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Comparative evaluation of acaricidal effect of *Azadirachta* indica crude extract and commercially used synthetic acaricides against *Boophilus Annulatus* ticks (Acari: Ixodidae)

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ABSTRACT: Tick infestation remains one of the major health problems that affect the productivity and comfort of cattle. The control of ticks mainly relies on using chemical acaracides. Limited information is available on the potential benefits and activity of various neem extracts on *Boophilus annulatus* ticks. The present study investigated the acaricidal activity of neem leaf extract at different concentrations against adult *Boophilus annulatus* ticks in comparison to Deltamethrin and Lambda-cyhalothrin. Adult *Boophilus annulatus* ticks were challenged with serialized solutions of different concentrations of both plant extract and synthetic chemical using immersion method in a Completely Randomised Design (CRD) and monitored for a period of 24, 48, 72, and 96 hours post treatment. Data were analysed using Log-probit regression and one-way Analysis of Variance (ANOVA). Results revealed dose and Time-dependent mortality and significant variations ($P<0.05$) among treatment concentrations. Extract of *Azadirachta indica* was more potent with the highest bioactive range (100%: 6:33±1.64) over the synthetic acaricides (Deltamethrin; 86.70%: 6.00±1.21, Lambda-cyhalothrin; 76.6%: 5.00±1.12). LD_{50} values for *Azadirachta indica*, Deltamethrin and Lambda-cyhalothrin are 53.56µl/ml, 22.47µl/ml and 60.0µl/ml, respectively, whereas the LT_{50} values for *Azadirachta indica*, Deltamethrin and Lambda-cyhalothrin are 63.66hrs, 76.13hrs and 66.69hrs respectively. In comparison, the acaricidal potential of the three treatments can be arranged in order of *Azadirachta indica* (100%)>Deltamethrin (86.70%)>Lambda-cyhalothrin (76.6%). All three treatments showed significant difference ($p<0.05$) and a positive correlation of mortality with treatment concentrations and time of exposure. Qualitative analysis revealed the presence of flavonoids (+), saponins(+), Steroids(+), terpenoids(+) as slightly present, alkaloids(++), phenols(++), glycosides(++) as moderately present while tannins(+++) as highly present. The quantitative analysis revealed the highest percentage of Phytochemical to be phenol (25.5%), saponins (16.8%), alkaloids (11.3%), tannins(10.8%), followed by flavonoids (5.5%), glycosides (4.8%), terpenoids (3.5%), steroids (2.7%) arranged in descending order of percentage presence. The study revealed a comparable bioefficacy and biopesticidal potential of acetone extract of *Azadirachta indica* over the synthetic acaricides and can be utilised as an effective tool for the control of *Boophilus annulatus* ticks in cattle within the study area and globally.

Keywords: Acaricidal properties, *Azadirachta indica, Boophilus annulatus*, cattle tick, deltamethrin, lambda-cyhalothrin.

Abbreviations: CRD = Completely Randomised Design, **ANOVA** = Analysis of Variance, **µl/ml** = Microlitre/millilitre, **LD_{50}** = 50% Lethal dosage, **LT_{50}** = 50% Lethal time, **LSD** = Least significant difference.

INTRODUCTION

Cattle are considered of great importance in the farming industry of different countries around the world as they are a major source of milk, meat and hides (Burger *et. al.,* 2019). However, their resulting product output or measure of productivity is affected by infestation of a wide range of external and internal parasites, resulting in considerable economic losses (Jaime Betancur Hurtado *et al.,* 2019; Rattan, 2020). Cattle are the primary host of the adult stage of *Boophilus annulatus,* however, it can also infest other domestic animals like sheep, goats and equids. The larvae and nymphs feed on birds and small burrowing animals, but nymphs can also infest large animals such as adults and complete its life cycle on two or three hosts (Abdel-Shafy, 2018).

Ticks are destructive, obligatory blood sucking ectoparasites with worldwide distribution and of great economic importance in both the tropics and subtropics (El Tigani *et. al.,* 2010; El-Gohary *et. al.,* 2016). They are known to be involved in the transmission of various bacterial, protozoal, rickettsial, viral and parasitic diseases of both medical and veterinary importance as potential vectors in animals and man (Brites-Neto *et. al.,* 2015). They are therefore considered great contributors to the issue of emerging and re-emerging diseases (Brites-Neto *et. al.,* 2015). Given its economic importance, tick infestation in cattle can result in symptoms such as loss of weight, reduction in milk production, tick worry, blood loss, tick toxicosis, tick paralysis, loss of appetite, skin deterioration, intense pruritus and mild to severe anaemia.

