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Research Article

Adulticidal, Larvicidal, and Repellent Potential of Ethyl Acetate Extract of *Moringa oleifera* against *Rhipicephalus microplus* Cattle Ticks

Rao Zahid ABBAS [1 (*)] Arslan Muhammad Ali KHAN [1] Mohammed M. MARES [2] Muhammad MOHSIN [3]

[1] University of Agriculture Faisalabad, Faculty of Veterinary Science, Department of Parasitology, Chemotherapy Lab, 38040 Faisalabad, PAKISTAN

[2] King Saud University, College of Science, Department of Zoology, P.O. Box 2455, Riyadh 11451, KINGDOM OF SAUDI ARABIA

[3] Shantou University Medical College, Department of Pharmacology, Shantou, Guangdong, CHINA

[(*)] **Corresponding author:**
Rao Zahid ABBAS
Phone: +92 300 6682811
Cellular phone: +92 300 6682811
E-mail: raouaf@hotmail.com

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Abstract

The emergence of tick resistance against synthetic and eco-toxic chemical acaricides has stressed the need to find target-specific, non-toxic, and more potent alternatives such as botanicals. The current study was designed to investigate the acaricidal and repellent potential of ethyl acetate extract of *Moringa oleifera* seeds against *Rhipicephalus microplus* cattle ticks. Adult immersion test, larval immersion test, and tick climbing repellent assay were carried out to evaluate the acaricidal and repellent potential of *M. oleifera* extract. Five different concentrations (20, 10, 5, 2.5, and 1.25 %) of the ethyl acetate extract of *M. oleifera* were prepared to evaluate the acaricidal and repellent potential. Different parameters like adult tick mortality, larval mortality, egg hatchability, inhibition of egg hatchability, oviposition reduction, reproductive index, reproductive efficiency index, tick repellency, and product effectiveness were determined. Ethyl acetate extract of *M. oleifera* showed 70% adult mortality after 24 h treatment when 20% concentration was used. Similarly, 73% larval tick mortality was observed at 20% concentration. The results also showed the dose-dependent response of ethyl acetate extract of *M. oleifera* seeds against egg hatchability, inhibition of egg hatchability, oviposition reduction, reproductive index, reproductive efficiency index, and tick repellency, confirming that 20% concentration is effective as an acaricide and repellent against *R. microplus*. Therefore, the tested extract can be considered a possible candidate for controlling *R. microplus* in cattle.

Keywords: Acaricides, *Moringa oleifera*, Seed extract, Repellents, *Rhipicephalus microplus*, Ticks, Cattle

Introduction

Rhipicephalus (Boophilus) microplus ticks are hematophagous ectoparasites of cattle *(Bos taurus)* and are mostly found in tropical and subtropical regions [1]. *R. microplus* ticks also infest other bovids including water buffalo *(Bubalus bubalis),* gaur *(B. frontalis),* and banteng *(Bos javanicus)* [2]. *R. microplus* cattle ticks are causing huge economic losses in terms of decreased milk and weight gain, morbidity, mortality, hide damage, and cost of disease control. The economic loss due to *R. microplus* is estimated at 22-30 billion dollars/annum [3]. They also transmit several pathogens including *Babesia bovis*, *Babesia bigemina*, *Anaplasma marginale*, and thrombocytopenia syndrome virus [4]. No doubt, *R microplus* (Acari: Ixodidae) infest livestock animals, but they also have zoonotic potential because they can transmit certain pathogens to humans [5,6]. For example, in the United States of America, 4151 cases of human granulocytic anaplasmosis (HGA) were recorded in 2016, and 2358 cases of babesiosis in 2017 [7,8].

