https://doi.org/10.46344/JBINO.2024.v13i06.09

IXODICIDAL *IN VITRO*ACTION OF A NEEM MACERATE ON *RHIPICEPHALUSMICROPLUS* TICKS

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ABSTRACT

The present study evaluates the mortality of *R. microplus* engorged female ticks exposed to a vegetal macerate of *A. indica* (neem). Twenty ticks were selected and assigned into threegroups: 96, 120 and, 144 h maceration. The specimens of each group were immersed for a 15-minute period in its respective macerate. Once the time was completed, the ticksof each group were removed from the solution, dried, and placed in Petri dishesto rest in an incubator at 28 C and 50% relative humidity. The ticks were observed every day in 24 hours periods until the last death was recorded. In all treatments mortality was registered, with a 90%, 95%, and 100% in T1, T2 and T3. It is necessary to carry out more studies with neem macerates of less than 96 hours to evaluate mortality in ticks.

Keywords: Engorged female ticks, mortality rates, neem macerate.

INTRODUCTION

Rhipicephalus microplus,it is a highly invasive ectoparasite of livestock that has spread throughout the world (Etiang et al., 2024). Some estimates indicate that 80% of cattle around the world are infested by these ticks, causing serious economic losses (Calvano et al., 2021). Skin irritation, discomfort, and stress are associated with the tick bites (Rodriguez-Vivas et al., 2018). Furthermore, severe infestations with this tick cause a decrease in productive parameters, as well as depression of immune function (Abbas et al., 2014). On the other hand, during the blood feeding, *R. microplus* could vector pathogens medical importance (Piloto-Sardiñas et al., 2021), which, when the infection manifests in animals, can translate into signs such as anemia, dyspnea, low feed conversion, drop in egg production and in severe cases, death (Nejash, 2016).

The main method around the world for tick controlis the application of chemical acaricides (de Aquino et al., 2024).However, the indiscriminate use of chemicals has severe effects on the environment, as well as the generation of tick resistanceto commercial acaricides (Rodriguez-Vivas et al., 2014, 2018). The above mentioned, motivates the search for alternatives or strategies for tick control (Cerqueira et al., 2022). Several plant products with repellent activity or acaricidal effects against ticks have been studied (Adenubi et al. 2016; Benelli and Pavela, 2018; Peniche-Cardeña et al., 2022). The plant *Azadirachta indica*(Neem) has been investigated and as a control of *R. microplus*ticks (Blando, 2024; Shakya et al., 2024). This study evaluates the mortality ofengorged female ticks *R. microplus*exposed to a vegetal macerate of *A. indica* (Neem).

MATERIALS AND METHODS

Study area, tick collection and management

The ticks were collected in a farm located in Cuajinicuilapa, Guerrero, México. The farm has dual-purpose cattle for milk and meat production. The experiment was carried out in the Multidisciplinary Laboratory of the Faculty of Veterinary Medicine and Zootechnics No. 2 of the Autonomous University of Guerrero. The municipality is located between 16°28'34" N and -98°25'46" W, at an altitude of 46 masl. The temperature has a range of 24 - 26°C, with an annual rainfall of 1,100 - 1,300 mm, with a warm sub-humid climate with rains in summer, with an average humidity of 97% (INEGI, 2010). Once in the laboratory, the ticks were identified according to the taxonomic keys as *R. microplus* females and, maintained in a room under controlled conditions at 30-32 °C, 50 % humidity, and 12-h photoperiod. For experimental purposes, the ticks were selected according to their body size, and mobility. In total, 60 ticks were selected, and 20 individuals were assigned for each treatment group: T1-T3.

Macerate preparation procedure

To prepare the neem macerates, a tree branch was cut, and the leaves were extracted. In brief, 100 grams of leaves, previously washed in distilled water, were placed in three different white plastic containers, with a capacity of 2 liters, which were labeled with the key for each treatment. On each container, 1,900 ml of distilled water was added, and they were kept at rest for different times. For T1, it was left to rest for 144 hours, T2 for 120 hours, and T3 for 96 hours. The ticks used in each treatment were previously disinfected with

1% benzalkonium chloride. They were immersed for one minute, removed from the solution and dried with paper. As required for the study, ticks were immersed in each of the treatment solutions for 15 minutes (Drummond et al., 1973). They were dried with paper and finally placed in duly identified Petri dishes.

Experimental model

The ticks in each group were placed in containers with their respective macerate, where they remained submerged for 10 minutes (Mendes et al., 2020). After the time had elapsed, they were removed from the containers and blotting paper was used to remove the excess solution. Finally, each group of ticks was placed in its respective Petri dish for observation.The specimens were observed over 24-hour periods, according to Domingues et al. (2013), to detect live or dead ticks.

Mortality measurement

The ticks were observed with the aid of a stereomicroscope to detect whether they were alive or dead, and those that did not react to exposure to light or to touching them with entomological tweezers were considered dead (Batiha et al., 2019).

Data analysis

Tick mortality was calculated using the formula described by Krishnaveni and Venkatalakshmi (2014), and the results are presented as descriptive statistics.