The control of ticks is mainly based on the direct application or injection of acaricides to animals. Several synthetic acaricides have been extensively used and recommended for the control of ticks, including organophosphates, carbamates, pyrethroids, and amidines (De meneghi *et. al.,* 2016). However, several major drawbacks were reported for the majority of acaricides, including alarming reports of tick resistance, residues in foods, and environmental pollution (Ghosh *et. al.,* 2013; De meneghi *et. al.,* 2016). It is therefore not surprising to mention that there is an urgent need to develop eco-friendly, effective alternatives for these chemicals. Interestingly, the use of herbal medications becomes a promising alternative approach for the treatment of various infectious agents because of their biodegradability, target efficiency, and cost-effectiveness (Drummond, 1983; Elmahallawy *et. al.,* 2014; Abdel-Ghany *et. al.,* 2021) and therefore, they gained a considerable interest in tropical and subtropical regions (Alkazzaz *et al.,* 2018; Elmahallawy *et al.,* 2020; Nugraha *et al.,* 2020; Elmahallawy *et. al.,* 2021; Ramez *et. al.,* 2021; Tahmasbi *et. al.,* 2021; Zheoat *et. al.,* 2021; Elmahallawy *et. al.,* 2022). Among others, plant-derived materials and their bioactive substances were proposed as substitutes for synthetic acaricides due to their activity against ticks (Abdel-Ghany *et. al.,* 2021). As compared to synthetic ones, several previous reports revealed that herbal acaricides caused little environmental pollution and a low toxicity level to non-target organisms, including humans (Torres *et. al.,* 1999; Kilani-Morakchi *et. al.,* 2021), apart from the rapid biodegradation of their residues and their role in the prevention of resistance development (Rattan, 2010; Singh *et. al.,* 2014).

The neem tree, *Azadirachta indica* A. Juss, is an evergreen tree that originates from India and other neighbouring countries (Raizada *et. al.,* 2001). It is considered one of the most reliable botanical sources of biopesticides with a wide range of biological activities (Rajput *et. al.,* 2006). Most active and functional ingredients of *Azadirachta indica,* including its oil, bark, and leaves, have been shown to exhibit promising therapeutic effects such as antifeedant, larvicidal, ovicidal, repellent action, growth deregulation, reduction in ecdysone levels, alterations in development and reproduction, sterility and damage in insects' moulting processes (Senguttuvan *et. al.,* 2014; Gareh *et. al.,* 2022; Baby *et. al.,* 2022). It has been reported in available literature that this plant and its extracts show potent activity against all stages (adult, nymph, and larva) of ticks (Gareh *et. al.,* 2022). There is limited information on the acaricidal potential of the acetone extract of *A. Indica* against *B. Annulatus* in comparison to that of commonly used synthetic acaricides. Therefore, this study seeks to provide information on the suitability of this plant extract for the control of these tick species and compare its activity to that of commonly used acaricides in Nigeria in order to ascertain their resistance status, which might provide new insights into the control of hard ticks in cattle.

MATERIALS AND METHODS

Study design

This investigation employed an experimental study design in a laboratory-based *in vitro* acaricidal activity of leaf extract of *Azadirachta indica* and two acaricides (Deltamethrin and Lambda-cyhalothrin) in different concentrations using the immersion method (IM) as described by Drummond *et al*. (1973). Cattle brought to the Amansea Cattle Market, Anambra State, Nigeria, were randomly selected for the study. Amansea Cattle Market is located in Awka North Local Government area of Anambra State, Nigeria, latitude of 6°21'40"N and a longitude of 6°51'38"E.

Collection of ticks

Engorged adult hard ticks were collected from naturally infested cattle (5- to 15-year-olds) from Amansea Cattle

Market, Anambra State, Nigeria. In order to minimise damage to the mouthparts and cuticle, the ticks were manipulated by rotating for easy removal with a pair of soft forceps. The collected ticks were then placed in a clean plastic container with perforated lids to allow ventilation, then immediately transported to the laboratory at the Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, for identification and authentication (NAU/DPE/16/056). Identification was carried out using standardised international keys and bioassays, as described elsewhere (Hoogstraal, 1956; Walker, 2003; Essa *et. al.,* 2022).

Plant preparation and extraction

Leaves of *Azadirachta indica* were collected from the Nnamdi Azikiwe University surroundings in Awka, then transported to the Herbarium, Department of Botany, Nnamdi Azikiwe University, Awka for identification and authentication (NAU/DBH/16/009). Plant leaves were washed with tap water, cut into small pieces, spread out on paper sheets, dried at room temperature for one week and were pulverised using a mortar and pestle. 500 g of the pulverised plant material was soaked in 5litres of distilled water for 24 hours at room temperature according to (Nejash, 2016). The mixture was filtered using a sieve, concentrated over the water bath at 50ºC and stored at 4°C in a refrigerator until when needed. Serial dilution of the leaf extract was carried out in acetone. The extract was regarded as 100% concentrate and subsequently diluted serially to 50, 25%, 12.5%, 6.25% and 3.125% yielding 500, 250, 62.5 and 31.25 µl/ml, respectively.

Preparation of acaricides

Deltamethrin (Suspend®) and Lambda-cyhalothrin (Demand®) were obtained commercially from a supermarket. Stock solution of desired concentration was prepared by diluting the acaricides independently in acetone according to Mohammed, (2011). 1ml of the stock solution of each acaricide was taken and serially diluted in acetone to obtain 50, 25, 12.5, 6.25, and 3.125%, respectively.

