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Mubarik Ali
Animal Science Institute, National Agricultural Research Center, Islamabad-54000-Pakistan, mubarikalicheema@gmail.com

Atta Ur Rehman
Faculty of Veterinary and Animal Sciences, Gomal University Dera Ismail Khan-29050-Pakistan, attreman77@gmail.com

Naimat Ullah
I3Institute of Biological Sciences (IBS) Gomal University Dera Ismail Khan-29050-Pakistan, drnaimat@gu.edu.pk

Muhammad Tariq
Faculty of Veterinary and Animal Sciences, Gomal University Dera Ismail Khan-29050-Pakistan, muhammadtariq90@gmail.com

Sonia Bibi
Botany Department, University Of Science and Technology Bannu-28100-Pakistan, sonia_gu@yahoo.com

See next page for additional authors

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Authors

Mubarik Ali, Atta Ur Rehman, Naimat Ullah, Muhammad Tariq, Sonia Bibi, Saeed Ullah, Anila Khan, Faiqah Ramzan, Muhammad Ayub, Attia Imam, and Muhammad Jamil

ANTI-TICK PROPERTIES/REPELLENCY OF NEEM, *AZADIRACHTA INDICA* ON *RHIPICEPHALUS SANGUINEUS* (ACARINA) UNDER LABORATORY CONDITIONS

MUBARIK ALI[1], ATTA UR REHMAN[2], NAIMAT ULLAH[3], MUHAMMAD TARIQ[2], SONIA BIBI[4], SAEED ULLAH[5], ANILA KHAN[3], FAIQAH RAMZAN[2], MUHAMMAD AYUB[6], ATTIA IMAN[7], AND MUHAMMAD JAMIL[8]

[1]*Animal Science Institute, National Agricultural Research Center, Islamabad-54000-Pakistan*
[2]*Faculty of Veterinary and Animal Sciences, Gomal University Dera Ismail Khan-29050-Pakistan*
[3]*Institute of Biological Sciences (IBS) Gomal University Dera Ismail Khan-29050-Pakistan*
[4]*Botany Department, University Of Science and Technology Bannu-28100-Pakistan*
[5]*Purebred Buffalo Breeding and Dairy Farm Dera Ismail Khan, 29050-Pakistan*
[6]*PARC Agricultural Research Station Skardu-16100-Pakistan*
[7]*National Agriculture Research Center, Islamabad-5400-Pakistan*
[8]*PARC Arid Zone Research Centre, Dera Ismail Khan-29050-Pakistan*

Corresponding author's email: jamilmatrah@parc.gov.pk

ABSTRACT

Animal production is highly affected due to ticks and diseases caused by ticks. Tick control is critical for the economic well-being of the animal industry. This study focused on anti-tick properties of the Neem, *Azadirachta indica* extracts against *Rhipicephalus sanguineus* in vitro conditions. *R. sanguineus* exhibited a high (P < 0.05) dose revolting reaction to *A. indica*. There were no significant differences in reaction magnanimity between both sexes. The essence also prominently ($p < 0.05$) retarded the shedding of 60 % of *R. sanguineus* engorged nymphs. It resulted in that mortality percentage increasing with an increase in the dose of *A. indica*. At 20 % of *A. indica,* 87.76 % of tick mortality was recorded, while 53.23 % at 15% concentration. Probit analysis showed a repellent EC_{50} of *A. indica* for male and female ticks was 4.87, and 4.87 mg/mL, respectively. The findings advocate to *A. indica* is potent enough to use for anti-tick action. Further studies are needed to test Neem extract on other developmental stages *i.e.* eggs, nymphs, and larvae of tick.

Keywords: Acaricides, *Azadirachta indica*, anti-tick activity, *Rhipicephalus sanguineus,* ticks.

INTRODUCTION

The tick is a blood-sucking ectoparasite belonging to the Ixodida order consisting of families (Ixodidae, Argasidae, and Nuttalliellidae (Djebir et al., 2019). Tick is a vector of various viral (Crimean-Congo Haemorrhagic Fever), protozoal (*Babesia, Theleria*), bacterial (*Pasteurella, Brucella, Staphylococcus, Spirochaetes*), and rickettsial (*Anaplasma, Ehrlichia*) pathogens to livestock, human, poultry and reptile resulting health problems of animals which caused significant economic losses (Ramzan et al., 2018; Jamil et al., 2021a, b; Jamil et al., 2021d,e; Rahman et al., 2022; Ullah et al., 2022). The enormous economic losses occurred on adopting the control strategies for ticks (Ramzan et al., 2020, 2021; Selles et al., 2021; Jamil et al., 2022a,b,c). The most dangerous and lethal disease spread in humans is CCHF (Crimean-Congo Haemorrhagic Fever). The first case of CCHF was found in Pakistan in 2014. In Pakistan, CCHF is becoming a serious health issue with a more than 24% fatality rate (Atif et al., 2017). The campaigns started in 2012 to prevent the outbreaks of

CCHF (Altaf et al., 1998; Hussain et al., 2016).

