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# The Effects of Aqueous Root Bark and Seed Extracts of *Azadirachta indica* in the Treatment of Ticks (*Hyalomma* spp) *in vitro*

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors NCO and MHCC designed the study, wrote the protocol and supervised the work. Authors MA and YAB carried out all laboratories work and performed the statistical analysis. Authors MHCC and OAN managed the analyses of the study. Author UCE wrote the first draft of the manuscript. Authors UCE and MHCC managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

The acaricidal effect of the aqueous extracts of neem (*Azadrichta indica*) seed and root bark on tick (*Hyalomma spp*) was investigated. Adult *Hyalomma* ticks of both sexes were incubated in aqueous extracts of neem seed and root bark. The extracts were made in varying concentrations (100%, 50% and 25%) with sand and sawdust media. Survival rate of the ticks in the extracts were monitored daily by recording of the ratio of dead to live ticks. Phytochemical screening of the extracts was also determined. The results showed the presence of tannins, saponins, anthraquinones, phlobatanins

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and cardiac glycosides. Both extracts contained alkaloids, phlobatanins and cardiac glycosides. Tannin occurred in larger concentration in the root bark than in the seed whereas the seed contained larger quantities of anthraquinones and saponins than the root bark. The results further showed that the seed and root extracts of neem have acaricidal effect against the ticks through death of the ticks incubated in them. However, the root bark extract was more effective than the seed extract as it produced higher killing effects of the ticks incubated in it.
Therefore, the aqueous extracts of the root bark and seeds of *Azadirachta indica* can be used as commercial acaricide to control ticks and tick infestation.

*Keywords: Azadirachta indica; tick; root bark; seed.*

## 1. INTRODUCTION

Ticks are ectoparasites of vertebrates with vital roles in the transmission of many disease causing agents to man and livestock [1-3]. Production losses in livestock production and quality has been attributed to tick infestation [4] and coupled with the increased risk to life threatening diseases in humans [5]. The control of tick infestations and transmission of tick-based-diseases remains an important huddle to the livestock industry [6]. Until recently, the indiscriminate use of acaricides has led to the fast development of tick resistance, environmental toxicity [7-9] and the possible appearance of chemical residues in products of animal origins [10].

The most common acaricides used in the control of ticks include pyrethroids, carbaryl, fipronils and amitraz [11]. Plant extracts have been employed to control some species of ticks [12-14]. Some of the advantages of phytotherapies that justify their use are the synergistic effects of its constituents and the combination of mechanisms for substances acting on various molecular targets among others [15,16]. Alternative insecticides derived from compounds in plants can act on parasites by reducing their development, reproductive capacity and survival [17]. Experiments on neem plant have indicted some acaricide activities of its extracts [18-20]. The current knowledge on the composition of the extract and the doses required for the proper control of ticks are scarce [21].

Therefore, this study was designed to evaluate the efficacy of aqueous root bark and seed extracts of *Azadrichta indica* on ticks (*Hyalomma* spp).

## 2. MATERIALS AND METHODS

### 2.1 Tick Collection

The ticks (*Hyalomma* spp.) used were collected from cattle and camels brought for slaughter in Maiduguri Metropolitan Abattoir, North Eastern Nigeria. The animals were brought for slaughter from different parts of Borno State and adjoining States. The ticks were collected from the different body parts of camels and cows of several breeds immediately after being slaughtered, placed directly into sample bottles covered with wire netting, and transported immediately to the laboratory for the study.

### 2.2 Collection of Standard Tick Acaricide (Pyrethrin)

Tick flea, a 5% pyrethrin based powder produced by Primose Veterinary (Victoria Island Lagos) was used as the standard acaricide for the study.

### 2.3 Collection and Extraction of Plant Materials

Fresh quantities of the root bark and seeds of neem (*Azadirichta indica*) were collected from the trees planted within the University of Maiduguri campus.