Various chemical drugs including pyrethroids (permethrin, flumethrine), arsenical preparations, chlorinated hydrocarbons (DDT, lindane), organophosphates (coumaphos), carbamates (carbaryl) macrocyclic lactones (ivermectin), formamidines (amitraz), phenyl pyrazoles (fipronil) and insect growth regulators (fluazuron) were used to

control *R. microplus* population [9,10]. Unfortunately, the continuous and irrational use of these chemical acaricides has led to the development of resistance in the tick population [11]. The resistance is developed because of the mediation of para-sodium channels and mutation in the acetylcholinesterase enzyme system [12]. These chemical acaricides are also not readily accessible to the farmers because of their high costs. However, they may have hazardous effects on human and animal health through the contamination of cattle milk and meat [13]. Due to the increasing emphasis on sustainable agriculture and the growing interest in organic farming practices, synthetic acaricides such as pyrethroids, organo-chlorines, and organophosphates have faced restrictions in the global market [14]. Consequently, scientists and researchers have changed their interest in the development of new agents and are focusing on alternative control strategies [15,16]. Therefore, many effective control strategies have been used, including pheromone-assisted control, vaccination, entomopathogenic fungi, nanoparticles, and botanicals [17,18].

Plant-derived products seek special attention among the alternate control strategies and can be promising as an acaricidal source [19]. The insecticidal and acaricidal potentials of these plant extracts are due to the presence of chemical components and their interaction among themselves [20,21]. Furthermore, plant extracts are biodegradable, eco-friendly, and target-specific to the species.

Moringa oleifera is a globally distributed plant and most people use it in their diet because of its therapeutic properties. It has been used to cure various chronic diseases for centuries because of its ethnomedicinal properties [22]. Because of its pharmacological properties, it has been used as an antioxidant, anti-inflammatory, antimicrobial, antiviral, antifungal, anthelmintic, and anti-parasitic [23]. These properties are due to the presence of several chemical compounds such as tannins, flavonoids, saponins, terpenoids, glycosides, steroids, coumarins, proteins, carbohydrates, and starches. Other phytochemical analysis revealed the presence of isoquercetine, astragalin, isorhamnetin, daidzein, apigenin, luteolin, genistein, epicatechin, ferulic acid, gallic acid, ellagic acid, sinapic acid, caffeic acid, chlorogenic acid, salicylic acid, vicenin-2, niazimicin, and niazirin [24].

In this study, we investigated the potential of ethyl acetate extract of *M. oleifera* on adult tick mortality, larval tick mortality, percentage egg hatchability, inhibition of oviposition, reproductive index, and reproductive efficiency index of *R. microplus*. In addition, we also investigated the repellent effect of *M. oleifera* against adult ticks of *R. microplus*.

Materials and Methods

Ethical Approval

This study was conducted with approval from the Departmental Ethical Review Board, Department of Parasitology, University of Agriculture, Faisalabad (Approval No. PAR/315-24, dated 03-06-2024).

Plant Material Preparation

The seeds of *M. oleifera* were purchased from the local herbal market in Karkhana Bazar Faisalabad, Pakistan. The seeds were then identified by an expert botanist at the Department of Botany, University of Agriculture, Faisalabad, Pakistan. The seeds were then air-dried for 48 h and ground into fine powder. The powder was kept in a clean and dry glass container (Imperial-G®). The plant extract was prepared by mixing 300g of *M. oleifera* powder into 2700mL of liquid ethyl acetate (UNI-CHEM®) in a 1:10 w/v ratio. The material was stirred continuously after regular intervals and filtered. The desired extract was obtained by reducing the vacuum under Xinchen®XZ-10L at 27ºC. The extract was then stored at -4ºC until the analysis. The percentage yield was then calculated by the formula given below:

$$\text{Percentage yield} = \frac{\text{weight of the dry extract}}{\text{weight of the dry seeds before extraction}} \times 100$$

Collection and Preparation of Ticks

A total of 300 *R. microplus* ticks were collected from different areas of Tehsil Chuk Chumbra, District Faisalabad, Pakistan. The ticks were collected in 100 mL plastic bottles (Merum®) from different body parts including the neck, groin, and udder of cattle with the help of blunt end forceps (Premium®). The plastic bottles were provided with tiny holes for proper aeration. The ticks were then transported to the Chemotherapy Lab, Department of Parasitology, University of Agriculture, Faisalabad, Pakistan. The ticks were washed and identified under a stereomicroscope (Nikon®) according to the identification guide [25,26]. Two groups of 300 ticks were made. One group contained 210 ticks for the adult immersion test and the remaining 90 ticks were placed in a separate group and incubated in biological oxygen demand (BOD at 27ºC and 85% relative humidity. The latter was used to evaluate, inhibition of oviposition (IO), egg hatchability (EH), reproductive index (RI), and reproductive efficiency index [27]. The effectiveness of the product was also investigated. For these purposes, the following formulas were used.

