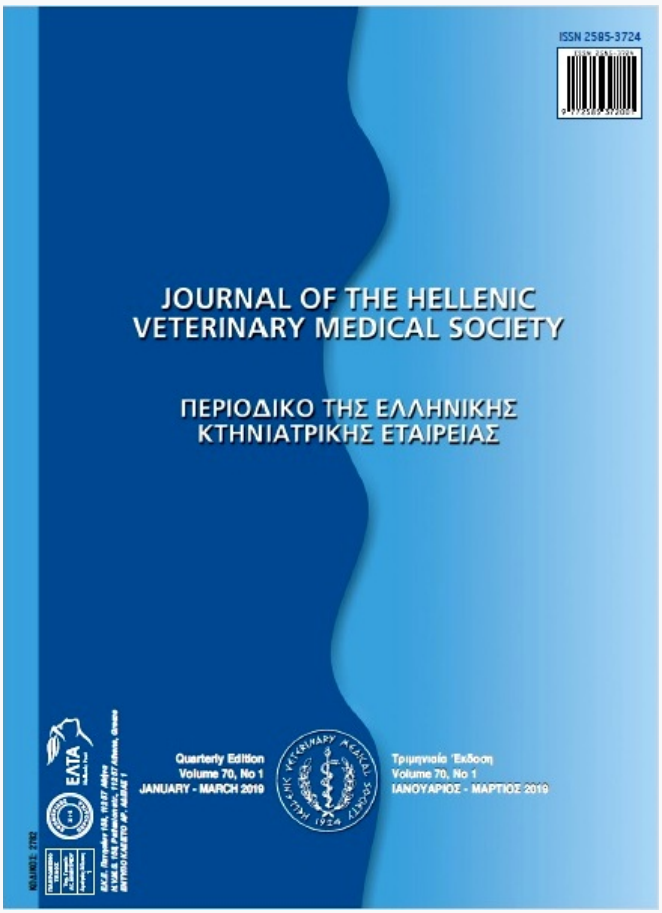
Journal of the Hellenic Veterinary Medical Society

Vol 70, No 1 (2019)



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doi: 10.12681/jhvms.20343

To cite this article:

ULLAH, N., AKHTAR, R., LATEEF, M., JAN, S. U., ZAHID, B., & DURRANI, U. F. (2019). Factors affecting the prevalence of ticks in cattle and acaricidal activity of Nicotiana tabacum extracts. *Journal of the Hellenic Veterinary Medical Society*, *70*(1), 1381–1386. https://doi.org/10.12681/jhvms.20343

J HELLENIC VET MED SOC 2019, 70(1): 1381-1386
ΠΕΚΕ 2019, 70(1): 1381-1386

Research article
Ερευνητικό άρθρο

Factors affecting the prevalence of ticks in cattle and acaricidal activity of *Nicotiana tabacum* extracts

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ABSTRACT. The present study was designed to determine factors affecting the prevalence of ticks *(Hyalomma and Rhipicephalus)* in cattle in district Loralai of Balochistan and to evaluate the acaricidal activity of chloroform and methanol extracts of tobacco *(Nicotiana tabacum)*. A total of 670 cattle of different breeds, age and gender were examined for tick infestation with overall prevalence of 21.49% in Loralai. Friesian was more infected (26.15%) as compare to non-descriptive (22%) and Sahiwal (12.80%) breeds. Similarly, cattle less than one year old were most infected (27.90%) followed by those between 1-2 year (26.88%); the least prevalence was in cattle more than 2 years of age (19.34%). Higher prevalence was noticed in female cattle (21.98%) as compare to male cattle (16.92%). Three concentrations of *(Nicotiana tabacum)* (12.5mg/mL, 25mg/mL and 50mg/mL) were prepared in chloroform and methanol. The acaricidal activity of these extracts was determined by egg laying index and percentage inhibition of egg laying. The decline in egg laying index was significantly more by chloroform extract (10.048%, 17.378% and 25.143%) as compare to methanol extract (6.367%, 13.152% and 20.827%). Hatchability of eggs in chloroform extract was less than that in methanol extract (67.5%, 43.5% 17% and 77.5%, 47.5% and 23%) respectively. We concluded that the prevalence of ticks in cattle is affected by their age, breed and gender and that chloroform extract of *Nicotiana tabacum* is more acaricidal as compared to the methanol extract.