The infestation rate of ticks is highest due to the high contact rate of animals on the occasion of Eid-UL-Adha. Different products of acaricides have been used broadly to manage the tick population to decrease the infestation rate in mammals and humans. The applications of acaricides reduce the tick population and cause environmental pollution (Klafke et al., 2017; Amer and Amer, 2020). Due to excessive use of acaricides not only causes pollution and acaricide resistance, but their residues come in milk, meat, and milk which become the cause of kidney and liver problems (Pignati et al., 2017).

To minimize these problems, an alternative, eco-friendly approach was needed in the country because before this study, no such work was established and applied against ticks neither in the field nor in lab conditions. The current study tested an eco-friendly and readily available product, *Azadirachta indica*, in lab conditions. The results will prove practical tools to control the country's tick population.

MATERIALS AND METHODS

Ticks culture, Plant materials and Preparation of Extracts

The population of *Rhipicephalus sanguineus* was bred on Angora rabbits at the Parasitology Laboratory in PARC Arid Zone Research Centre, Dera Ismail Khan. Specimens were retained in cotton sacks committed to the host's back to being infested (Magano, Els and Chown 2000). Three (3.00) kg flesh leaves of *A. indica* were detached in June 2017 from the host and brought to the laboratory for further processing. Collected leaves were washed with flowing water for 10 minutes, then air-dried for an hour. Dried leaves were sliced, finely powdered, sieved, and stored separately in air-tight containers at 4ºC until further used. 20 g of leaf powder was dissolved in 100 ml distilled water. The mixed powder was stirred for 30 minutes at 6000 rpm for proper mixing, kept overnight at 4°C, and the supernatant was collected and tested on an adult tick (Bian et al. 2011). There were five treatments including control with three replications *i.e.* T_1= 5 % *A. indica;* T_2= 10 % *A. indica;* T_3= 15 % *A. indica;* T_4= 20 % *A. indica* and T_5= Control. Ten engorged female ticks were selected randomly from mass culture, immersed into a treated extract for five minutes, and then deposited into a petri dish. Data were noted on the daily basis to check the repellency.

Bioassay

Repellency bioassay was determined by vertically installing two similar-length glass rods on a polystyrene platform of 5, 6, and 3.5 cm in length, width, and height, respectively. Each glass rod stood 20 centimeters overhead its stand. The two stands with implanted glass poles were mounted distinctly inside of a 35 cm long, 24 cm wide, and 8 cm high plastic container. Water was poured into the container until it surrounded each platform and nearly reached its height. 100 liter of the test solution was placed on a Whatman No. 1 (2 cm x 5 cm). A Whatman No. 1 test filter paper strip of 2 cm x 5 cm was used to sieve 100 litres of *A. indica* distillate (Bian et al. 2011). The same kind and equal size strips were dipped in water and considered a control. The solvent was evaporated by air-drying, and both test and control filter paper strips were used to cover the top 5 cm of the glass rods. To meet the edges of filter papers, two other extra strips were fixed below both (control and test) filter papers on the glass rods. A total of ten male and female *R. sanguineus* adults were unconstrained individually on the test and control. There were five replications in each treatment. After one hour of tick release, repellency was recorded on the position base of ticks. Ticks present on the top filter paper were deemed non-repellant. Those on the bottom neutral filter paper, the naked part of the glass rod, and the platform were thought to be repulsed. Ticks that grew into the water were air dried and replaced. The repellency was determined by using the given formula by Jantan and Zaki (1998).

Statistical Analysis

The most effective dose required to repel half of the tick population (50 %) and the confidence interval (CI) of ticks repelled by the extracts was calculated using probit analysis and each replication was assessed discretely. The 95 % confidence intervals of the EC50 were used to govern the variance in rejoinder among male and female ticks. Before undergoing one-way independent ANOVA (analysis of variances), the data was transformed into the arc sin square root of the number of ticks prevented or subdued from moulting (Hammer et al., 2001). No significant differences in repellent responses between both sexes of tick were at all *A. indica* concentrations, the repellent rejoinders of both sexes were pooled together for ANOVA; but, the mean (SE) of unchanged data is testified. By using a student t-test, a growth inhibition bioassay was determined.

RESULTS AND DISCUSSION

A multifaceted tick control and management approach may be the most effective way to eliminate ticks and tick-borne pathogens. The residual activity and toxicity of neurotoxic acaricides mostly depend on the mode of application and active ingredient. Avoiding tick bites using repellents may also benefit a tick management program. Different acaricides control tick populations in animals in laboratory and field conditions.