The fresh samples of the root bark of *Azadrichta indica* were dried under room temperature for about two weeks. The dried root barks were pulverized using pestle and mortar into coarse particles and then grounded finely with a mechanical grinder. Also, the fresh ripped seeds were separated from the seeds, washed and then shade dried. The seeds were cracked to remove the kernels.

Two hundred and fifty grams (250 g) of the powdered root bark extract and 350 g of the grounded seed were each respectively extracted in 1000 mL of distilled water with intermittent shaking at intervals of 6 hours. The extract was filtered using What-man size 1 filter paper and concentrated in vacuo using vacuum rotary evaporator. The percentage yield was calculated as described by Omoja et al. [22] and the extracts were each kept in the refrigerator at 4℃ for use in the study.

### 2.4 Processing of the Sawdust and Sand Samples

The sawdust was dried at room temperature for ten days. The sand sample collected was sharp porous sand without dust. One hundred grams of the sand sample was sterilized at 100℃ for 10 min to destroy all living microorganisms that may be present in the sand.

### 2.5 Phytochemical Screening

Basic phytochemical screenings were carried out on the seed and root bark extracts using the methods of Shellard [23] and Trease and Evans [24].

### 2.6 *In vitro* Acaricidal Efficiency Test

The ticks collected were divided into several groups each of which were treated either with the extract, the conventional acaricide or remained untreated as control as stated below. The ticks were grouped into twelve groups for each extract. For each experiment, 30 ticks were placed in the sample bottle containing either extracted powder, acaricide or their mixture with the media. The bottles were then covered with a wire netting to prevent the ticks from escaping. The experimental groups were kept in the laboratory at room temperature. The experimental set-up was monitored every day to assess the survival of the ticks in each of the extract powder, acaricide and their mixture with the various media. On each day, the number of dead ticks or live ones were determined and recorded. Dead ticks were removed from the experimental group during each observation period. The sand and sawdust served as litter for the ticks to rest.

Group A control (Untreated).
Group B extract 100%
Group C extract 50% + sawdust 50%
Group D extract 25% + sawdust 75%
Group E extract 50% + sand 50%
Group G acaricide 100%
Group H acaricide 50% + sawdust 50%
Group I acaricide 25% + sawdust 75%
Group J acaricide 50% + sand 50%
Group K acaricide 25% + sand 75%
Group L sawdust 100%
Group M sand 100%

### 2.7 Statistical Analysis

Data obtained were presented as graphs and table. The data were analyzed using ANOVA followed by the student t-test. The acceptable level of significance was $P<0.05$.

## 3. RESULTS

The results of the phytochemical constituents of the aqueous seed and root bark extracts of the plant is presented in Table 1. The results show that the aqueous root bark extract of the plant contained appreciable quantities of alkaloids, tannins, phlobatannins and cardiac glycosides, moderate quantities of saponins and a little quantities of cardiac glycosides. In comparison, the two extracts appeared to contain the same amounts of alkaloids and phlobatanins. However, the root bark had more tannin than the seed extract while the seed extract contained more anthraquinones and saponins than the root bark extract.

**Table 1. Phytochemical analysis of the aqueous root bark and seed extracts of *Azadirachta indica***

| Phytochemical test | Root bark extract | Seed extract |
|---|---|---|
| Alkaloids | +++ | +++ |
| Tannins | +++ | ++ |
| phlobatanins | +++ | +++ |
| anthraquinones | + | ++ |
| saponins | + | ++ |
| Steroids | +++ | +++ |
| Cardiac glycosides | ++ | + |

*+++ = very positive, ++ = moderately Positive, + = mild positive*

The results of incubating the ticks in 100% standard acaricide (5% pyrethrin), 100% root bark extract, and seed extract is shown in Fig. 1. The results show that incubation in the 100% standard acaricide killed all the ticks after 4 days post incubation while the 100% root bark and 100% seed extract killed all the ticks after 8 days post incubation respectively. Also, incubation in 100% sawdust and 100% sand produced 100% lethality after 8 and 14 days post incubation respectively. Generally there was a gradual death from day 6 post incubation in the untreated control group during the 21 days period of experiment.