Keywords: Egg laying index, Hatchability, *Nicotiana tabacum* extracts, Ticks.

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Date of initial submission: 04-12-2018
Date of revised submission: 29-08-2018
Date of acceptance: 25-09-2018

INTRODUCTION

Ticks act as a vector for various diseases of livestock including the zoonotic diseases. In Pakistan, many species of ticks have been reported to affect livestock and decrease their production (Sajid et al., 2008). Keeping in mind the importance of different types of ticks present in different geographical distribution there are many reports about the types of ticks present in different areas of Pakistan. However, there is not a single study about the types of ticks present in Lorali District of Balochistan. The geographical location of Lorali in centre of Balochistan and its livestock population makes this area important for evaluation of ticks types and factors affecting the prevalence of ticks in this area. This laid the basis for the present study.

Another objective of present study to evaluate acaricidal activity of *Nicotiana tabacum* plant extracts for the control of ticks. As the control of ticks primarily depends upon synthetic acaricides (Adenubi et al., 2018) but it has been complicated due to drug resistance (Banumathi et al., 2017). The development of resistance and presence of drug residues in milk, meat, and the environment has created the need for search of less toxic substances for tick control. Keeping in view the above-mentioned facts there is a dire need to investigate alternative acaricides and new approaches for tick control.

The use of herbal plants against ticks is getting popular in developing countries (Wu et al., 2017). A number of easily available herbal plants have been evaluated for their acaricidal effect in Pakistan (Tabassum et al., 2008). Zaman et al. (2012) explained the synergistic effect of *Nicotiana tabacum* leave extract in combination with three other plants against ticks. In India Chaudhary et al. (2004) studied the in vitro effect of *Nicotiana tabacum* aqueous extract on Rhipicephalus haemaphysaloides ticks. As there have been no studies to evaluate the chloroform and methanol extract of *Nicotiana tabacum* against ticks therefore we evaluated the acaricidal effect of methanol and chloroform extract of *Nicotiana tabacum* plant. This research was particularly designed to study the effect of *Nicotiana tabacum* against most commonly occurring ticks district Loralai, Balochistan. We also hypothesize that breed, age and gender could be the most important determinants to infestation of ticks and evaluated the link of these parameters on tick prevalence.

MATERIALS AND METHODS

The prevalence of tick infestation and associated risk factors were also determined in 670 cattle in three tehsils of district Loralai including Bori, Dukki, Makhtar and their villages. The breed, age and sex of cattle were studied as associated risk factors for tick prevalence.

The *Nicotiana tabacum* plant was collected from the area of district Loralai and dried for 8 to 10 days. The leaves were grinded mechanically into powder form in Soxhlet apparatus (Iqbal et al., 2005) and stored in refrigerator at 4°C until used. The powder extracts were used to make three different concentrations of 12.5, 25 and 50mg/mL for both extracts (chloroform and methanol). The results from these concentrations were compared to positive and negative control groups. The buffer PBS used as negative control.

Adult immersion test (AIT)

The female engorged ticks collected from study area were washed with phostphate buffer saline (PBS) and dried by using paper towel. After that the ticks were weighed and then immersed into the formulated solution for five minutes. After immersion, ticks were incubated at 30ºC with relative humidity 80-90%. After that the ticks were placed in incubator for oviposition for (16-18 days). The reproductive index (RI) and % age Inhibition of oviposition (IO%) was calculated on the basis of following parameters (Sabatini et al., 2001).

- Mortality was recorded up to 14 days post treatment (dpt) when normal ticks complete egg laying.
- The egg masses laid by the live ticks were recorded.
- Reproductive index (RI) = egg weight (EW) engorged female weight (IFW).
- Percent Inhibition of oviposition (IO%) =RI control−RI treated RI control×100

Egg hatchability test

Approximately 10mg (200 embryonated eggs) were treated in each concentration for five minutes. After that ticks were incubated at 30ºC with relative humidity 80-90% until the eggs hatched (Ribeiro et al., 2008). Hatchability percentage was calculated as the number of hatched larvae divided by the total number of incubated eggs.