$$\text{IO (\%)} = \frac{\text{IE control} - \text{IE treated}}{\text{IE control}} \times 100$$

$$\text{EH (\%)} = \frac{\text{Number of eggs hatched}}{\text{total number of eggs}} \times 100$$

$$RI\ (\%) = \frac{\text{Weight of eggs laid}}{\text{Weight of adult females before treatment}} \times 100$$

$$REI = \frac{\text{egg mass weight} \times \%\ \text{egg hatching}}{\text{engorged female tick weight}} \times 20000$$

$$PE\ (\%) = \frac{\text{RE control} - \text{RE treatment}}{\text{RE control}} \times 100$$

Bioassays

Adult immersion test, larval immersion test, and tick repellent assay were used to evaluate the acaricidal and repellent potential of ethyl acetate extract of *M. oleifera* seeds.

Adult Immersion Test (AIT)

The adult immersion test was carried out to determine the adult engorged *R. microplus* tick mortality. A total of 210 engorged female cattle ticks were divided into seven subgroups which include five subgroups for five different concentrations along with positive (cypermethrin 0.1% of K-CYPER®) and negative (distilled water) control groups. Each subgroup was further subdivided into three replications and each replication contained 10 ticks. Five concentrations (20, 10, 5, 2.5, and 1.25) of *M. oleifera* were prepared. 5 mL of each concentration was taken out in separate Petri dishes (SMART LAB®). The collected ticks were then immersed, removed, washed with distilled water, and placed on filter paper to dry. All dried ticks were then transferred into a desiccator and placed in a BOD incubator where temperature and relative humidity were maintained at 27ºC and 85% respectively. The adult tick mortality of all groups was calculated after 6, 12, and 24 h treatment. The mortality of *R. microplus* ticks was confirmed by the loss of motility or sensitivity and the absence of motion of malpighian tubules and pedal reflex.

Percentage mortality was calculated by the following formula:

$$\text{Percentage mortality} = \frac{Number\ of\ dead\ ticks}{Total\ number\ of\ ticks} \times 100$$ [28]

Larval Immersion Test

The LIT was used to evaluate the larval mortality [29]. Similar to the adult immersion test five concentrations (20, 10, 5, 2.5, 1.25%) concentrations were taken in 1.5 mL microcentrifuge tubes. 100 *R. microplus* larvae were placed in each 1.5 mL microcentrifuge tube. The positive control group was treated with 1 mL of 0.1% cypermethrin while the negative control was treated with distilled water. After adding the larvae, the tubes were sealed and shaken vigorously for 30 sec and then gently for 5 min. Each treated larva was then transferred to filter paper to dry. After that, the larvae were placed on Whatman filter paper No. 1 (8.5×7.5 cm), folded, and sealed with hairpins to form a packet. These packets were then incubated at 27°C and 85% relative humidity. Live and dead larvae were counted after 48 h treatment. The experiment was repeated three times and larval mortality was counted by the following formula:

$$\text{Percentage mortality} = \frac{Number\ of\ dead\ larvae}{Total\ number\ of\ larvae} \times 100$$ [30]

Tick Climbing Repellent Assay (TCRA)

The TCRA was used to investigate the repellent activity of ethyl acetate extract of *M. oleifera*. The same protocol was followed as adapted by [31]. For this procedure, two vertical aluminium rods were taken which were spaced 7cm from each other and attached to an aluminium base. Each aluminium rod was encased in a glass tube with a piece of filter paper wrapped around it. The top of each tube was sealed with wet cotton wool. *M. oleifera* concentrations (20, 10, 5, 2.5, 1.25 %) were applied to aluminum rods and 30 ticks were placed for each concentration. A similar procedure was done for positive (0.1% cypermethrin) and negative control (distilled water). Some ticks were unable to climb up due to the repellent effect of ethyl acetate of *M. oleifera* and data were recorded for all groups. The percentage repellency was calculated by using the following formula:

$$\text{Percentage Repellency} = \frac{100 - (\text{mean number of ticks not repelled})}{\text{mean number of ticks not repelled by control}} \times 100$$

Statistical Analysis

Results of *R. microplus* adult cattle ticks (adult tick mortality, larval mortality, reproductive index, reproductive efficiency, inhibition of oviposition, percentage larval hatching, and product effectiveness) were analyzed using Tuckey's test ($P>0.05$). Lethal concentrations (LC_{50} and LC_{90}) and repellent concentrations (RC_{50} and RC_{90}) were calculated by using Statistical Product and Service Solutions (SPSS) software.