The results of incubating the ticks in 50% root bark extract, 50% seed extract, acaricide (5% pyrethrin), sawdust and sand is shown in Fig. 2. The incubation in 50% acaricide combination with sawdust and sand respectively produced 100% lethality by 2 and 4 days post incubation

respectively while the incubation in 50% root extract in combination with sawdust and sand produced 100% lethality on the ticks by day 9 post incubation. Also, the combination of 50% seed extract with sawdust and sand produced 100% lethality after 14 and 16 days post incubation.

The results of incubating the ticks in 25% root bark extract, 25% seed extract, acaricide (5% pyrethrin), sawdust and sand is shown in Fig. 3. The results showed that the combination of 25% acaricide with sawdust or sand killed all the ticks after 4 days post incubation while the incubation in root bark extract with sawdust and sand respectively produced 100% lethality on the 21 and 13 days post incubation. The treatment in 25% seed extract in combination with sawdust and sand produced 100% lethality after 9 and 21 days respectively.

## 4. DISCUSSION

The results of the phytochemical analysis of the root bark and seed extract of *Azadirichta indica* showed that both extracts have the same level of alkaloids. The root extract contained more tannins than the seed extract while the seed extract contained more of saponins. It can be inferred from the results in Table 1 that these plant extracts contained copious amounts of phythochemicals acting synergistically and contributing to the observed acaricidal property.

In comparison, the two extracts appeared to have acaricidal effect that was manifested in a concentration dependent manner. Higher concentration of the extracts produced earlier killing of the ticks incubated in them than those produced by lower concentration of the extracts with either sand or sawdust. The results are in line with Giglioti et al. [9] that observed that the effectiveness of neem seed extract on Rhipicephalus microplus was concentration dependent. Also, the report of Ndumu et al. [25] observed that the efficacy of the neem seed extract on Amblyomma variegatum was dependent on the time of exposure to oil and quality of oil in the extract. The results also showed that at the 100% or 50% mixture with sand or sawdust acaricidal effect of the extracts (seed, and root bark) were comparable to those