Egg laying index (IE) = mean weight of eggs laid (g)/ weight of females (g)

% inhibition of egg laying = IE control group - IE treated group/ IE control group x 100

Statistical analysis was made by SPSS version 22.0 using mean ± S.D. Qualitative variables were presented with help of frequency tables, pie charts and bar charts. Comparative analysis was done using one-way ANOVA and P value <0.05 was considered significant.

RESULTS AND DISCUSSION

The results of present study revealed that tick infestation was common in cattle of district Loralai, Baluchistan with 21.49% prevalence. Out of total 670 cattle 526 were positive for ticks. We found increased incidence of Hyloma ticks (380) as compare to Rhipicephalus (146). The geographical importance of district Loralai, Balochistan cannot be neglected due to its central position in Balochistan province. To the best of our knowledge this is the first study about the tick prevalence in Loralai, Balochistan. This prevalence is much higher than the tick prevalence in Quetta city of same province reported as low as 10% by Kakar et al. (2008).

Our results are different from another study conducted in Peshawar district (KPK) where Manan et al. (2007) found four types of ticks including Boophilus (46%), Hyalomma (31%), Rhipicephalus (18%) and Amblyomma (5%) in cattle with overall prevalence of 20%. However, our results are in agreement with the studies of Sajid et al. (2008) who found highest prevalence of Hyalomma followed by Rhipicephalus in cattle of district Layyah and Muzaffargarh.

The cross tabulation test showed significant difference (P<0.05) in prevalence of tick infestation in different cattle. Friesian was significantly more infected (P<0.05) (26.15%) as compare to non-descriptive (22%) and Sahiwal (12.80%) breeds. The more tick resistance of Sahiwal cattle (P<0.05) may be due to their thick leathery and naturally shivering skin. However, this is not in agreement with previous studies conducted in Lahore, Pakistan (Sadaqat et al., 2016) that reported highest tick infestation in Sahiwal (23.8%) followed by Friesian (16.3%) and non-descript cattle (12.8%) respectively. However, these differential results in both studies could be due to the difference in geographical location, sample size and different seasons.

Similarly, the cattle less than one year were most infected (27.90%) followed by cattle between 1-2 years (26.88%) of age. The least prevalence was in cattle more than 2 years (19.34%). This may be linked with development of immunity with increasing age. This is in agreement with the studies of Rehman et al. (2017) who described increased tick prevalence in older animals as compare to younger.

Higher prevalence was noticed in female cattle (21.98%) as compare to male cattle (16.92%). The production and reproduction of female may be important risk factors involved in high incidence of disease in female. This is in contrast with the studies of Rehman et al. (2017) that indicated almost three times more tick prevalence in male cattle (60.77%) as compare to female cattle (20%).

Acaricidal effects of chloroform extracts compared with control group

The ticks in group A1, A2 and A3 were treated in vitro with chloroform extract at the concentration of 12.5mg/mL, 25mg/mL and 50mg/mL respectively. The egg laying index ± SD calculated was 0.4782800±0.02789077, 0.4388300±0.05119868 and 0.3963600±0.03380405. In Post Hoc LSD multiple comparison tests the results of group A were significantly different (P<0.05) from the ticks in group C (untreated control group) with egg laying index± SD 0.5331200±0.02757486 (Table 1). The percentage inhibition of egg laying was group A1, A2 and A3 10.048, 17.378 and 25.143 in respectively.

Acaricidal effects of methanol extracts compared with control group

The ticks in group B1, B2 and B3 were treated in vitro with methanol extract at the concentration of 12.5mg/mL, 25mg/mL and 50mg/mL respectively. The egg laying index± SD calculated was 0.4991200±0.00948646, 0.4614300±0.03917896 and 0.4205800±0.04183098. In Post Hoc LSD multiple comparison tests the results of group B were significantly different (P<0.05) from the ticks in group C (control) with egg laying index± SD