Evaluation of acaricidal effect of extract of neem and synthetic acaricides

The acaricidal activity of *Azadirachta indica* leaf extract and the two synthetic acaricides against *Boophilus annulatus* ticks was determined using *invitro* immersion method. The design was completely randomised with three replicates; each one contained 10 adult ticks. The ten ticks were dipped in 5ml of the respective dilutions of each treatment for 5 minutes. After immersion, the ticks were then subjected to *invitro* residual effect or contact application. Whatman filter paper (No. 5, 9cm in diameter) was placed in each of the petri dishes used for the experiment and aliquots of 1ml of each concentration was evenly dispensed onto the filter paper and then left for 30 minutes to ensure proper spreading of the solution. The acetone solvent was allowed to evaporate completely. Controls with only acetone were included. Mortality counts were taken after 24, 48, 72 and 96 hours. Mortality was considered if the ticks do not respond to probing after pricking with fine forceps and also if, after 24 hours, no form of movement was observed.

Phytochemical analysis of the plant

Qualitative Phytochemical analysis was conducted according to standard procedures described by Senguttuvan *et al*., (2014), to test for the following secondary metabolites: alkaloids (Mayer's test), tannins (Tannins test), saponins (Froth test), flavonoids (Alkaline Reagent test), glycosides, steroids (Salkowski test), Phenol and terpenoids (Chloroform and sulfuric acid test). The qualitative grading system of the phytochemicals was described in the following manner: (+++) as heavily present, (++) as moderately present, (+) as traces or slightly present, (-) as absent. Quantitative phytochemical determination was carried out using the gravimetric method and Spectrophotometer (Model: Spectrumlab 752s UVS V9S) according to standard procedures as described by Aguoru *et al.* (2015) and Khanal (2021).

Statistical analysis

Data obtained were analysed using log-probit regression analysis (Finney, 1971) to determine lethal dosage (LD_{50}) and lethal time (LT_{50}). Subsequently, data were further analysed using a factorial analysis of variance model in the Genstat package 9.2 (9th edition). Treatment with significant differences were compared and separated at 5% level of significance using Newman Keuls (SNK) with the least significant values tested at $p<0.05$.

RESULTS

The study compared the acaricidal effect of the acetone crude leaf extract of the Neem plant and two synthetic acaricides, Deltamethrin and Lambda-cyhalothrin, at different concentrations using the immersion method after exposure for 96hours. Mortality rate of *Azadirachta indica* extract and selected acaricides revealed dose and Time-dependent mortality responses with significant variations across treatments. The control with acetone recorded no mortality after 96hours of exposure.

Data presented in Tables 1, 2, and 3 are the mortality

Table 1. Mean and percentage mortality of *Boophilus annulatus* to exposure to Acetone crude leaf extract of *Azadirachta indica*.

Concn. (µl/ml)	Log. Concn.	Time (hr)				Mean mortality	% Mortality	Probit value
		24	48	72	96			
500	2.699	2.33	5.33	7.66	10.0	6.33±1.64	100.0	8.8979
250	2.298	2.00	4.33	6.33	8.66	5.33±1.42	86.60	6.0803
125	2.097	1.67	3.67	6.00	7.33	4.67±1.25	73.30	5.6128
62.5	1.796	1.00	2.33	4.32	6.00	3.42±1.10	60.0	5.2533
31.5	1.496	0.33	1.00	2.00	4.00	1.83±0.20	40.00	4.7467
0.00	0.00	0.00	0.00	0.00	0.00	0.00±0.00	0.00	
Mean Mortality		1.47±0.36	3.33±0.76	5.2±0.97	7.20±1.04			

**LSD for Conc. = 3.45; *P-value Conc. = 0.012.*
**LSD for Time = 2.480; *P-value Time = 0.001.*

Table 2. Mean and Percentage Mortality of Deltamethrin on adult ticks of *Boophilus annulatus* after 96 hours of exposure.

Concn. (µl/ml)	Log. Concn.	Time (hrs)				Mean mortality	% mortality	Probit values
		24	48	72	96			
500.00	2.699	3.00	5.33	7.00	8.67	6.00±1.21	86.70	6.1125
250.00	2.298	2.33	4.00	5.33	6.33	4.50±0.87	63.30	5.3398
125.00	2.097	1.33	3.00	4.33	5.33	3.50±0.87	53.30	5.0828
62.50	1.796	1.00	2.33	3.66	4.99	3.00±0.86	49.90	4.9975
31.25	1.496	0.67	1.67	3.00	4.67	2.50±0.87	46.70	4.9172
0.00		0.00	0.00	0.00	0.00	0.00±0.00		0.00
Mean Mortality		1.67±0.43	3.27±0.64	4.66±0.70	6.00±0.72.			

**LSD for Conc. = 2.559; *P-value = Conc. = 0.003.*
**LSD for time = 1.906; *P-value = Time = 0.001.*

Table 3. Mean and percentage mortality of Lambda-cyhalothrin on adult ticks of *Boophilus annulatus* after 96 hours post treatment.

