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In vitro testing of the acaricidal activity of *Moringa oleifera* Lam. aqueous root bark extract on adult *Hyalomma* ticks

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Abstract

Natural products are gradually replacing synthetic products as acaricides. The *in vitro* acaricidal activity of *Moringa oleifera* aqueous root bark extract on adult *Hyalomma* ticks was investigated. Graded concentrations of the aqueous extract of *Moringa oleifera* root bark were tested on 135 adult *Hyalomma* ticks, weighing between 160 – 300mg. The ticks were randomly assigned into nine groups of five ticks per group in three replicates. The treatment for the groups included concentrations of the extract ranging from 5 – 25 mg/ml, and controls (Distilled water and Normal saline/Petri dish Controls, and Amitraz Positive Control). Acaricidal activity was assessed using the adult immersion test (AIT) at exposure time of 24 and 48 hours. Ticks were considered dead following loss of pedal reflex and motility with darkened cuticles. Results showed a concentration dependent adult tick percentage mortality pattern ranging from 6.7% to 73.3% (highest) recorded at extract concentration of 25mg/ml after 48 hours of exposure, which though lower than the recorded percentage mortality of 100% for Amitraz (the positive control), compared favourably with it. It was concluded that the aqueous root bark extract of *Moringa oleifera* as used in this study exhibited a concentration dependent acaricidal activity against adult *Hyalomma* ticks.

Keywords: *In vitro* testing; Acaricidal activity; *Moringa oleifera*; Root bark extract; *Hyalomma* ticks.

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Introduction

Locally available plants could constitute the nucleus of drug development (Habeeb, 2010), and in the last few decades, there has been an exponential growth in herbal medicines worldwide (Mishra *et al*., 2011). Natural products obtained from plants commonly offer farmers a lower cost and less toxic alternative to synthetic acaricides. *Moringa oleifera*, also known as horse radish tree, belongs to the family *Moringaceae*, and is widely distributed in sub-Saharan Africa. It is commonly used in North Eastern Nigeria for the treatment of inflammation, fever, malaria, stomach pain, liver and kidney disorders (Anwar *et al*., 2006; Furo and Ambali, 2012).

Ticks are ectoparasites of vertebrates in the order *Acari*. Some ticks are known to transmit important viral, rickettsial, bacterial and protozoan diseases of humans and animals. The cost of tick control is estimated to be several billions of money worldwide. *Hyalomma* species are hard ticks of the family *Ixodidae* reported as vectors of *Theileria annulata* in ruminants and Congo haemorrhagic fever virus in humans (Thembo *et al*., 2010).

Plant products such as *Senna italica* have been reported to have acaricidal activity against *Hyalomma marginatum rufipes* ticks (Magano *et al*., 2007). Also, there had been other reports of the effects of plant products on ticks, including that of Islam *et al* (2018) on mortality of ticks using *Ricinus communis* in Bangladesh; Abdul-Shafy and Zayed (2002) on neem seed oil activity against eggs, nymph and adult stages of *Hyalomma ainatolicum excavatum;* Benelli *et al* (2016) on tick repellants and acaricides of botanical origin; Alborzi *et al* (2022) on ovicidal and lavicidal activity against *Hyalomma anatolicus*; Hatzade *et al* (2022) on acaricidal efficacy of *Azadirachta indica* and *Melia azedarach* against *Hyalomma anatolicum*; and Pirali-Kheirabadi *et al* (2009) on botanicals that inhibited the feeding process, moulting, fecundity and egg hatch of ticks. This present study investigated the adult tick mortality pattern following the immersion of *Hyalomma* ticks in graded concentrations of the aqueous extract of *Moringa oleifera* root bark.

Materials and Methods

Collection, Identification and Rearing of the *Hyalomma* Ticks: The adult *Hyalomma* ticks used for the study were collected by handpicking and with smooth forceps from naturally infested livestock at Maiduguri livestock market (Kasuwan shanu, Maiduguri, Borno State, Nigeria), and placed in wide mouthed sample bottles plugged with cotton wool and taken to the Veterinary Parasitology and Entomology Laboratory, Department of Veterinary Parasitology and Entomology, University of Maiduguri where they were identified using their morphological features as described by Walker *et al.* (2003). The ticks were maintained in desiccators at room temperature, controlled relative humidity of 75 – 80% using saturated KOH solution and a 12-hour photo-period.

Collection, Identification and Extraction of *Moringa oleifera:* *Moringa oleifera* was identified at the University of Maiduguri using botanical features described by Ramasubramania *et al*. (2016). A herbarium reference copy was authenticated and deposited at the Department of Biological Sciences, University of Maiduguri. The root bark was collected, thoroughly washed with clean water, air dried under shade and ground into fine powder using pestle and mortar to obtain a 25g powder which was Soxhlet-extracted using 700 ml of distilled water at 60^{o}C for 8 hours. The extraction process yielded 4g of the aqueous crude extract, giving a percentage yield of 16% weight/weight.