Results

Yield of the Ethyl Acetate Extract of *M. oleifera*

Using the hydrodistillation process, a yield of 12.8% was determined for the ethyl acetate extract of *M. oleifera*.

Adult Tick Mortality

Effect of Ethyl Acetate Extract of M. Oleifera Seeds on Adult Tick Mortality: The results of the adult immersion test for various concentrations (20, 10, 5, 2.5, 1.25%) of ethyl acetate of *M. oleifera* seeds are shown in *Table 1* and *Fig. 1*. Value for adult tick mortality was lowest at AX=1% concentration whereas 70% mortality was seen at EX=20% treatment. The results demonstrated that a 20% concentration of *M. oleifera* showed a significant ($P<0.05$) acaricidal effect against *R. microplus* adult ticks when compared with negative control. LC_{50} and LC_{90} values were calculated as shown in *Table 2*.

Table 1. *Percentage mortality of adult Rhipicephalus microplus ticks treated with various concentrations of ethyl acetate extract of M. oleifera seeds under laboratory conditions (27°C and RH = 85%)*

Concentrations of *M. oleifera*	Mortality After 6 h	Mortality After 12 h	Mortality After 24 h
AX	10 ± 0^{d}	16.6 ± 5.77^{c}	23.33 ± 5.77^{d}
BX	23.33 ± 5.77^{cd}	33.33 ± 5.77^{c}	43.33 ± 5.77^{d}
CX	36.66 ± 5.77^{cd}	46.66 ± 5.77^{c}	56.66 ± 5.77^{cd}
DX	46.66 ± 5.77^{bc}	56.66 ± 5.77^{bc}	66.66 ± 5.77^{bc}
EX	53.33 ± 5.77^{b}	63.33 ± 5.77^{b}	70 ± 10^{b}
PC	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
NC	0 ± 0^{e}	0 ± 0^{d}	0 ± 0^{e}

AX: *M. oleifera 1.25%;* ***BX:*** *M. oleifera 2.5%;* ***CX:*** *M. oleifera 5%;* ***DX:*** *M. oleifera 10%;* ***EX:*** *M. oleifera 20%;* ***PC:*** *Positive Control;* ***NC:*** *Negative Control. Mean±SD along with the same superscripts have a non-significant difference (P>0.05) from each other*

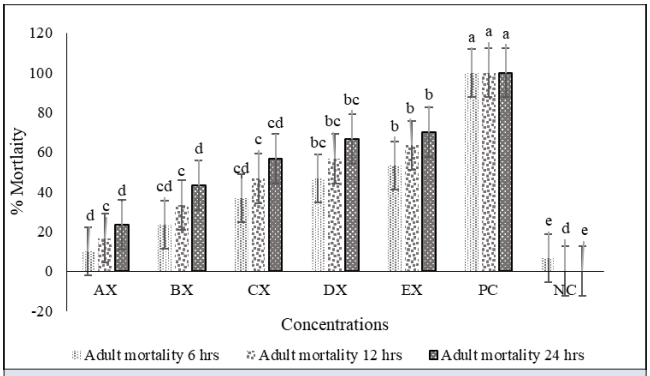

Fig 1. Effect of different concentrations of *M. oleifera* on adult *R. microplus* ticks. AX: *M. oleifera* 1.25%; BX: *M. oleifera* 2.5%; CX: *M. oleifera* 5%; DX: *M. oleifera* 10%; EX: *M. oleifera* 20%; PC: Positive Control; NC: Negative Control. Mean±SD along with the same superscripts have a non-significant difference (P>0.05) from each other

Table 2. *LC_{50} and LC_{90} values with 95% Confidence interval (C.I) of ethyl acetate extract of M. oleifera seeds against adult tick mortality at different time intervals*