Concn.	Log. of Concn.	Time (hrs)				Mean Mortality	% Mortality	Probit values
		24	48	72	96			
500.00	2.699	2.33	4.33	3.66	7.66	5.00±1.12	76.60	5.7257
250.00	2.298	2.00	4.32	5.66	6.99	4.75±1.06	69.90	5.5215
125.00	2.097	2.00	3.67	5.34	6.34	4.34±0.95	63.40	5.3425
62.50	1.796	1.33	3.33	5.00	6.00	3.92±0.97	60.00	5.2533
31.25	1.496	1.00	2.33	4.33	5.33	3.25±0.97	53.30	5.0828
0.00		0.00	0.00	0.00	0.00	0.00±0.00	0.00	
Mean mortality		1.73±0.24	3.60±0.37	5.20±0.25	6.50±0.40			

**LSD for Conc. = 2.791; *P-value Conc. = 0.015.*
**LSD for Time = 0.973; *P-value Time = 0.001.*

responses of adult engorged cattle ticks, *Boophilus annulatus,* to acetone crude extract of *Azadirachta indica,* Deltamethrin and Lambda-cyhalothrin, respectively, after 96 hours of exposure. The results indicated dose and time-dependent mortality responses to treatment concentrations; mortality increased with treatment concentrations and time of exposure. The highest mortality of 100, 86.7, and 76.6% was obtained at 500 µl/ml for *Azadirachta indica,* Deltamethrin and Lambda-cyhalothrin, whereas the least mortality of 40, 46.7, and 53.3% was recorded for *Azadirachta indica,* Deltamethrin and Lambda-cyhalothrin at 31.25µl/ml, respectively. Extract of *Azadirachta indica* was more potent, had the highest bioactivity range (1.83±0.20-6.33±1.64) compared to Deltamethrin (2.50±0.87-6.00±1.21) and Lambda-cyhalothrin (3.25±0.97-5.00±1.12). No mortality was recorded in the control group after 96 hours of exposure. There is a significant difference in mortalities across treatments ($p<0.05$).

Mortality rate of the acetone extract of *Azadirachta indica* leaves on adult *Boophilus annulatus* ticks increased with

Table 4. Time exposures and percentage mortality of adult cattle ticks, *Boophilus annulatus* to acetone crude extracts of *Azdirachta indica* after 96 hours of exposure.

Time (hrs)	Log. of time	% Mortality	Probit values
24	1.380	14.7	3.9506
48	1.681	33.3	4.5684
72	1.851	52.6	5.0652
96	1.982	72.0	5.0828

**P-value = 0.001.*

Table 5. Time exposures and percentage mortality of adult cattle ticks, *Boophilus annulatus* to exposure to Deltamethrin after 96 hours post treatment.

Time (hrs)	Log. of Conc.	% Mortality	Probit values
24	1.380	16.7	4.0839
48	1.681	32.7	4.5518
72	1.851	46.6	4.9147
96	1.982	60.0	5.2533

**P-value = 0.001.*

Table 6. Time exposures and percentage mortality of adult cattle ticks, *Boophilus annulatus* to exposure to Lambda-cyhalothrin after 96 hours post-treatment.

Time (hrs)	Log. of time	% Mortality	Probit values
24	1.380	17.3	4.0576
48	1.681	36.0	4.6415
72	1.851	52.0	5.0502
96	1.982	65.0	5.3855

**P-value = 0.001.*

an increase in time of exposure, as seen in Tables 4, 5, and 6. Mortality percentage ranges of 14.7-72.0, 16.7-60, and 17.3-65% were obtained for *A. indica,* Deltamethrin, and Lambda-cyhalothrin, respectively. *Boophilus annulatus* ticks showed more susceptibility to the extract of *Azadirachta indica* (1.47±0.36-7.20±1.04) as observed from the mean±SD values obtained compared to Deltamethrin (1.67±0.43-6.00±0.72), Lambda-cyhalothrin (1.73±0.24-6.50±0.40) post-treatment (See Tables 1 to 3). Results showed a highly significant difference ($p<0.05$) in mortality responses across treatment concentrations recorded at different time exposures (Tables 4 to 6).

Qualitative analysis revealed the presence of all tested phytochemicals with flavonoids, saponins, Steroids, terpenoids as slightly present, alkaloids, phenols, glycosides as moderately present, while tannins are highly present (Table 7). A creamy yellow precipitate indicates the presence of alkaloids, a persistent foam of mass of small bubbles which escapes with surface of liquid indicates the presence of saponins, a brick red precipitate indicates the presence of glycosides, a greenish coloration indicates tannins, a yellow color which disappears on standing indicates flavonoids, a red/dark colour indicates steroids, yellow to bright orange colour indicates phenols, a reddish brown colouration at interface indicates terpenoids. The quantitative analysis revealed the highest percentage of phytochemical to be phenol (25.5%), saponins (16.8%), alkaloids (11.3%), tannins (10.8%), followed by flavonoids (5.5%), glycosides (4.8%), terpenoids (3.5%), steroids (2.7%) arranged in descending order of percentage presence.

