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Original Article

Acaricidal efficacy of ethanol extract of *nicotiana rustica* (wild tobacco) against *hyalomma anatolicum* (Acari: Ixodidae)

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Abstract. Ticks are important ectoparasites of livestock, causing considerable economic losses beyond their role as vectors of various diseases. While chemical acaricides are commonly used for tick control, their application is associated with risks such as toxicity, environmental hazards, and resistance development. These challenges necessitate the exploration of alternative solutions, that are safer and more environmentally compatible. This study evaluates the acaricidal activity of ethanol extracts of *Nicotiana rustica* (wild tobacco) against all life stages of the hard tick *Hyalomma anatolicum* using egg hatchability test and the immersion method. The extract was tested in three replicates of 2, 4, and 8% concentrations. Complete inhibition of egg hatching occurred at the 8% concentration. The extract exhibited lethal effects across all flat stages of *H. anatolicum.* The LC_{95} on flat stages of larvae, nymphs and adults was 5.5, 7.59 and 8.41, respectively. The engorged larvae completely failed to molt into nymphs. The extract significantly ($P \leq 0.05$) reduced molting of engorged nymphs and inhibited egg laying of the engorged female, with a notable lethal effect (33.8%) observed at the 2% concentration. These findings demonstrate the potential of *N. rustica* extract as a promising botanical acaricide, offering an eco-friendly and effective alternative for tick management. Further research is needed to elucidate its mode of action and evaluate its long-term viability in field applications.

Keywords: Acaricidal activity, Nicotiana rustica, ticks, Hyalomma anatolicum, botanical extract.

Introduction

Hyalomma anatolicum is one of the most prevalent hard tick species in Sudan, infesting various hosts and acting as a vector for several debilitating diseases, including bovine tropical theileriosis, malignant ovine thieleriosis and equine thieleriosis [1-4]. In addition to disease transmission, it decreases weight gain, milk production, and the quality of skin and hide in livestock [5, 6]. Tick infestation is primarily controlled worldwide through chemical acaricides. However, these chemicals pose significant risks, including misapplication, intoxication of humans and animals, and adverse impacts on non-target organisms and the environment [7]. They also lead to contamination of meat and milk products with chemical residues [8]. Moreover, chemical acaricides are expensive [9], and resistance to them is increasingly developing. In addition, the evolution of resistance to chemical acaricides is increasingly developing [10]. Given these challenges, it is critical to explore new compounds with satisfactory properties for controlling the target ticks as well as having less negative impact on the environment, animal welfare and public health.

Various medicinal plants have been screened for acaricidal activity against different tick species including *H. anatolicum* such as *Azadirachta indica (neem*) seeds [11] and *Guiera senegalensis* (*Algubeish*) leaves [12]. The wild tobacco herb, *Nicotiana rustica* (*Solanaceae*), is a rainforest plant originating from Central and South America [13]. It grows in Sudan and is locally known as *tombac*, constituting the main cash crop in the Darfur States [14]. *N. rustica* contains a large amount of alkaloids; the most predominant is nicotine [15]. While the acaricidal properties of *Nicotiana tabacum* have been documented [16], the efficacy of *N. rustica* against ticks remains largely unexplored.

This study investigates the acaricidal activity of ethanol extracts of *N. rustica* against all developmental stages of *H. anatolicum*. The findings aim to establish *N. rustica* as a viable botanical acaricide and an eco-friendly alternative for sustainable tick management.

2. Materials and Methods

2.1. Preparation of *Nicotiana rustica* extract.

Dried leaves of *Nicotiana rustica* (wild tobacco) were obtained from local mills in El Fashir, Northern Darfur

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State, Sudan. Ethanol extraction was conducted using a modified protocol based on (17). Briefly, 100 g of powdered leaves were soaked in 250 ml of 95% ethanol in a tightly sealed flask for three days. The mixture was filtered using Whatman No.1 filter paper, and the remaining plant material was re-soaked in ethanol for an additional day to maximize yield. The combined filtrates were concentrated using a rotary evaporator at 40°C under reduced pressure, and the resulting extract was stored in glass vials at 4°C until use. Test solutions of 2%, 4%, and 8% were prepared by dissolving the extract in distilled water and thoroughly mixing to ensure homogeneity.

2.2. Tick Collection and Identification

Engorged female ticks were collected manually from naturally infested cattle on dairy farms near North Omdurman, Khartoum State, Sudan (15.64°N, 32.47°E). The ticks were transported to the Parasitology Laboratory, Faculty of Veterinary Medicine, University of Khartoum, where they were identified as Hyalomma anatolicum using standard morphological criteria (18).