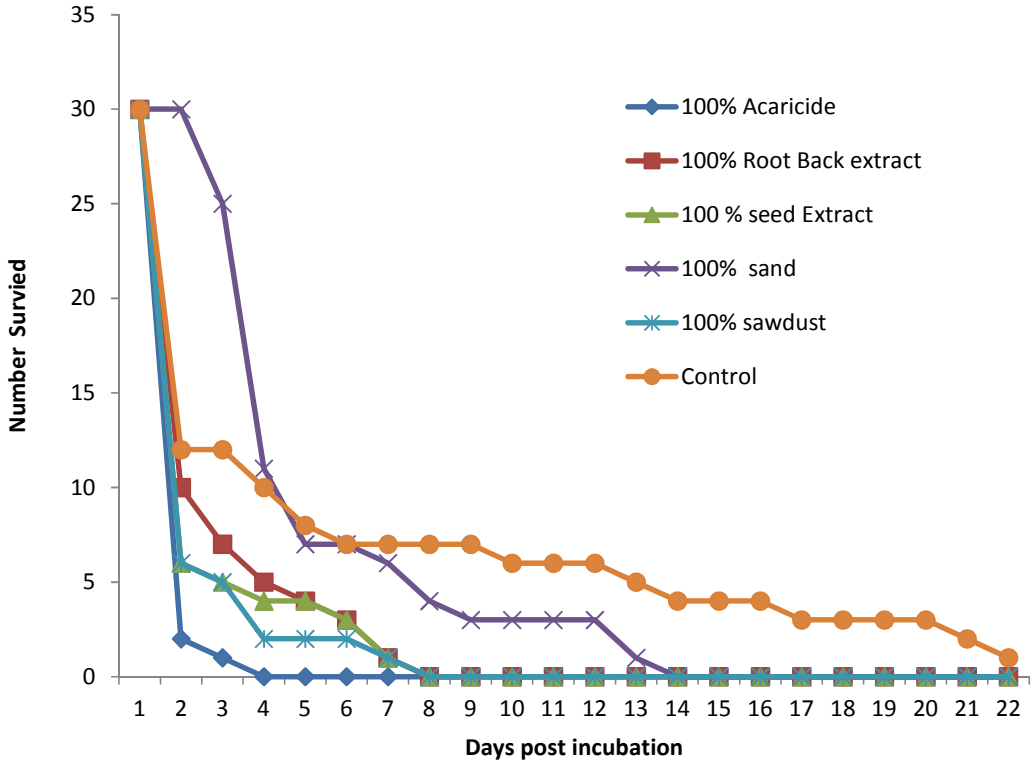


**Fig. 1. The survival of ticks incubated in 100% root bark, seed extract and acaricide**

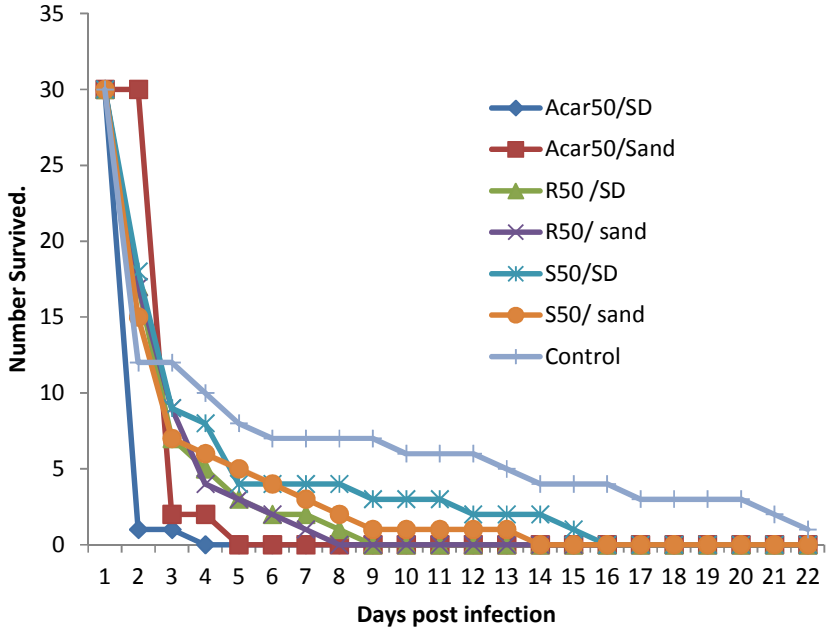


**Fig. 2. The survival of ticks incubated in 50% root bark, seed extract, and acaricide**

*Acar50/SD = 50% acaricide +50% sawdust, Acar50/Sand=50%acaricide +50% sand, R50/SD=50% root extract +50% sawdust, R50/sand=50% root extract +50% sand, S50/SD=50% seed extract +50% sawdust, S50/sand=50% seed extract +50% sand*