Illustrated in Figures 1 and 2 are the combined effects of the respective regression line log-probit analysis of *Azadirachta indica* extract, Deltamethrin and Lambda-cyhalothrin. Lethal dose values (LD_{50}) of *Azadirachta indica* extract, Deltamethrin and Lambda-cyhalothrin are 53.56µl/ml, 60.0 µl/ml and 22.47µl/ml, respectively (Figure 1). The least dosage was recorded for Lambda-cyhalothrin against the *Boophilus annulatus* ticks, followed by *Azadirachta indica* extract and Deltamethrin. Lethal time values (LT_{50}) of *Azadirachta indica* extract, Deltamethrin and Lambda-cyhalothrin are 63.66, 76.13 and 66.69 hours, respectively. *Azadirachta indica* extract recorded the least lethal time value against the *Boophilus annulatus*

Table 7. Qualitative and quantitative analysis of extract of *Azadirachta indica.*

Phytochemical constituents	Qualitative analysis	Quantitative analysis (%)
Flavonoids	+	5.5
Saponins	+++	16.8
Alkaloids	++	11.3
Steroids	+	2.7
Phenol	+++	25.5
Glycosides	+	4.8
Terpenoid	+	3.5
Tanins	++	10.8

Note: (+++) = highly present, (++) = moderately present, (+) = slightly present, (-) = absent.

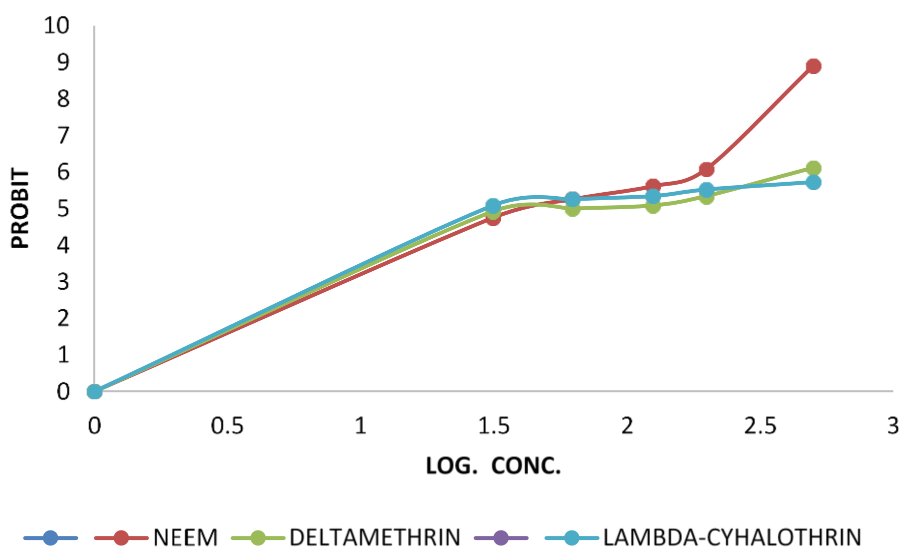

Figure 1. LD_{50} values and Combined Logistic regression line graph of *Azadirachta indica*, Deltamethrin and Lambda-cyhalothrin.
Neem…………………….. y=3.0355x + 0.2479, R^2=0.7856, LD_{50}=53.56µl/ml
Lambda-cyhalothrin……. y=0.5166x + 4.3017, R^2=0.985, LD_{50}=22.42µl/ml
Deltamethrin…………….. y=0.9086x + 3.3844, R^2=0.7895, LD_{50}=60.0µl/ml

ticks, followed by Lambda-cyhalothrin and Deltamethrin, which indicated a quicker action against the test organism compared to the acaricides (Figure 2). Mortality rates of *Boophilus annulatus* ticks were found to be positively associated with time of exposure of treatments (Figure 2: R^2 = 0.9762, 0.9949 and 0.9907) and treatment concentrations (Figure 1: R^2 = 0.7856, 0.985 and 0.7895) for *Azadirachta indica* extract, lambda-cyhalothrin and deltamethrin, respectively.

DISCUSSION

Control of ticks over time has faced a lot of challenging issues due to tick resistance, effects on non-target organisms, the environment and human health in general. Plant products, on the other hand, are rich source of bioactive organic agents that offer lots of advantages over synthetic insecticides as they are generally less toxic, easily biodegradable and less prone to the development of resistance. This study was therefore performed to compare the *in vitro* acaricidal effect of the acetone crude extract of *Azadirachta indica* and two synthetic acaricides, namely Deltamethrin and Lambda-cyhalothrin, against adult cattle ticks, *Boophilus annulatus*. Results revealed time and concentration-related mortality responses with significant variations ($p<0.05$) among treatments. Mortality rates of all toxicants increased with an increase in treatment concentrations and time of exposure of *Boophilus annulatus* ticks to the three treatments. This is in consonance with previous studies; for instance, Abdel-Ghany *et al*. (2021) reported increased mortality of *Hyalomma anatolicum execatum* ticks with increased extract concentrations, with no significant effect on the

