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Neem Leaf Extract: An Acaricide Against Adult Cattle Tick (*Boophilus microplus)*

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Abstract

This study was conducted to evaluate the acaricidal effect of Neem Tree Leaf Extract (Azadirachta indica) using varying levels of concentrations at different lengths of exposure and to compare its efficacy with Organophosphate. The Experiment was laid out in Completely Randomized Design (CRD) with five treatments, replicated three times. The treatments evaluated were as follows: T1 –Amitraz (Control); T2 – 25% Neem Tree Leaf Extract; T3 – 50% Neem Tree Leaf Extract; T4 – 75% Neem Tree Leaf Extract; and T5 – 100% Neem Tree Leaf Extract. Filter papers were dipped with the extracts and matted in petridishes. The ticks were placed in the treated filter papers with corresponding levels of Neem Tree Leaf Extract. There were three observations (60, 90 and 120 minutes) used to determine the effect and the killing duration of the extract. All the levels of the Neem tree leaf extract concentration used could kill ticks of cattle, however, the killing depends upon the level of concentrations and the duration of exposure. At higher concentrations, it was proven that the killing effect of the Neem tree leaf extract to adult ticks were faster than the extract at lower concentration.

Based on the result of the study, the use of Amitraz (control) was statistically similar to that of 100 percent Neem tree leaf extract in killing ticks at a maximum length of one hundred twenty minutes exposure.

Keywords: Aracidal effect, Neem tree (Azadirachta indica), leaf extract, cattle tick (Boophilus)

1. Introduction

Beef cattle are the third most numerous terrestrial farmed animals worldwide [1] and consequently their husbandry involves a wide range of health and animal-welfare challenges. About 80% of the world's cattle are affected by ticks and tick-borne diseases, both of which cause significant production losses [2].

Ticks are ectoparasitic blood-sucking arthropods that have the ability to spread parasitic, bacterial, and viral infections. Rhipicephalus (Boophilus) spp., Ixodes spp., and Hyalomma spp. are all found around the world. infest domestic animals and livestock [3].

As obligate parasites, ticks can transmit numerous pathogens to both humans and animals [4]. The most significant ectoparasite concern for cow productivity in tropical and subtropical locations worldwide is infestations with the cattle tick, Rhipicephalus microplus, which causes significant financial losses. The main components of microplus are conventional acaricides and macrocyclic lactones. However, due to the frequent usage of such substances, tick populations now show resistance to every major chemical class used in acaricides [5].

In the field, tick infection is frequently seen in cattle and water buffalo, resulting in hide damage and pest anxiety. Numerous infections that these ticks spread have a bad effect on the health of large ruminants. The protozoan parasites Babesia and Theileria, as well as the bacteria Anaplasma and hemotropic Mycoplasma (hemoplasma), which were all previously documented in the Philippines, are among the most economically significant tick-borne pathogens [6]. Cattle host resistance to ticks is the most important factor affecting the economics of tick control, but it is largely neglected in tick-

control programs due to technical difficulties and costs associated with identifying individual-animal variation in resistance [2].

The tick Rhipicephalus (Boophilus) microplus is a major carrier of bacterial and protozoan illnesses, resulting in significant direct and indirect economic losses in the animal husbandry industry. The use of synthetic acaricides and repellents has resulted in non-target consequences on human health and the environment as well as the rapid emergence of resistance in targeted vectors, which have made it difficult to effectively reduce tick populations in recent years. The majority of medicinal plants were used to make eco-friendly pesticides for treating and controlling animal parasites like ticks, making them the most abundant source of materials for medicine production. Even if there are efforts undertaken to discover dependable plant-based acaricides to control ectoparasites in animal husbandry, the effective control of R. (B.) Microplus ticks continue to be a significant problem in modern veterinary entomology [7].

The need to provide essential drugs has become more burdens to our small raisers in the remote rural areas. This circumstance necessitates the use of alternate tick management strategies that cause less environmental harm [8]. Tick-toxic plant-based products are the subject of growing scientific investigation. Acaricidal activity of crude extracts from different plants against ticks has been reported [9]. This is mainly due to the fact that numerous scientists have identified plants as potential sources of anti-tick compounds [10]. Their biodegradability is one of the frequently mentioned benefits that may result from the use of botanicals for tick management [11].

