% at 12 hrs whereas 1% mortality was observed in control group treated with distilled water after 24 hours of treatment. The Mortality of ticks treated with 50% tobacco and 40% herbal extract was 100 % whereas 1 % mortality was observed in control group treated with distilled water. After 24 hours of post treatment 75 % nymphal mortality and 70% mortality in adult stages of *Rhipicephalus*(*Boophilus*) and *microplus*. (Table.1).The observations were taken by observing the number of ticks died per hour and percent mortality was calculated.

The Controlling of ticks by using herbal medicines should be ecofriendly, safe, cheap and easy to use. The herbal acaricide are beneficial to overcome the problems of acaricidal resistance, side effect in animals and environmental pollution. The *Nicotiana tabacum* (Tobacco) is commonly used acaricidein rural areas in India and found to be more effective against all the stages of ticks. Choudhary *et al.*, (2004) observed the paralysis and absence of pedal reflex in *Rhipicephalus haemaphysaloides* ticks after application of aqueous leaf extract of *Nicotiana tabacum*. The *in vivo* acaricidal efficacy of *Nicotiana tabacum* leaf extract studied by Neira *et al.*, (2009) and reported that the high mortality rate in adult *Rhipicephalus* ticks were found in dogs. The aqueous leaf extract of *Nicotiana tabacum* against *Rhipicephalus* spp. of ticks was effective to cause paralysis and mortality in adult ticks (Moharana, 2014). *In vitro* acaricidal efficacy mixture of *N. tabacum* leaves and a mineral salt was evaluated by Dipeolu and Ndungu (1991) and they 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 70 -75 % mortality of both the mature and immature stages of ticks was observed within 24 hours post treatment along with other herbal extract. Lan

et al., (2016) 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 and tobacco leaf extracts of different alkaloid contents exhibited tick repellency. The *in vitro* acaricidal efficacy of the aqueous leaf extract of *Nicotiana tabacum* was evaluated on brown dog tick, *Rhipicephalus*

sanguineus was evaluated by Jeyathilakan *et al.* (2019) using the concentration of 25%, 50% and 100% in comparison with the standard acaricide deltamethrin and water. The extract at 50% and 100% concentration causes 100% mortality in adult ticks on 10th day of exposure whereas standard drug deltamethrin caused immediate mortality.

Table 1. *Invitro* assay for the effect of herbal mixture combination over the ticks.

Duration of treatment	Condition of ticks	Concentrations of <i>Nicotiana tabacum</i> leaf extract			50% of tobacco leaf extract and 20% of annona leaf extract, 20% of neem leaf extract and 10% of turmeric 50%: 20 % :20%:10%	Control group Distilled water
		30%	40%	60%		
0 hrs	Nymph (exposed)	20	20	20	20	20
	% mortality	0	0	0	0	0
	Adult (exposed)	20	20	20	20	20
	% mortality	0	0	0	0	0
12 hrs	Nymph (exposed)	20	20	20	20	20
	% mortality	10	15	25	40	0
	Adult (exposed)	20	20	20	20	20
	% mortality	0	10	20	60	0
24 hrs	Nymph (exposed)	20	20	20	20	20
	% mortality	15	20	35	75	5
	Adult (exposed)	20	20	20	20	20
	% mortality	10	15	25	70	1%



Figure 1. Fresh annona tree leaves used for preparation of acaricide.



Figure 2. Preparation of herbal mix for ticks.



Figure 3. Application of 50% tobacco extract, 20% annona leaf extract, 20% neem leaf extract and 10%



Figure 4. Application of 50% tobacco extract, 20% annona leaf extract, 20% neem leaf extract and 10%

turmeric mix into the head,neck, ear and face of the animal



Figure 5. Paralysis and detachment of ticks after 2hrs of application.

turmeric mix into the all over the body of animal



Figure 6. Preparation of aqueous extract of tobacco for *in vitro* study.



Figure 7. *Invitro* assay for adult tick mortality with different concentration of herbal preparation.



Figure 8. *Invitro* assay for nymph mortality with different concentration of herbal preparation.

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 (Magadum *et al.*, 2009). Zaman *et al.*, (2012) reported that the anti-tick efficacy of combined aqueous herbal extracts of *Azadirachta indica* leaves, *Nicotiana tabacum* leaves, *Calotropis procera* flowers and *Trachyspermum ammi* and they observed reduced tick infestation on the infested calves at 45% concentration. Waber *et al.*, (2019) reported that different alkaloid contents of tobacco leaf extracts which exhibited tick repellency and the tobacco leaf extracts with high nicotine levels were causes death to ticks and the alkaloid anatabine present in tobacco exhibited the highest tick repellency. The fresh leaves of annona were spread in poultry houses and are left until they are dried in order to achieve the control of parasites such as fleas and lice (Salifou *et al.*, 2012). In this present study showed tobacco leaf extract along with other herbal mixture causes high mortality both adult and young stage of ticks however in depth detailed further study is needed.

ACKNOWLEDGEMENT

The authors thankful to the Director, Centre for Animal Health Studies, Madhavaram Milk Colony, Chennai-51 for providing necessary facilities

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