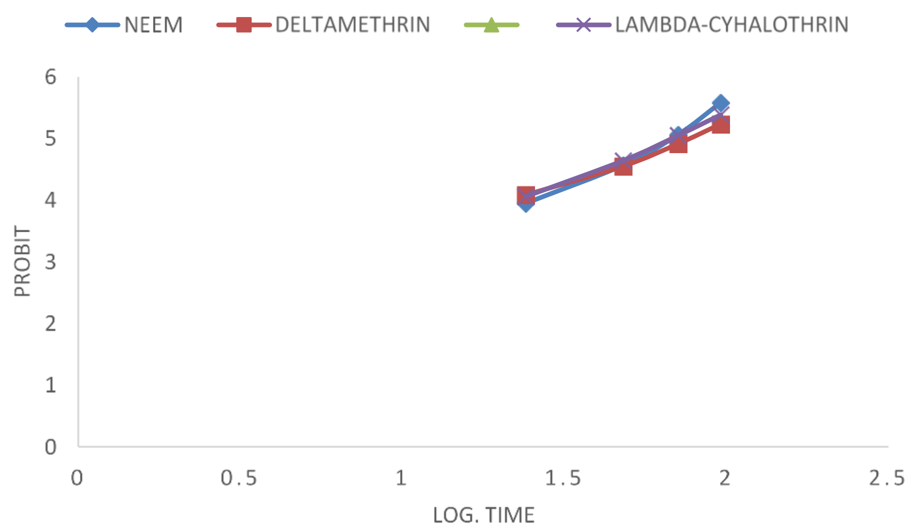

Figure 2. LT_{50} values and combined Logistic regression line graph of *Azadirachta indica*, Deltamethrin and Lambda-cyhalothrin.
Neem…………………….. y=2.638x + 0.2402, R^2=0.9762, LT_{50}=63.66 hours
Lambda-cyhalothrin……. y=2.1843x + 1.0157, R^2=0.9949, LT_{50}=66.69 hours
Deltamethrin…………….. y=1.9902x + 1.2553, R^2=0.9907, LT_{50}=76.13 hours

mortality rates of fed nymphs of the ticks. Avinash *et al*. (2017) also reported on increased mortalities of *Rhipicephalus microplus* ticks with increased concentrations of Deltamethrin, hexane, chloroform and aqueous leaf extracts of *Azadirachta indica* with significant variations among treatments. The increased mortality observed at higher concentrations could be attributed to the presence of a high amount of certain bioactive agents in the plant, higher concentration of acaricides and also prolonged exposure to such active ingredients could be responsible for its seemingly quick activity against the test organism.

The immersion of *Boophilus annulatus* ticks in different concentrations of *Azadirachta indica* extract resulted in 100% mortality of adult ticks at 96 hours post-treatment, which, in comparison to Deltamethrin and lambda-cyhalothrin, induced mortality of 86.70% and 76.60% respectively, at 96 hours post-treatment. This indicates the efficacy of the plant extract over the acaricides. The two acaricides recorded 87.70% and 76.60% mortality at the maximum concentration of 500µl/ml, unlike the plant extract. Deltamethrin and lambda-cyhalothrin are pyrethroids, which are most commonly used as acaricides for the control of tick infestation in Nigeria and Awka, Anambra state. Due to their wide usage, *Boophilus annulatus* and other tick species have developed a certain level of resistance to the synthetic acaricides. In the present study, 100% mortality was never obtained even at the maximum concentration of 500µl/ml with the two acaricides at 96 hours post-treatment. The inability of the acaricides to cause 100% mortality at the highest treatment concentration and 96 hours of exposure is indicative of a measure of resistance of the ticks to the acaricides. This resistance could be attributed to misuse by some locals or personnel who are ignorant of application procedures as it relates to dosage. It could also be due to widespread usage, increased α/β esterase activity and target site insensitivity (mutation para-sodium channel gene) (Macchioni *et al.,* 2004). This observation is in agreement with the result of Avinash *et al*. (2017), who observed a level of resistance of *Rhipicephalus microplus* to Deltamethrin acaricide.

Parte *et al.* (2014) reported acaricidal activity of Aqueous extracts of *Azadirachta indica, Mangifera indica, Polyalthia longifolia, Annona squamosa, and Ficus benghalensis* against *Rhipicephalus microplus*. They observed that the combination of five plant extracts showed 100% mortality as compared to a single plant extract. They concluded that the effect of each plant extract required maximum time in order to attain 100% mortality. Giglioti *et al.* (2011) detected acaricidal activity of Neem seed extracts against *Rhipicephalus microplus* in Brazil. Zaman *et al.* (20127) evaluated the anti-tick efficacy of combined Aqueous herbal extracts of *Azadirachta indica* leaves, *Nicotiana tabacum* leaves, *Calitropis procera* flowers and *Trachy spermumanoni* seeds against *Rhipicephalus microplus* using adult immersion, larval packet test and ear bag method. According to Ali-Akbar *et al.* (2013), highest concentration (0.62%) of Neem seed oil mixture was considered highly effective against ear mite, *Otodectes cynotis* with 86% mortality rate at 12 hours exposure, the mortality was 98% at 24 hours exposure and was comparable to 2% Ivermectin at 100%. The acaricidal activity of the Neem seed oil was attributed to the presence of the secondary metabolites as bioactive compounds that were found during qualitative phytochemical analysis, which includes alkaloids, flavonoids and saponins (Ali-Akbar *et al.,* 2013). In comparison to existing literatures on work done on this plant extract, it has been observed that even though other studies revealed 100% mortality of Neem extract over other acaricides, more longer days of post treatment were observed as compared to this study for instance Abdel-Shafy and Zayed (2002) reported 100% mortality of 10% Hexane seed extract of *Azadirachta indica* on *Rhipicephalus microplus* ticks in comparison to Butox 5.0 and Diazinon-60 synthetic acaricides at 5, 10, 15, 20 days post treatment.