The Meliaceae family tree Neem (Azadirachta indica), also known as loamy, lilac of India, and Neem in English (from the Sanskrit Nimba Sarva red nivarini (curator of all diseases), is a medium to large tree that can grow to 30 m tall. It has a short and straight shaft, a wrinkled bark of dark brown to gray, and a dense and rounded crown with leaves. Pinnate, adapted to semi-arid climates, tolerates 0˚C to 49˚C temperatures and is grown in over 50 countries, retaining its features and offering several uses [12]

Due to its extensive biocidal action, notably against arthropods, the neem tree (Azadirachta indica) is one of the most extensively studied plants. The tick-controlling ability of extracts mostly made from its leaves and seeds, which are thought to be a source of biodegradable acaricides, has been extensively researched [13]. Medicinal plants with in vitro acaricidal or tick-repellent properties against juvenile and adult stages of ticks were the subject of a review of published scientific studies. [14] evaluated the efficacy of 66 plants species used to protect animals against ticks. Among the species include Azadirachta indica and found to had good acaricidal and larvicidal effects with 90–100% efficacy, comparable to those of currently used acaricides.

Undoubtedly, it will help preserve beneficial organisms in the environment as well as reduce pesticide hazards to our Filipino farmers. As an effective tool in pest control, the Neem Tree will certainly reduce the farmer's expenses with less use of insecticides and, at the same time, improve farm productivity and increase profits. Hence, this study compares the efficacy of Neem Tree Leaf Extract with that of Amitraz, a synthetic acaricide commonly used in tick control. While some studies may focus solely on natural remedies, this research provides a direct comparison between a traditional synthetic pesticide and a natural alternative, offering valuable insights into the potential of NTLE in practical applications.

2. Material and Methods

2.1 Material

2.1.1 Collection and Preparation of Neem Tree Leaf Extract

The neem leaves, preferably young, were harvested from a single Neem tree that was grown in our backyard. The preparation of the neem tree leaf extract was carried out at the Animal Science Laboratory, College of Agriculture, Quirino State University, Dipintin, Maddela, Quirino. The

preparation of extract followed the methods of [15]. Fresh Neem tree leaves were washed with running water, and subsequently dehydrated in the oven at a temperature of 40 ^{o}C for a duration of 10 days. Dried leaves that have been pulverized using a blender were sifted to produce powder. The powdered leaves were extracted using a 70% ethanol solution. The Neem leaf ethanol extract powder was prepared using the specified concentration levels of 25 percent, 50 percent and 75 percent. The 100 percent pure extract was placed in separate container.

2.1.2 Collection of Adult Ticks

Adult cattle ticks that were used in this study were collected in a private farm in Maddela, Quirino. Four hundred fifty adult cattle ticks of either sex were collected directly from cattle skin and were taken mostly on the neck, udder and medial aspect of the thigh. They were placed in container with good ventilation.

2.1.3 Exposure of Ticks to Neem Tree Leaf Solution and Control

Forty-five petri dishes were used in the study. Time interval for each length of exposure were as follows: 60 minutes, 90 minutes and 120 minutes. Each petri dishes representing each observation was matted with filter paper impregnated with drops of the treatments solution to keep the filter paper moist. Ten tick samples were placed in each petri dishes for observation.

2.2 Method

2.2.1 Experimental Design and Treatments

This study employed an experimental study design: an *in vitro* immersion method as described by [16].

Five treatments were used in the study and each treatment was replicated three times. It was laid out following the Completely Randomized Design (CRD).

The different treatments were:

Treatment 1 – Amitraz (Control)
Treatment 2 – 25% Neem Tree Leaf Extract
Treatment 3 – 50% Neem Tree Leaf Extract
Treatment 4 – 75% Neem Tree Leaf Extract
Treatment 5 – 100% Neem Tree Leaf Extract

2.2.2In Vitro Evaluation and Observation

The ticks in the petri dishes were observed with the maximum length of 120 minutes. Observation was done for a period of 60 minutes, 90 minutes and 120 minutes. Ticks were considered as alive if they responded normally to pressure or physical stimulation from wooden dowels. Dead ticks were those that lacked the capacity to move, maintain upright posture, coordinate their legs, correct themselves, or exhibit any signs of life [17].