2.3. Rearing of Ticks

Engorged female ticks were individually placed in glass tubes (5.2 × 2.2 cm) sealed with cotton plugs and maintained at 85% relative humidity using a saturated potassium chloride solution and the tubes were kept at 28 ± 1°C to facilitate oviposition [19]. Eggs laid by these females were collected and incubated under the same conditions until hatching. One- to two-week-old larvae were used for larval immersion tests. A subset of the larvae was fed on rabbits using ear bags [20] to obtain nymphal and adult stages for subsequent tests.

2.4. Bioassays for Acaricidal Activity

The acaricidal efficacy of the N. rustica ethanol extract was assessed through a series of bioassays, including egg hatchability and immersion tests, to evaluate its effects on various life stages of *H. anatolicum*.

2.4.1. Egg Hatchability Test (EHT)

The effect of the extract on egg hatchability was assessed following the method of [21] with minor modifications. Approximately 12 mg of embryonated eggs (~200 eggs) were placed in glass tubes. Three milliliters of extract at concentrations of 2%, 4%, and 8%, as well as distilled water (control), were added to each tube. After 30 seconds of exposure, the solutions were decanted, and the eggs were air-dried. The tubes were sealed and incubated at 28 ± 1°C and 85% relative humidity for 14 days. The percentage of hatching inhibition was calculated as described below:

$$\text{HI\%} = \frac{\text{Total No. of treated eggs}}{\text{Emerged larvae}} \text{x100}$$

2.4. 2. Larval Immersion Test (LIT)

Flat larvae aged 1–2 weeks were used to assess larvicidal activity following Shaw [22]. Groups of 100 larvae were exposed to 3 ml of extract (2%, 4%, or 8%) and distilled water (control) on Whatman No. 1 filter paper in Petri dishes. After 30 seconds, the larvae were transferred to clean filter paper for drying and then incubated in desiccators at 85% relative humidity and 28 ± 1°C for 24 hours. Mortality rates were recorded. Immobile larvae were considered dead.. Engorged larvae were also immersed in the same concentrations as described by Mwangi et al. (1995) and observed for moulting inhibition over 14 days.

The Larval mortality and moulting rate were calculated as described below:

$$\text{Mortality\%} = \frac{\text{Dead larvae}}{\text{Total larvae}} x100$$

$$\text{Moulting\%} = \frac{\text{No. of moulted larvae}}{\text{Total No. of treated engorged larvae}} x100$$

2.4. 3. Nymphal Immersion Test (NIT)

Flat and engorged nymphs were immersed in 3 ml of extract at 2%, 4%, and 8% and distilled water (control) for 30 seconds, as described by [23]. After drying on filter paper, the nymphs were incubated under the same conditions as larvae and monitored for mortality and moulting inhibition over 14 days. The nymphal mortality and moulting rate were calculated as follow:

$$\text{Mortality\%} = \frac{\text{Dead nymph}}{\text{Total nymph}} x100$$

$$\text{Moulting\%} = \frac{\text{No. of moulted nymph}}{\text{Total No. of treated engorged larvae}} x100$$

2.4. 4. Adult Immersion Test (AIT)

Flat and engorged adult ticks were subjected to immersion tests following the procedure of [24]. Mortality, egg production index (EPI), and inhibition of oviposition (IO) were recorded. Eggs laid by surviving females were collected, weighed, and incubated under the same conditions to determine egg production index (EPI) and inhibition of oviposition (IO).

$$\text{EPI} = \frac{\text{Weight of engorged female (g)}}{\text{Weight of eggs produced (g)}} x100$$

$$\text{IO \%} = \frac{\text{EPI of control group} - \text{EPI of treated group}}{\text{EPI of control group}} x100$$

2.5. Statistical Analysis

The data on tick mortality, egg hatchability inhibition, moulting inhibition, and oviposition inhibition were analyzed using one-way analysis of variance (ANOVA) to compare the efficacy of each concentration and identify differences among sample means using Statistical Package for Social Science (SPSS 20) according to the method of Finney [25]. Statistical significance was determined at a probability level of $P \leq 0.05$.