The lethal dose (LD_{50}) values for acetone leaf extract of *Azadirachta indica*, Lambda-cyhalothrin and Deltamethrin are 53.56, 22.42, and 60.0 µl/ml, respectively, at 96 hours post-treatment. Despite *Azadirachta indica* extract showing more potency over the two acaricides in terms of mean mortality, percentage mortality and lethal time recorded, Lambda-cyhalothrin recorded the least LD_{50} values against *Boophilus annulatus* ticks over *Azadirachta indica* and Deltamethrin, which indicated a higher toxicity on the test organism. This observation could be due to chance, certain prevailing experimental conditions or chemical constituents' status. This shares a striking similarity with the result of Abdel-Shafy and Zayed (2002), in which two synthetic acaricides, namely Butox5.0 and Diazinon-60 at 5% concentration, recorded 100% mortality and the least LD_{50} values at 3days post-treatment over *Azadirachta indica* seed oil extract. The LD_{50} value for Deltamethrin (60.0 µl/ml) against *Boophilus annulatus* recorded in this study shares a close value with that recorded against *Rhipicephalus microplus* ticks at 62.29 µl/ml for Deltamethrin by Avinash *et al*. (2017).

Lethal time (LT_{50}) values of *Azadirachta indica* extract, lambda-cyhalothrin and Deltamethrin against adult ticks of *Boophilus annulatus* are 63.66, 66.69 and 73.13 hours, respectively. Though slight differences exist in their lethal time of exposure, the extract of *Azadirachta indica* had the least lethal time of exposure, which indicated its quick activity and mortality responses against the cattle ticks. This quick response could be due to solvent extract and/or bioactive agents present in the plant. This could also be attributed to the presence of substances present in the extract other than Azadirachtin, which could likely influence the efficacy of the plant, especially to a certain lethal concentration and time range (Giglioti *et al.,* 2011).

Logistic regression analysis revealed a significant ($p<0.05$) and positive correlation between mortality rates of adult *Boophilus annulatus* ticks and treatment concentrations of *Azadirachta indica* extracts, Deltamethrin and Lambda-cyhalothrin. This is inconsistent with the result of Abdel-Shafy and Zayed (2002), who reported no significant association between acaricidal activity of 5% concentrations of Butox 5.0 and Diazinon-60 against *Hyelomma dromedarii* ticks, whereas in that same study, all *Azadirachta indica* extracts in comparison to the activity of these synthetic acaricides revealed significant associations in their activity. The efficacy of Neem plant extract based on ticks mortality at 96 hours of exposure was found to be more effective and comparable with the commercially available acaricides: Deltamethrin and lambda-cyhalothrin. The efficacy based on the mortality might be the effect of the secondary metabolites that were identified during the qualitative grading system of the phytochemical tests, wherein tannins, phenols, glycosides, steroids, flavonoids, alkaloids, terpenoids and saponins were detected.

Qualitative analysis of the acetone leaf extract of *Azadirachta indica* revealed all tested phytochemicals to be present with flavonoids(+), glycosides (+), terpenoids (+), Steroids (+) as slightly present, alkaloids (++), tannins (++) phenols (++) as moderately present while saponin (+++), phenol (+++) are highly present. A creamy yellow precipitate indicated the presence of alkaloids, a persistent foam of mass of small bubbles which escapes with surface of liquid indicates the presence of saponins, a brick red precipitate indicates the presence of glycosides, a greenish coloration indicates tannins, a intense yellow color which disappears on standing indicates flavonoids, a red/dark colour indicates steroids, yellow to bright orange colour indicates phenols, a reddish brown colouration at interface indicates terpenoids. The quantitative analysis revealed the highest percentage of phytochemical to be phenol (25.5%), followed by saponins (16.8%), alkaloids (11.3%), tannins (10.8%), flavonoids (5.5%), glycosides (4.8%), terpenoids (3.5%), steroids (27.5%), arranged in descending order of percentage presence. This agrees with the results of previous studies (Avinash *et. al.,* 2017; Nwali *et al.,* 2018; Seriana *et al*., 2021; Khanal, 2021) and disagrees with Biu *et al.* (2009), Aguoru *et al*. (2015), who reported low percentages and absence of some of these phytochemicals in the leaf extract of *A. indica.* Differences observed with other studies in the composition of plant phytochemicals could be attributed to the differences in geographical locations, soil factors, humidity and rainfall levels (Gahukar, 2014). Environmental factors such as temperature, light, soil salinity, fertility and general geographical locations have been documented to greatly affect plant chemical composition (Gahukar, 2014).