	Time Interval	LC50 (95% C.I)	LC90 (95% C.I)	Slope ± SE	X^2	R^2
***M. oleifera* Seed Extract**	6 h	0.057 (0.019-1.070)	6.246 (3.019-8.302)	0.91±0.62	0.176	0.947
	12 h	0.047 (0.017-1.009)	3.721 (1.171-5.223)	1.12±0.69	0.471	0.909
	24 h	0.019 (0.007-0.461)	0.702 (0.039-3.121)	1.68±0.91	0.588	0.886

LC: *lethal concentrations;* ***X^2:*** *Chi-square;* ***R^2:*** *coefficient of determination;* ***SE:*** *standard Error*

Larval Mortality

Effect of Ethyl Acetate Extract of M. oleifera Seeds on Larval Mortality: The larvicidal effect of ethyl acetate extract of *M. oleifera* seeds at various concentrations (20, 10, 5, 2.5, 1.25%) was evaluated against the larvae of *R. microplus* ticks. The results demonstrated that a 20% concentration of *M. oleifera* showed a significant (P<0.05) effect when compared with negative control. LC_{50} and LC_{90} values were also calculated which showed toxicity at 12.41 and 2.41% doses respectively. *Fig. 2* indicates that as extract concentration increased, larval mortality also increased.

Effectiveness of the *M. oleifera* Extract

The efficacy of ethyl acetate extract of *M. oleifera* seeds against *R. microplus* ticks was determined through the product effectiveness parameter. Different concentrations of ethyl acetate of *M. oleifera* seeds were used and proved to have different results concerning effectiveness against

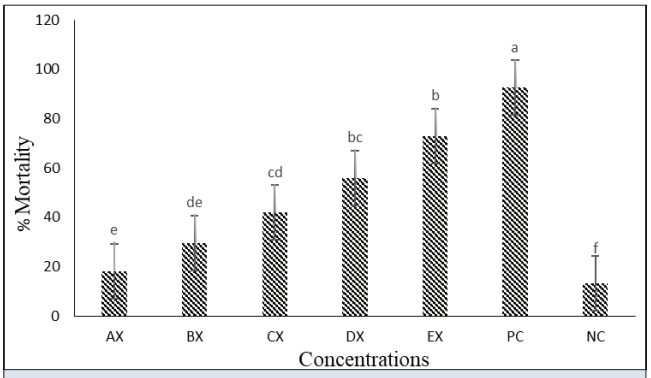

Fig 2. Effect of different concentrations of *M. oleifera* on larvae of *R. microplus* ticks. AX: *M. oleifera* 1.25%; BX: *M. oleifera* 2.5%; CX: *M. oleifera* 5%; DX: *M. oleifera* 10%; EX: *M. oleifera* 20%; PC: Positive Control; NC: Negative Control. Mean±SD along with the same superscripts have a non-significant difference (P>0.05) from each other

Table 3. *Mean values of egg hatchability, % inhibition of hatchability, oviposition reduction, reproductive index, and reproductive efficiency index engorged females of R. microplus treated with various concentrations of ethyl acetate extract of M. oleifera seeds under laboratory conditions (27°C and RH = 85%)*

Concentrations of *M. oleifera*	Egg Hatchability	%Inhibition of Hatchability	Oviposition Reduction	Reproductive Index	Reproductive Efficiency Index
AX	80.55±1.50^{a}	19.44±1.50^{a}	54.73±0.90^{d}	33.59±0.71^{b}	54.12±2.05^{b}
BX	75.11±3.20^{a}	24.88±3.20^{a}	62.87±1.70^{cd}	27.54±1.26^{bc}	41.4 ±3.64^{bc}
CX	63.11±5.27^{b}	36.88±5.27^{b}	69.05±1.64^{cd}	22.96±1.21^{bc}	29.07±3.92^{cd}
DX	42.44±1.67^{c}	57.55±1.67^{c}	79.94±4.92^{bc}	14.88±3.65^{cd}	12.71±3.55^{de}
EX	27.22±2.54^{c}	72.77±2.54^{c}	89.15±1.74^{b}	8.04±1.29^{d}	4.4±1.04^{ef}
PC	13.11±2.77^{d}	86.88±2.01^{77d}	93.06±10^{a}	5.14±0.74^{e}	1.34±0.32^{f}
NC	88.22±2.52^{a}	11.77±2.52^{a}	40.66±0.95^{e}	44.03±0.7^{a}	77.7±3.36^{a}