Preparation of Graded Concentrations of the Aqueous Crude Extract of *Moringa oleifera* Root Bark: A 10% dimethyl sulphoxide (DMSO) solution, prepared by diluting 10 ml of 99.9% DMSO stock with 90 ml of distilled water was used as solvent. The 4 g aqueous crude extract was dissolved in 20 ml of the 10% DMSO solution in a reagent bottle to obtained 200 mg/ml stock solution from which graded concentrations of 5, 10, 15, 20 and 25 mg/ml were prepared.

***In vitro* Testing of the Acaricidal Efficacy of the Extract:** One hundred and thirty five adult *Hyalomma* ticks weighing between 160 – 300 mg were thoroughly washed with clean water, dried up for three minutes using filter paper and randomly assigned to 9 groups (A - I) of 5 ticks per sub-group, with each group having 3 replicates (15 ticks per group in all). The Groups and their specific treatments were as follows: Group A – Distilled water (Control); Group B – 10% DMSO (Control); Group C – Normal Saline or Petri dish Control; Group D – 5 mg/ml *Moringa oleifera* aqueous root bark extract (MOARBE); Group E – 10 mg/ml MOARBE; Group F – 15 mg/ml MOARBE; Group G – 20 mg/ml MOARBE; Group H – 25 mg/ml MOARBE; and Group I – 12.5 % Amitraz (Positive Control).

All the test groups were immersed in Petri dishes containing their different solutions for 10 minutes; this was followed by gentle agitation, removal and transfer of the ticks into new Petri dishes padded with filter paper, and incubation in a desiccator maintained at room temperature, 75 - 80% relative humidity and a 12-hour photoperiod for 24 and 48 hrs respectively. The ticks were observed and monitored for mortality. Ticks were considered dead following loss of pedal reflex and motility, with darkened cuticles.

Data obtained were subjected to descriptive statistics, and presented as bar charts of percentage mortality per group.

Results

The results of *in vitro* testing of the acaricidal efficacy of the extract on the *Hyalomma* ticks are presented in Figures 1 and 2. Adult tick percentage mortality recorded for the specific groups after 24 hours were as follows: 0% for 5mg/ml MOARBE and the three controls (Normal saline, Distilled water and 10% DMSO); 6.7% for 10 and 15 mg/ml; 13.3% for 20 and 25 mg/ml; and 100% for 12.5% Amitraz (Figure 1). After 48 hours however, the percentage tick mortality recorded for the specific groups were 0% for Normal saline and Distilled water controls, 7% for 10% DMSO control, 27% for 5 mg/ml MOARBE, 46.7% for 10 mg/ml MOARBE, 53.3% for 15 and 20 mg/ml MOARBE, 73.3% for 25 mg/ml MOARBE, and 100% for 12.5% Amitraz (Figure 2). Adult tick percentage mortality was observed to be extract concentration dependent.

Discussion

Over the past decades, plant extracts and essential oils have both gained wide acceptability for use against phytophagous pests, and some have been reported to exhibit acaricidal, ovicidal, repellent, anti-feedant and biocidal activities on various arthropod vectors (Pirali-Kheirabadi and Teixeria da Silva, 2011). This present study has shown that exposure of adult *Hyalomma* ticks to certain doses of aqueous extract of the root bark of *Moringa oleifera* led to significant mortality 24 and 48 hours after exposure. This finding concurs with the reports of Kemal *et al.* (2010) using *Rhipicephalus decoloratus* and *Rhipicephalus pulchellus.* At 25 mg/ml dose, the *Moringa oleifera* extract used in the present study compared favourably with the positive control synthetic acaricide (12.5% Amitraz®).

Though the phytochemical analysis of *Moringa oleifera* aqueous root bark extract was not performed in the present study, the stem and the root bark of *Moringa oleifera* have been

reported by Anwar *et al*. (2006) to contain alkaloids, moringine and moringinine, and other bio-active compounds such as flavonoids, saponins, tannins, anthraquinones, coumarins, carotenoids and cyanogenic glycosides, which has been proven to have knockdown and inhibitory effects on ticks and other acarines (Heinz-Castro *et al*. 2021). The acaricidal effects recorded in this study are thought to be due to the additive or combined activities of these phytochemical constituents.