RESULTS AND DISCUSSION

This study was performed to evaluate the *in vitro* mortality of *R. microplus* engorged ticks with a neem macerate. During the study, all the macerates showed an ixodicide effect over the ticks. The highest mortality rates were observed in T3 until day 12, while in T1 and T2 was observed on day 13. All the specimens of the T1 and T2 dead on day fourteen. The start of mortality was observed at 96 h post-treatment in T2 and T3, while in the T3 the specimens started to die at 144 h post-treatment. None of the ticks started oviposition process during the time of observation. The number of dead specimens is presented with respect to the hours of treatment in the Figure 1.

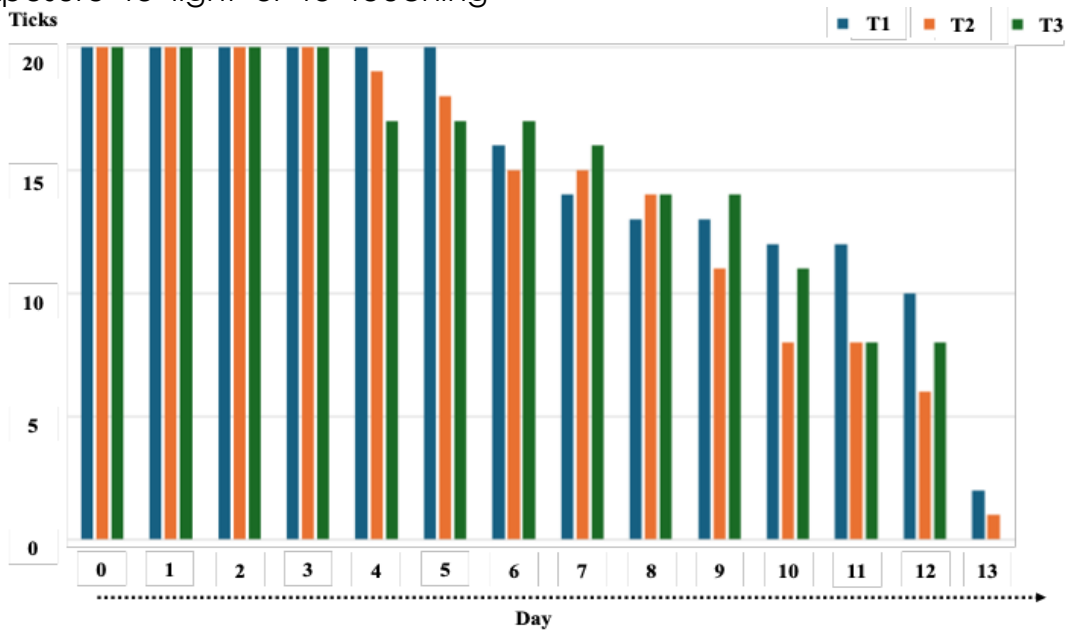

Figure 1. Observed tick mortality during observation of the threated groups.

The use of substances such as plant extracts are promising tools for controlling tick populations (de Oliveira et al., 2016).Tick repellents or acaricides have mechanisms of action that are not fully known (Adenubi et al., 2018). However, plant extracts have been shown to have various toxic effects, interfering with vital processes such as feeding, molting and fertility on ticks (Habeeb, 2010). *Azadirachta indica* extracts influence the reproductive physiology and mortality of female ticks, the action is attributed to compounds known globally as azadirachtin (Abdisa, 2017). In our study, during the observation period, the specimens showed progressive inactivity as the days passed after immersion in the different macerates, it may be attributed to neurotoxic effects (Quadros et al., 2020).In addition, they showed a change in color from brown to dark gray, as reported by Blando (2024), after the application of neem leaf extract.

Several studies reported the use of neem extracts, and researchers reported variable results over *R. microplus* ticks. Punia et al. (2022) evaluated the efficacy of *A. indica* (seed, leaves and bark) in aqueous extracts in adult immersion tests at concentrations of 70%, 50%, 25% and 10%; however, no mortality of ticks was observed at any concentration.This contrasts with our study, where mortality began to be observed from the fourth day post treatment. Diaha-Kouamé et al. (2017) used neem leaves extract at concentrations of 25 mg/ml, 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml; however, for all treatments, only five of 640 ticks died before laying. While in our study, all ticks died before laying. On the contrary,Parte et al. (2014) used aqueous extract of neem leaf powder at a concentration of 5 mg/ml, immersed the specimens and measured the time for acaricidal activity, observing that all ticks died four minutes after immersion. Kumar-Das et al. (2020)compared the in vitro acaricidal properties against *R. microplus* of aqueous, ethanolic, hydroethanolic and methanolic extracts; reporting the highest mortality in aqueous extract during 24 hrs in-vitro screening. These researchers attribute that the greater availability of biomolecules in this extract contributed to the best for the acaricidal property. The various reports mention differences in their results, these can be attributed to the extraction method, type of solvent used, as well as the impact of the time and temperature maintained for the extraction, which directly affect the concentration of azaridictin and consequently its acaricidal effects.

All treatments caused 100% mortality over the ticks subjected to the experiment, even in the lowest concentration solution. Neem oil extracts is a viable alternative for *R. microplus* engorged female ticks *in vitro*. It is necessary to carry out studies on its efficacy in macerates of less than 96 hours of storage, and to prove the efficacy of the product *in vivo*. Tick mortality showed important effects, in this sense, the evaluation of the product is required to determine the potential of its ovicidal and larvicidal capacity.

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