Azadirachtin, which is known to be the main component of the Neem plant, has been reported to have adverse effects on more than 200 insect species. It is a complex tetra or triterpenoid limonoid that is known to act as an anti-feedant, an insect growth regulator, sterilant against a broad range of insect pests and toxic to different organisms (Wink, 2000; Raizada *et al.,* 2001). Neem extracts are considered to be very suitable insecticides for pests as ticks because of its potential to inhibit insect cellulases. This inhibition has been identified to be caused by active components in the plants, such as flavonoids and saponins, which are good insect repellents. Flavonoids have been established to affect insects, bacterial microorganisms (Havsteen, 2002), Its insecticidal activity has to do with mainly involves inhibition of enzymatic pathways, such as the action of a cytochrome P-450 dependent oxidase (Macchioni *et al.,* 2004). It has also been reported to demonstrate acaricidal activity against *Psoroptes cunicula* (Mohammed, 2011). Alkaloids can act as defence compounds of plants, which are efficient against pathogens due to their toxicity (Anitha Sri, 2016). It produces multiple toxic effects on insects by inhibiting choline acetyltransferase, affecting neurotransmission and several other neuroreceptors and DNA synthesis (Wink, 1998). Also, in a review of Chowański *et al*. (2016), alkaloids were reported to be effective against insects. Saponins act on nervous enzymes of insects and show a broad range of biological and pharmacological activities (Subrahmanyam *et. al.,* 1989) such as insect enzymes inhibitory activity, insect repellent potential, pesticidal and insecticidal potential (Chaieb, 2010). Its insecticidal activity is due to its interaction with cholesterol, causing a disturbance of the synthesis of ecdysteroids. Saponins are also found to be anti-feedants, protease inhibitors, and affect the growth, reproduction in lower animals, and protect against microbes and fungi (Foerster, 2006). They are known to be toxic to cold-blooded organisms and insects at a particular concentration (Ribeiro *et. al.,* 2011) or cytotoxic to certain insects. The toxicity of saponins to various organisms is linked to their interaction with biological membranes. Some saponins form complexes with proteins, and by this action, they inhibit proteases and affect digestion in the insect gut. Tannins play an important role as pesticides (Liener, 1980).

GC-MS analysis of hexane leaf extract of *Azadirachta indica,* as reported by Avinash *et al.* (2017), revealed the identification of 12 different groups of organic compounds. These include diphenylmethane, benzophenone, benzenemethaniminealpha-phenyl, 4,8,12,16-tetramethylheptadecan-4-olide, tetratracontane, octadecane, 3-ethyl-5-(2-ethylbutyl), nonacosane glyceryl monoricinoleate, dl-alpha-tocopherol alpha-tocopherolquinone, stigmasterol and gamma-sitosterol, whereas the Chloroform extracts revealed Benzenemethanimine, alpha-phyl-3-heptanol, 3,5-dimethyl and phytol. Seriana *et al.* (2021) reported 20 active compounds in the ethanol leaf extract of *A. indica,* with linolenic acid and n-hexadecanoic acid as the main compounds. These identified compounds, especially linolenic acid and n-hexadecanoic acid, have been reported to show good acaricidal activity against ticks and other insects, and the result obtained in this study is traceable to the rich store of chemicals in this plant. Therefore, the acetone extract of *Azadirachta indica* has potent acaricidal activity and an eco-friendly nature compared to the synthetic acaricides, Deltamethrin and Lambda-cyhalothrin, against *Boophilus annulatus*. Therefore, application of *Azadirachta indica* and its products on affected animals might offer many advantages for the control of ectoparasites such as ticks in cattle without the risk of toxicity to them and the environment.

Conclusion

This study assessed the *in vitro* acaricidal effect of the acetone crude extract of *Azadirachta indica* in comparison to that of Deltamethrin and Lambda-cyhalothrin, which are synthetic and commercially purchased acaricides commonly used in Nigeria for the control of ticks. This study, therefore, concludes that the application of *Azadirachta indica* extracts against cattle ticks of *Boophilus annulatus* proved to be potent as compared to that of the synthetic acaricides. More importantly that the extract exhibited 100% mortality at 72 hours post-treatment, which is a shorter time recorded compared to other studies, and a considerably low lethal dose and time of exposure. Phytochemical analysis of Neem revealed the presence of alkaloids, saponins, tannins, steroids, terpenoids, and glycosides that are known for various biological effects. The present results of this study do not reveal which chemical compound is responsible for the different activity. Therefore this study revealed the effectiveness and richness of *Azadirachta indica* as a potent plant for ticks control over synthetic acaricides commonly used in Nigeria, provide a platform and baseline information for the application and development of environmental friendly, non-toxic and bio-degradable substances against ectoparasites such as *Boophilus annulatus* ticks which could be carried out on a large scale in animal farms.

Recommendations

However, further studies are needed to identify and isolate pure chemicals from this plant, study the effect of these isolates, toxicity studies of these toxicants on ticks should be carried out and also an *in vivo* study on the acaricidal activity before their therapeutic application in veterinary practice. Molecular bioactivity and optimisation of the studied pure chemical isolates or extracts of the plant is recommended for further studies.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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