AX: *M. oleifera 1.25%;* ***BX:*** *M. oleifera 2.5%;* ***CX:*** *M. oleifera 5%;* ***DX:*** *M. oleifera 10%;* ***EX:*** *M. oleifera 20%;* ***PC:*** *Positive Control;* ***NC:*** *Negative Control. Mean±SD along with the same superscripts have a non-significant difference (P>0.05) from each other*

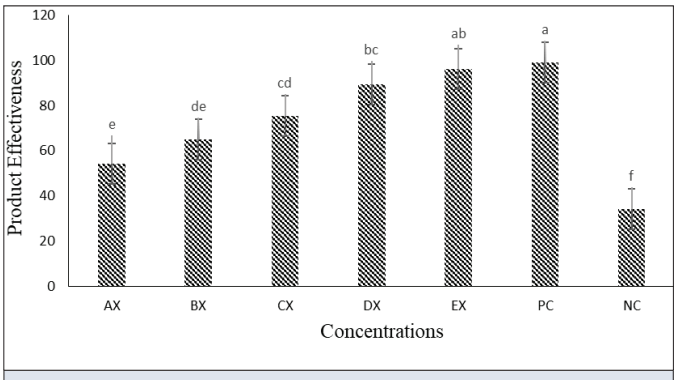

Fig 3. Effectiveness of different concentrations of *M. oleifera* against *R. microplus* ticks. AX: *M. oleifera* 1.25%; BX: *M. oleifera* 2.5%; CX: *M. oleifera* 5%; DX: *M. oleifera* 10%; EX: *M. oleifera* 20%; PC: Positive Control; NC: Negative Control. Mean±SD along with the same superscripts have a non-significant difference (P>0.05) from each other

R. microplus cattle ticks. The 20% concentration showed significant (P<0.05) results when compared with the negative control and had non-significant (P>0.05) results from the positive control (0.1% cypermethrin) as shown in *Table 3* and *Fig. 3*.

Percentage Repellency

The results of tick repellency of ethyl acetate extract of *M. oleifera* seeds at different concentrations (20, 10, 5, 2.5, 1.25%) were shown in *Table 4* and *Fig. 4*. Tick repellency was calculated at 4 h and 8 h post-treatment. The results

Table 4. *Percentage Repellency of adult Rhipicephalus microplus ticks treated with various concentrations of ethyl acetate extract of M. oleifera seeds under laboratory conditions (27°C and RH = 85 %)*

Concentrations of *M. oleifera*	Repellency After 4 h	Repellency After 8 h
AX	19.62±1.69^{de}	14.94±2.13^{d}
BX	34.81±5.59^{d}	29.89±6.4^{c}
CX	50.74±4.62^{c}	43.05±2.22^{c}
DX	64.81±3.39^{bc}	49.1±4.89^{b}
EX	77.4±4.2^{b}	60.49±4.04^{b}
PC	97.77±2.22^{a}	91.45±4.31^{a}
NC	7.4±2.79^{e}	4.27±2.82^{e}

AX: *M. oleifera 1.25%;* ***BX:*** *M. oleifera 2.5%;* ***CX:*** *M. oleifera 5%;* ***DX:*** *M. oleifera 10%;* ***EX:*** *M. oleifera 20%;* ***PC:*** *Positive Control;* ***NC:*** *Negative Control. Mean±SD along with the same superscripts have a non-significant difference (P>0.05) from each other*

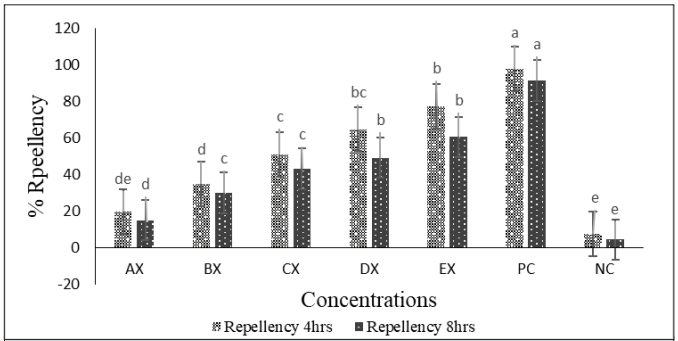

Fig 4. Effect of different concentrations of *M. oleifera* on repellency of *R. microplus* ticks after 4 and 8 h interval. AX: *M. oleifera* 1.25%; BX: *M. oleifera* 2.5%; CX: *M. oleifera* 5%; DX: *M. oleifera* 10%; EX: *M. oleifera* 20%; PC: Positive Control; NC: Negative Control. Mean±SD along with the same superscripts have a non-significant difference (P>0.05) from each other