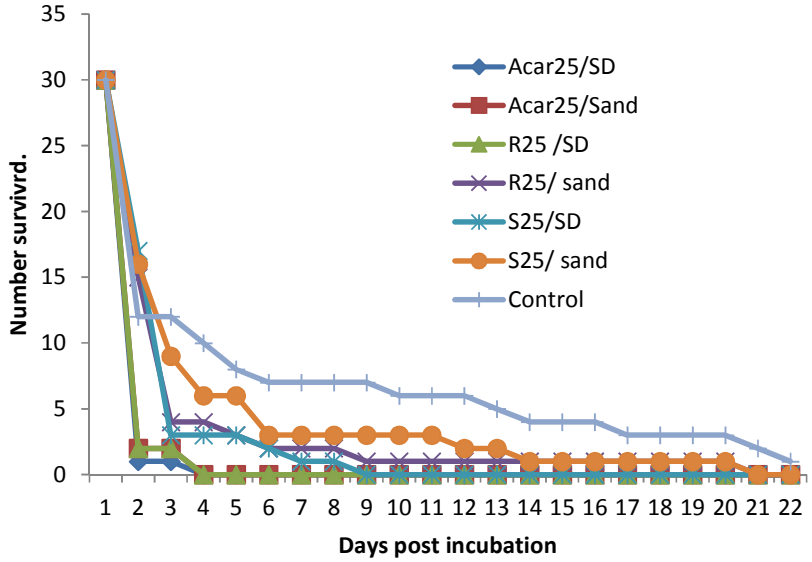


**Fig. 3. The survival of ticks incubated in 25% root bark, seed extract, and acaricide**

*Acar25/SD = 25% acaricide +75% sawdust, Acar25/Sand=25%acaricide +75% sand, R25/SD=25% root extract +75% sawdust, R25/sand=25% root extract +75% sand, S25/SD=25% seed extract +75% sawdust, S25/sand=25% seed extract +75% sand*

produced by the standard acaricide at the same level of mixture with the two media. However, the standard acaricide produced significantly ($p<0.05$) greater effect than the extracts at the 25% mixture with the media. The results suggest that the use of the extract at 25% concentration in combination with sand or sawdust could not produce acaricidal effects comparable to those

caused by 25% mixture of the standard acaricide with sand or sawdust. The results from the present study were in agreement with reports of previous studies on ticks. Mohsin et al. [26] reported the anti-tick effect of *A. indica* against Rhipicephalus microplus which was time dependent. Other studies reported on the severity of *A. indica* extracts on Rhipicephalous annulatus reproduction and mortalities [27,28]. The reports of Giglioti et al. [9] showed that the main toxic effects of neem seed extract on engorged female Rhipicephalus microplus was to inhibit its reproduction. The commercial neem seed oil (Nemm Azal F) has been shown to inhibit the reproduction of tick Hyaloma anatolicum excavatum [12] while Mwangi et al. [29] reported that the oil extracts from the leaves of Ocium suave killed all the stages of Rhipicephalus appendiculatus.

At 100% concentration both extracts produced the same pattern of mortality and killed all the ticks incubated in them within 8 days. These effects appeared comparable to those produced by the acaricide. However, at the 50% combination with sawdust and sand, the root bark extract produced similar effect by causing 100% mortality by day 9 post incubation in both cases. On the other hand, 50% mixture of the seed extract produced a more gradual killing effect and the ticks survived up to 14 and 16 days post incubation in combination with sand and sawdust respectively. Therefore, the root bark extract appeared to produce earlier killing than the seed extract in mixture with either sawdust or sand. At 25% combination with sand or sawdust, the root bark produced a similar mortality pattern. However, 100% death was produced earlier (day 14) post incubation with sand than with sawdust (day 21) post incubation. In the case of the seed extract in 25% combination with sawdust or sand, the mortality pattern differed significantly while death was earlier (Day 9) with saw dust it was much more delayed with sand(day 21). Other plants extracts have been reported to be toxic to adult ticks [3]. The report of Cohen et al. [30] showed that other limonoids (nimbolide and epoxiazadiradion) in neem extracts appeared to possess cytotoxic property and were proved to be toxic to parasites. It will be difficult to determine the exact effect of the different types of neem extracts in the control of livestock pests. This could be attributed to the complex nature of its constituents and their different mode of action which make it rather hard to explain the mechanism of action involved [9,31]. The aqueous extracts were used in the study to justify the use in traditional medicine.

## 5. CONCLUSION

The results of the study showed that the aqueous root bark and seed extracts of *Azadirachta indica* have acaricidal effect on ticks (*Hyalomma* spp) through the death of the ticks incubated in them. The effect was manifested in a concentration dependent manner with higher concentrations of the extracts producing earlier killings of the ticks than those produced by lower concentrations. Therefore, the aqueous root bark and seed extract of *Azadirachta indica* can be a source of commercial acaricide to control tick infestation in livestock. Characterization and optimization of the extract is required.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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