Table 1: Classification of Neem, *Azadirachta indica.*

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Rutales
Suborder	Rutinae
Family	Meliaceae
Subfamily	Melioideae
Tribe	Melieae
Genus	*Azadirachta*
Species	*Indica*
Scientific name	*Azadirachta Indica*

Table 2: Dose-dependent repellent response of *R. sanguineus* to *A. indica*

Treatments	Mean ± SE	Repellency percentage
5%	19.88 ± 2.00	29.69
10%	22.43 ± 1.98	42.57
15%	33.95 ± 2.45	53.23
20%	45.67 ± 2.64	87.76
Control	0.98 ± 0.87	0.00

SE= Standard error; df = 4.42; $F_{observed} = 69.98$; $F_{critical} = 4.29$ *(at 0.05).*

Excessive applications of acaricides have resulted in the development of insect resistance to acaricides, harmful to other biological fauna, and harmful effects on the host and associated persons like humans.

Table 3: The effective dose used to repel 50 % of ticks (EC_{50})

Botanical	Sex of tick	EC50 (mg/mL)	Lower-Upper CI (95 %)
Azadirachta indica	Male	4.87	3.56-5.65
	Female	4.87	3.98-5.45
	Both (male and female)	4.87	3.76-5.99

CI= Confidence interval

Neem (A. indica) tree belongs to the family Meliaceae, which is mostly found in tropical and subtropical regions of the world. This 20-23 m tall fast-growing tree is widely spread in Pakistani areas. The extract of this tree has insect repellent potential and antifeedant properties. It is ecofriendly, does not develop resistance, is non-toxic, and not only kills the pests but also disturbs the life cycle of the pests, especially tick stages (Benelli et al., 2017; Kumar and Navaratnam, 2013; Alzohairy, 2016; Rahmani et al., 2018). The classification of A. indica is given in Table 1. An experiment was performed to check the efficacy of neem, A. indica on eggs of R. microplus by Bharkad et al. (2018). They resulted that A. indica reduced the percentage of egg hatchability (71.20 %). It has been reported that neem oil is the greatest powerful herbal Acaricide than others like karanj oil. The current study findings are in line with their findings. It was observed that mortality percentage increased with an increase in the dose of A. indica. At 20 % of A. indica, 87.76 % of adult mortality was recorded, while 53.23 % at 15 % concentration (Table 2). A significant difference in dose-repellent-response of ticks was recorded.

Nchu et al., (2012) performed an experiment to check the efficacy percentage of the essential oil of *Tagetes minuta* on a hard tick, *Hyalomma rufipes,* under controlled conditions. They looked into that adult of *Hy. rufipes* showed a highly substantial ($p < 0.05$) dose revolting rejoinder to *Tagetes minuta* essential oil. In the current study, probit analysis showed a repellent EC_{50} of *A. indica* for male and female ticks to be 4.87, and 4.87 mg/mL, respectively, while probit analysis of *T. minuta* showed 0.07 ml/ml for male and female as investigated by Nchu et al. (2012).

A study was conducted to compare neem extracts and ivermectin on calves in Bangladesh in 2016 (Islam et al., 2016). Based on tick count, the neem extracts were recorded as 68.8 % effective against ticks at 28 days of application. They reported that body weight, packed cell volume (PCV %), hemoglobin concentration (Hb), and total erythrocyte count (TEC) improved meaningly (P < 0.05) while erythrocyte sedimentation rate (ESR) reduced (Rahman et al., 2009). *A. indica* had shown significant anti-tick activity in Africa and Pakistan (Abdel-Shafy and Zayed, 2002; Nawaz et al., 2015; Thakur et al., 2020). Similar findings are observed in the current study. A similar experiment was performed by Kalwar et al. (2014) in Pakistan to govern the relative effects of some botanical extracts and ivermectin on ticks. They reported that ginger was the more toxic extract among tested botanicals, followed by *A. indica*, and garlic extracts. The combined application of botanical extracts with Ivermectin gave 100 % results.

Due to various factors such as high medicine prices, non-availability of medicine/drugs, and hospitals in the vicinity of poor farmers, farmers have no enough money to buy the contemporary drugs for the handling of cattle in Pakistan. So, we should develop an old-style scheme of medication in Pakistan; will be a benefit to farmers and livestock in general. Recently, there has been a surge in global interest in botanical extracts. Many countries have already recognized botanical extracts as a potential therapeutic agent, as well as their accessibility and low cost.

CONCLUSION AND RECOMMENDATION

Tick infestations are common in commercial farms and domestically raised animals. Farms have traditionally cast-off acaricides to indulgence tick infiltrations; nevertheless, new outcomes, inexpensive, and alternative sources of acaricides is a top priority. Botanical pesticides are the main source of tick management. In the current study, only *A. indica* is tested against adults, not on nymphs, larvae, and eggs. The extracts of this tree have high tick repellent potential. So, further studies must be performed to govern the botanical possessions of *A. indica* and many others extracts on different developmental stages such as eggs, nymphs, and larvae.

AUTHORS CONTRIBUTION

All authors have equal contribution in this article.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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