Table 1: Least significant difference and multiple comparison test values of group A compared to other treated and untreated groups.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Index of egg laying 12.5mg/mL of chloroform extract	Index of egg laying 12.5mg/mL of methanol extract	-.02084000	.01574539	.190	-.0523046	.0106246
	Index of egg laying 25mg/mL of methanol extract	.01685000	.01574539	.289	-.0146146	.0483146
	Index of egg laying 50mg/mL of methanol extract	.05770000*	.01574539	.001	.0262354	.0891646
	Index of egg laying 25mg/mL of chloroform extract	.03945000*	.01574539	.015	.0079854	.0709146
	Index of egg laying 50mg/mL of chloroform extract	.08192000*	.01574539	.000	.0504554	.1133846
	Control untreated	-.05484000*	.01574539	.001	-.0863046	-.0233754

0.5331200±0.02757486 (Table 2 & 3). The percentage inhibition of egg laying in group B1, B2 and B3 was 6.367, 13.152 and 20.827% respectively.

The previous studies have already explained the better anthelminthic effect of alcoholic extract of Nicotiana tαbacum as compare to aqueous extract. (Nouri et al., 2016). But the present study for the first time compared the effect of alcoholic extracts (methanol and chloroform) against ticks.

Bioactive compounds from *Nicotiana tαbacum* most-

Table 2: Least significant difference and multiple comparison test values of group B compared to other treated and untreated groups

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Index of egg laying 12.5mg/mL of methanol extract	Index of egg laying 25mg/mL of methanol extract	.03769000*	.01574539	.020	.0062254	.0691546
	Index of egg laying 50mg/mL of methanol extract	.07854000*	.01574539	.000	.0470754	.1100046
	Index of egg laying 12.5mg/mL of chloroform extract	.02084000	.01574539	.190	-.0106246	.0523046
	Index of egg laying 25mg/mL of chloroform extract	.06029000*	.01574539	.000	.0288254	.0917546
	Index of egg laying 50mg/mL of chloroform extract	.10276000*	.01574539	.000	.0712954	.1342246
	Control untreated	-.03400000*	.01574539	.035	-.0654646	-.0025354

Table 3: Least significant difference and multiple comparison test values of group C compared to other treated groups

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control untreated	Index of egg laying 12.5mg/mL of methanol extract	.03400000*	.01574539	.035	.0025354	.0654646
	Index of egg laying 25mg/mL of methanol extract	.07169000*	.01574539	.000	.0402254	.1031546
	Index of egg laying 50mg/mL of methanol extract	.11254000*	.01574539	.000	.0810754	.1440046
	Index of egg laying 12.5mg/mL of chloroform extract	.05484000*	.01574539	.001	.0233754	.0863046
	Index of egg laying 25mg/mL of chloroform extract	.09429000*	.01574539	.000	.0628254	.1257546
	Index of egg laying 50mg/mL of chloroform extract	.13676000*	.01574539	.000	.1052954	.1682246

ly include alkaloids. In present study it was noted that chloroform extract showed better acaricidal activities against *Rhiphicephalus* and *Hyloma* ticks as compare to methanol extract. Although methanol is more commonly used for good extraction various bioactive compounds due to its amphiphilic properties but it might be possible that alkaloids react better with chloroform as compare to methanol.

Hatchability of each treated group was calculated by dividing the number of eggs hatched with number of egg laid. Hatchability of group A1, A2 and A3 was 67.5%, 43.5% and 17% respectively. While for group B1, B2 and B3 it was 77.5%, 47.5% and 23% respectively. Hatchability of the ticks in group C (control group) not treated with any chemical only dipped in distilled water for five minutes was100%.

The egg laying index of ticks treated with lowest concentrations of both chloroform and methanol extracts of *Nicotiana tabacum* was significantly less ($P<0.05$) than the control group. This could be due to active ingredients of *Nicotiana tabacum*, which are helpful in controlling egg production in ticks. Chloroform extract with its highest concentration (50mg/mL) of the plant suppressed the hatchability of the egg to maximum extent but this difference was not significantly different ($P>0.05$) from the ticks treated with extracts of methanol.

CONCLUSIONS

The present study concluded that the use of methanolic and chloroform extract of *Nicotiana tabacum* plant is effective against ticks and egg laying characteristic of the tick is significantly inhibited by the extracts particularly chloroform extract. We also concluded that the tick prevalence is affected by various factors including age, breed and gender of infected cattle.

ACKNOWLEDGMENTS

This research was funded by Department of Parasitology, UVAS.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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