TABLE 1

THE EFFECT OF ETHANOL EXTRACT OF N. RUSTICA ON PRE-ADULTS STAGES OF H. ANATOLICUM

Developmental stage	Eggs	Flat larvae	Engorged larvae	Flat nymphs	Engorged nymphs
Parameters	IH% (Mean ± SE)	M% (Mean ± SE)	IM% (Mean ± SE)	M% (Mean ± SE)	IM% (Mean ± SE)
Control	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]	00.00 ± 0.00[a]
2%	87.31± 6.53[b]	98.28 ± 1.71[b]	100.00 ± 0.00[b]	79.19 ± 8.85[bc]	55.00 ± 21.79[b]
4%	96.37 ± 3.62[b]	100.00 ± 0.00[b]	100.00 ± 0.00[b]	61.18 ± 7.63[b]	41.66 ± 14.24[ab]
8%	100.00 ± 0.00[b]	99.65 ± 0.34[b]	100.00 ± 0.00[b]	90.55 ± 9.45[c]	51.66 ± 16.91[ab]

IH = inhibition of egg hatching. M = mortality. IM = inhibition of molting. Means within a column followed by the same letter are not significantly different ($p \leq 0.05$).

TABLE 2

THE EFFECT OF ETHANOL EXTRACT OF N. RUSTICA ON MORTALITY, EGG PRODUCTION INDEX (EPI) AND INHIBITION OF OVIPOSITION (IO) OF ADULT STAGE OF H. ANATOLICUM.

Developmental stage	Flat adult		Engorged adult	
Parameters	M% (Mean ± SE)	IO% (Mean ± SE)	EPI (Mean ± SE)	IO% (Mean ± SE)
Control	00.00 ± 00.00[a]	00.00 ± 00.00[a]	00.00 ± 00.00[a]	00.00 ± 00.00[a]
2%	76.82 ± 5.79[b]	33.81± 17.41[b]	0.488 ± 0.03[a]	16.66 ± 01.85[ab]
4%	84.66 ± 4.12[b]	5.17± 5.17[ab]	0.385 ± 0.11[a]	37.33 ± 18.26[b]
8%	72.97 ± 11.57[b]	1.18 ± 0.67[a]	0.508 ± 0.04[a]	15.33 ± 03.84[ab]

CM = mortality. EPI = egg production index. IO = inhibition of oviposition. Means within a column followed by the same letter are not significantly different ($p \leq 0.05$).

Lethal concentrations required to achieve 50% and 95% mortality (LC50 and LC95) and inhibitory concentrations required to achieve 50% and 95% inhibition of molting or hatching (IC50 and IC95) were calculated using probit analysis of a cumulative percentage [26].

All percentage data, such as mortality, egg hatchability inhibition, and oviposition inhibition, were corrected using Abbott's formula [27] to account for natural mortality observed in the control group.

$$\text{Corrected effect \%} = \frac{\text{Observed effect in treatment} - \text{Observed effect in control}}{100 - \text{Observed effect in control}} x100$$

Result

The effect on egg hatching of *H. anatolicum* treated with different concentrations of *N. rustica* extract is presented in (Table 1). The extract significantly ($p \leq 0.05$) reduced egg hatchability in comparison with the control group and completely inhibited egg hatching at 8% concentration. The calculated LC 50 and LC 95 were 1.5 and 5.79%, respectively (r2= 0.56). Ethanol extract of *N. rustica* significantly caused high mortality ($p \leq 0.05$) in flat larvae of *H. anatolicum* compared with the control group (Table 1). The calculated LC 50 and LC95 were 1.05 and 5.54%, respectively, (r2= 0.47). The engorged larvae completely failed to molt into flat nymphs at all concentrations. The calculated concentration that inhibits 50 and 95% of engorged larvae to molt (IC 50 and IC 95) was 1 and 5.5%, respectively, (r2= 0.46).

Ethanol extract of *N. rustica* significantly caused high mortality ($p \leq 0.05$) in flat nymphs of *H. anatolicum* compared with the control group (Table1). The calculated LC 50 and LC 95 were 2.65 and 7.59%, respectively (r2= 00.59). The molting of engorged nymphs was significantly reduced ($p \leq 0.05$) at all concentrations. The calculated concentration that inhibits 50 and 95% of engorged nymphs to molt (IC 50 and IC 95) was 6.14 and 15.36%, respectively (r2= 0.43). The extract of *N. rustica* induced significant mortality ($P \leq 0.05$) in flat adult of *H. anatolicum* in comparison with control group (Table 2). The calculated LC 50 and LC 95 were 2.31 and 8.48 %, respectively. (r2=0.40). The highest mortality of 84.66% occurred at 4% concentration. Ethanol extract of *N. rustica* against engorged females of *H. anatolicum* caused significant ($p \leq 0.05$) mortality of 33.81 % at 2% concentration (Table2). The calculated LC50 and LC95

were 29.17 and 65.96%, respectively, (r2=0.068). Significant Inhibition of oviposition (37.33%) in survival females was achieved at 4% concentration however there is no significant differences in egg production index.