The number of live and dead ticks was counted after each observation. The viability of ticks was checked regularly by stimulation with a needle, and ticks were recorded as dead if no reaction was shown. The percentage mortality was calculated by using a formula given by [18] as follows:

$$\text{Mortality \%} = \frac{\text{Number of dead ticks}}{\text{Total number of ticks}} \times 100$$

2.2.3 Data Collection and Analysis

Percentage mortality of adult cattle ticks and killing duration per treatment were statistically analyzed using the Completely Randomized Design (CRD). And the Least Significant Difference was used for the comparison of treatment means.

3. Results and Discussion

3.1. Results

3.1.1 Effect of neem leaf extract against cattle tick at sixty minutes of observation.

During the first-time schedule of observation, which was sixty (60) minutes, significant differences ($p<0.05$) on mortality rate were recorded. The highest mean tick mortality was 63.33 percent which was yielded by the use of Amitraz (T1). However, this was comparable with the effect of 100 percent Neem Tree Leaf Extract (T5) and 75 percent Neem Tree Leaf Extract (T4) with a mean tick mortality of 56.66 percent and 43.33 percent respectively. In addition, the 25 percent and 10 percent Neem Tree Leaf Extract was significantly different among all the treatments obtaining the lowest mean tick mortality of 16.66% and 10%, respectively (Table 1).

Table 1.
Mortality rate of ticks sixty minutes after imposition of treatment (%).
The number in parenthesis is a transformed data using √x.

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	III		
1 – Amitraz (Control)	60 (7.74)	60 (7.74)	70(8.36)	190(13.78)	63.33(7.95)a
2 – 25% NTLE	10 (3.16)	10 (3.16)	10 (3.16)	30 (5.47)	10(3.16)b
3 - 50% NTLE	20 (4.47)	10 (3.16)	20 (4.47)	50 (7.07)	16.66(4.08)b
4 – 75% NTLE	40 (6.32)	50 (7.07)	40 (6.32)	130 (11.40)	43.33(6.58)a
5 – 100% NTLE	60 (7.74)	50 (7.07)	60 (7.74)	170 (13.03)	56.66(7.52)a
GRAND TOTAL				570.00 (23.87)	
GRAND MEAN					37.99(6.16)

Note: Means with common letters are not significantly different with each other using LSD.

3.1.2 Effect of neem leaf extract against cattle tick at ninety minutes of observation.

After ninety (90) minutes of exposure (Table 2), the comparison of results from all the treatments showed that the control (T1) and the 100 percent Neem Tree Leaf Extract (T5) obtained the same mortality rate of 93.33 percent. This was followed by the 75 percent Neem Tree Leaf Extract (T4) and 50 percent Neem Tree Leaf Extract (T3) which obtained a mortality rate of 56.66 percent and 43.33 percent, respectively. The 25 percent Neem Tree Leaf Extract (T2) remained to have the least mortality rate of 13.33 percent.

Table 2.
Mortality rate of ticks ninety minutes after imposition of treatment (%).
The number in parenthesis is a transformed data using √x.

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	III		
1 - Amitraz (Control)	90(9.48)	90(9.48)	100(10.00)	280(16.73)	93.33(9.66)a
2 – 25% NTLE	10(3.16)	20 (4.47)	10(3.16)	40(6.32)	13.33(3.65)c
3 - 50% NTLE	40(6.32)	40(6.32)	50(7.07)	130(11.40)	43.33(6.58)b
4 – 75% NTLE	50(7.07)	60 (7.74)	60 (7.74)	170(13.03)	56.66(7.52)b
5 – 100% NTLE	90(9.48)	100(10)	90(9.48)	280(16.73)	93.33(9.66)a
GRAND TOTAL				900(30.00)	
GRAND MEAN					60.03(7.74)

Note: Means with common letters are not significantly different with each other using LSD.

3.1.3 Effect of neem leaf extract against cattle tick at one hundred twenty minutes of observation.

A significant increase ($p<0.05$) in the mortality rate of ticks at one hundred twenty minutes (120) exposure was observed. The results showed that control (T1) still gained the highest mean tick mortality of 100 percent which is statistically comparable with the use of 100 percent Neem Tree Leaf Extract (T5) with a mean of 93.33 percent.

On the other hand, the 75 percent Neem Tree Leaf Extract (T4) and 50 percent Neem Tree Leaf Extract (T3) registered mortality rate of 76.66 percent and 43.33 percent, respectively. While the 25 percent Neem Tree Leaf Extract (T2) consistently gained the least percentage mortality of 20 percent (Table 3).