There have been several reports in available literature on the potential acaricidal activity of various plant extracts (Giglioti *et al*., 2011; Hanifah *et al*., 2011; Kabore *et al*., 2012; Sanis *et al*., 2012; Singh *et al*., 2014). Biocidal molecules such as lectins, zeatin, quercetin, beta-sito sterol, cafteoylquinic acid, limonene, beta-bourbonene, terpinolene, delta-careen, cloven and kaempferol, reported to be contained in *Moringa oleifera* (Fahey, 2005; Kasolo *et al*. 2010), have also been shown to reduce tick populations by preventing egg hatch and larval survival of *Hyalomma anatolicum* ticks (Alborzi *et al*., 2022).

Based on the results of the study, it was concluded that aqueous *Moringa oleifera* root bark extract, as used in the study, exhibited a concentration dependent *in vitro* acaricidal activity against adult *Hyallomma* ticks, with 25 mg/ml dose giving acaricidal activity fairly comparable to the Amitraz control after 48 hours of exposure.

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Conflict of Interest

There was no conflict of interest as regards this research and the manuscript.

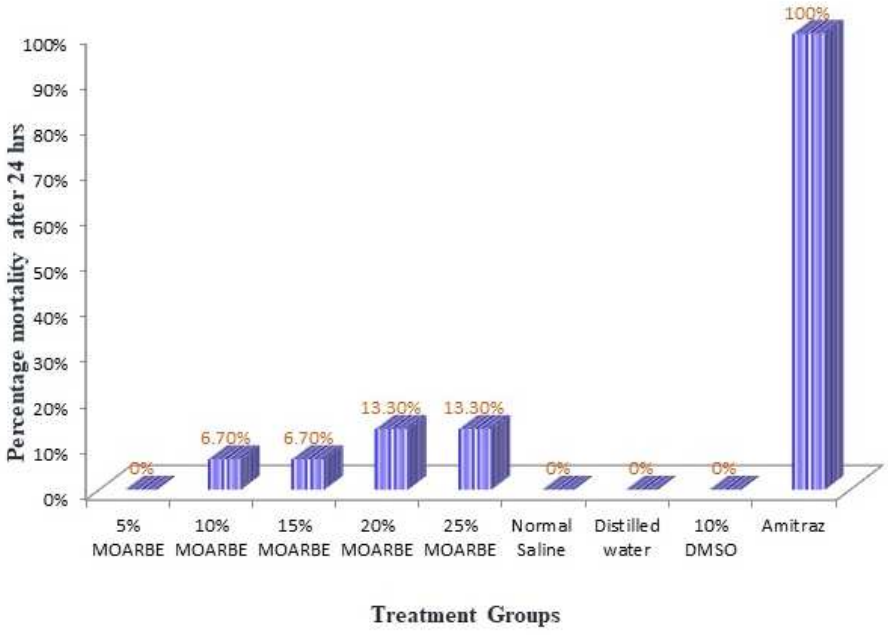

Figure 1: Percentage mortality of adult *Hyalomma* ticks 24 hours after exposure to graded concentrations of *Moringa oleifera* aqueous root bark extract (MOARBE).

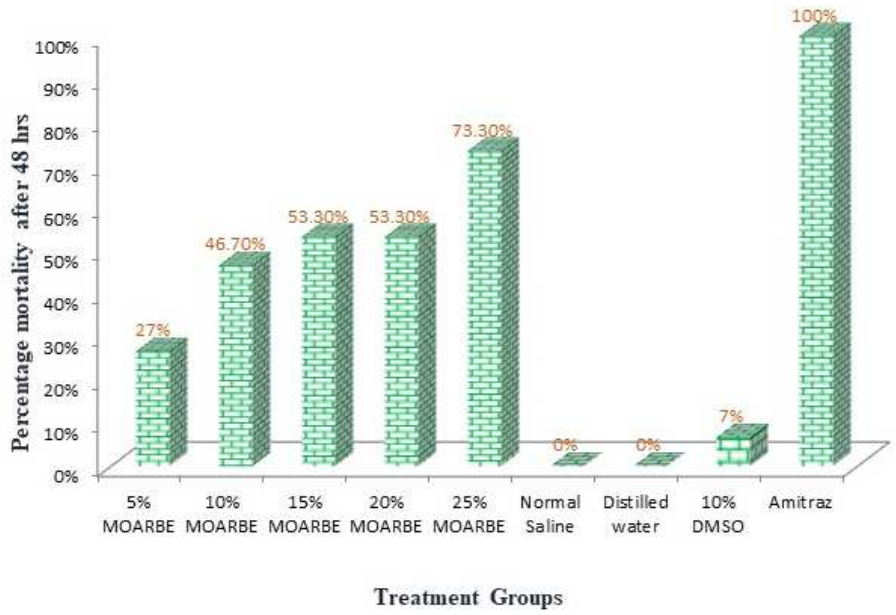

Figure 2: Percentage mortality of adult *Hyalomma* ticks 48 hours after exposure to graded concentrations of *Moringa oleifera* aqueous root bark extract (MOARBE).

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