Discussions

Ticks have economic importance; thus, their control with eco-friendly acaricides is required. It is shown in this study that the ethanol extract of *N. rustica* completely inhibited hatching of *H. anatolicum* eggs at 8% concentration. This effect is similar to that observed on *Rhipicephalus microplus* caused by *Illicium verum* extract [28], and in line with that reported by [29], where *Nicotiana tabacum* inhibited eggs hatching of *Hyalomma species*, with hatchability rates between 24% and 25% at 10% concentration of treatment. The action of hatching inhibition may be linked to the porosity of the egg shell of this tick. [30] Found micropyle- like regions in egg- shell of *Dermacenter andersoni*. Thus, it remains a possible feasible off- host tick control tactic to utilize the ovicidal property of this botanical extract as it would evidently satisfy the demands of the environment, animal welfare and public health. It was found in this study that *N. rustica* was lethal to flat stages of *H. anatolicum*. This finding is close to the studies conducted by [31] using alcoholic extracts of *Vitex agnus-castus* and *Populus euphratica*.

A similar effect on flat larvae and adults of *H. anatolicum* and *H. excavatum* was caused by extracts of *Azadirachta indica* and Guiera senegalensis as reported by [11] and [12], respectively. The presented results show that treated engorged larvae of *H. anatolicum* completely failed to molt into nymphs. Such high efficacy in prohibition of ecdysis of the engorged larval stage has not been reported before for any acaricidal compound as far as we know. In comparison, pyriproxyfen, a pyridine- based pesticide, partially decreased molting of engorged larvae of *Amblyomma americanum* by only 35.4- 68.4% [32]. Moreover, molting of the engorged nymphs of *H. anatolicum* was significantly inhibited by treatment with extracts of *N. rustica*. Similarly, *G. senegalensis* significantly reduced molting of engorged nymphs of *H. anatolicum* [12]. It is worth noting that the findings of this study showed that the juvenile life stages of *H. anatolicum* are more susceptible to the extracts of *N. rustica* than the late life stages. This is in agreement with the finding of [33] who demonstrated that treatment of larvae, nymphs and adults of *Rhipicephalus sanguineus* with fluralaner, a novel isoxazoline, showed that the juvenile life stages were more susceptible than adults; and among the juvenile stages larvae were slightly more sensitive than nymphs.

The data of the present study indicated that *N. rustica* caused lethality against flat adults of *H. anatolicum* reaching 85% at the concentration of 4%. A similar effect was induced by *Vitex castus*, and *Zingiber officinale* against the camel tick, *Hyalomma dromedarii*[34]. For engorged females of *H. anatolicum*, *N. rustica* significantly diminished oviposition. The same impact on oviposition was exhibited by *Artemisia absinthium* [35]. It is also observed that the concentration of 2% is significantly more potent than 8%. A similar effect on *Rhipicephalus microplus* has been caused by *Ageratum conyzoides* [36] where 5% was more effective than 10%.

It is probable, though further documentation is needed, that *N. rustica* affects ticks through its effect on the tick nervous system. Homogeneously, *N. rustica* may prevent tick molting through disturbance of molting hormones which are regulated by the nervous system [37]. It is worth noting that ticks possess a highly condensed, fused nerve mass where the cerebral ganglia and ventral nerve cord, with its associated segmental ganglia, have coalesced into a peri-oesophageal synganglion [38]. Peripheral nerves branch laterally from the synganglion and innervate all organs throughout the body. It is known that *N. rustica* contains nicotine as a toxic agent [15]. The symptoms of nicotine poisoning are caused by excessive stimulation of nicotinic cholinergic neurons. Nicotine is an antagonist at nicotinic acetylcholine receptors which are present in the central and autonomic nervous systems and the neuromuscular junction. At low doses, nicotine causes stimulatory effects on these receptors, however, higher doses or more sustained exposures can cause inhibitory effects leading to neuromuscular blockade [39, 12]. It can be concluded that *N. rustica* (wild tobacco) is potentially potent as an anti-tick agent.

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Conflict of interest

The authors declare no conflicts of interest.

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