Table 3.
Mortality rate of ticks one hundred minutes after imposition of treatment (%).
The number in parenthesis is a transformed data using √x.

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	III		
1 - Amitraz (Control)	100(10.00)	100(10.00)	100(10.00)	300(17.32)	100(10.00)a
2 – 25% NTLE	20 (4.47)	20 (4.47)	20 (4.47)	60 (7.74)	20(4.47)d
3 - 50% NTLE	40(6.32)	40(6.32)	50(7.07)	130(11.40)	43.33(6.58)c
4 – 75% NTLE	80(8.94)	80(8.94)	70(8.36)	230(15.16)	76.66(8.75)b
5 – 100% NTLE	90(9.48)	90(9.48)	100(10)	280(16.73)	93.33(9.66)a
GRAND TOTAL				1000(31.62)	
GRAND MEAN					66.66(8.16)

Note: Means with common letters are not significantly different with each other using LSD.

3. 2. Discussion

It was noticed that during the period of observation the ticks became inactive upon subjecting them in the different levels of concentration of Neem Tree Leaf Extract. Prior to imposition of the extract, these ticks were normal in size, active and brownish in color. However, ticks in all treatments regardless of concentration and length of exposure became dark brown in color and the body size shrunk after imposition of Neem tree leaf extract.

The results of the present study show that the different levels of concentration of Neem tree leaf extract were all effective acaricide against adult cattle tick. This effect however, varied with the different levels of concentrations as well as the length of time that the ticks were exposed into the extract. It was observed that as the concentration and time of exposure increase, mortality rate also increases. This finding of the present study is in consonance with the study conducted by [19], when they evaluated the acaricidal effect of neem leaves and *Citrullus colocynthis* extracts on engorged adult females, eggs hatchability and larvae of *Hyalomma dromedarii* ticks. Similar result was also observed by [20], stating that with the increase in concentration level of Azadirachta indica and deltamethrin, the percent mortality rate also increased.

The chemical that most plant entomologists believe to be the Neem tree's most potent element, azadirachtin, was not thought to be the reason ticks died. According to [21], neem possesses specific compounds that cause it to have diverse effects, such as the ecdysone, which modifies the behavior of insects in their essential biological processes. Additional component is salanine, which is known to possess repellent properties or activity. Furthermore, neem has been found to possess acaricidal, nematicidal, and fungicidal properties when applied to oily preparations. Moreover, azadirachtin, a complex tetranortriterpenoid limonoid present in seeds, is the key constituent responsible for both antifeedant and toxic effects in insects [22].

An extensive literature research, covering neem (Azadirachta indica) and 230 other plants, has revealed their bioactive properties, such as toxicity, repellency, anti-feeding effects, anti-oviposition effects, and the capacity to immobilize several types of ticks. Therefore, they show potential in the development of sustainable, efficient, and powerful substances that are well-suited for rural farmers to fight against ectoparasites [23]. Various Neem-based products have been repeatedly demonstrated by multiple scientists and have been deemed environmentally safe, characterized by minimal non-target effects, diverse modes of action, affordability, and ease of manufacture in regions with inadequate industrial infrastructure [24]. Previous reports have shown that herbal acaricides cause minimal environmental pollution and have low toxicity to non-target organisms, including humans. Additionally, these acaricides rapidly biodegrade and help prevent the development of resistance [25].

4. Conclusion

The present study concluded that Neem tree leaf extract provided evidence of acaricidal effect against adult cattle tick. The use of 100 percent concentration of Neem tree leaf extract at one hundred twenty (120) minutes exposure is considered promising which is comparable with that of the use of commercially available acaricide. Based on the findings of the study, the used of synthetic pesticides (T1) gained the highest mean tick mortality of 63.33%, 93.33% and 100% at 60, 90 and 120 minutes of exposure respectively. This result is statistically comparable with the use of 100 percent Neem Tree Leaf Extract (T5) with a mean of 56.66, 93.33 and 33.33 percent at 60, 90 and 120 minutes of exposure respectively. The indiscriminate use of synthetic pesticides, which may be harmful to both people and the environment, could be greatly reduced by using neem tree leaf extract, a low-cost natural source of acaricide. Thus, NTLE had a great potential as acaricide, and its effectiveness increased with increasing concentration and exposure time.

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