revealed that the highest repellencies 77.4 and 60.49% were observed after the 4th and 8th h of treatment respectively when a 20% concentration of the extract was used.

Discussion

Livestock have significant economic importance worldwide. Various synthetic chemical drugs have been used to control *R. microplus* but the continuous, frequent, misuse of these drugs has led to the development of *R. microplus* resistance [11]. Scientists are moving towards alternate sources such as vaccination, entomopathogenic fungi, nanoparticles, and botanicals [32]. Due to their pharmacological properties, botanicals have been extensively used as antioxidants, antibacterials, antivirals, antifungals, and antiparasitic [33-35]. Research on plant extracts revealed their insecticidal and acaricidal potential and they could be used to control ticks as an alternative replacement for synthetic compounds [36-38].

M. oleifera plant is widely distributed and mostly present in South Asian countries like Pakistan, India, and Bangladesh. In different countries, it is often used for medicinal purposes, mainly as a laxative and analgesic. These properties are due to the presence of tannins, phenolics, flavonoids, starch, carbohydrates, fats, vitamins, alkaloids, and minerals [39]. The present study was conducted to evaluate the *in vitro* acaricidal and repellent potential of ethyl acetate extract of *M. oleifera* seeds on *R. microplus* engorged females and larvae. Adult and larval immersion tests were carried out against *R. microplus* ticks and results showed maximum adult (70%) and larval mortality (73%) at 20% concentration. The LC_{90} values were also calculated which showed that 6.246, 3.721, and 0.702% values are toxic at 6, 12, and 24 h for adult tick mortality. The present study was in line with the same study investigated by Radwan et al.[40] that demonstrated the acaricidal potential of *Aloe vera* and *Rheum rhabarbarum* against *R. microplus*. A similar study was conducted by Alborzi et al.[41], in which the acaricidal potential of *M. oleifera* against *Hyalomma dromedarii* ticks was evaluated. The results also showed that the hydroethanolic extract of *M. oleifera* caused the mortality of adults and larvae and reduced the ability of ticks to lay eggs.

Our study also revealed the detrimental effects of ethyl acetate extract of *M. oleifera* seeds against reproductive parameters. It is evident by decreased egg-laying ability, egg weight, and reproductive index. Egg hatchability and

reproductive efficiency index have also been reduced. This decrease in reproductive parameters is associated with the presence of phytochemicals in *M. oleifera*. These phytochemicals act synergistically on the salivary and reproductive glands of ticks and act as antitick agents [42]. Different studies confirmed the presence of a variety of phenolics and flavonoids in *M. oleifera* which include syringic acid, caffeic acid, p-coumaric acid, benzoic acid, vallic acid, gallic acid, luteolin, galanin, quercetin, and kamferol. Bustos-Baena et al.[43] confirmed that caffeic acid and vallic acid obtained by *Randia aculeata* had been found effective against *R. microplus* and they are involved in inhibiting the synthesis and functioning of different hormones critical for the reproduction process, leading to decreased egg production and decreased egg hatchability. Zhang et al.[44] investigated the potential of gallic acid against *Ectropis obliqua* and found its application as an anti-insect agent. In a similar study, Baz et al.[45] determined the fumigant and adulticidal effect of garlic, mustard, radish, and rosemary against insects and found them to be an anti-insect agent. The previous study also demonstrated that quercetin present in plant extracts can target specific proteins in insects and hinder their feeding activity [46]. The phenolics particularly quercetin in plant extracts can bind with different amino acids at specific sites and denature the protein structure, hence leading to the disintegration of cuticular membrane [47]. The previous studies also revealed that the phenolics such as vallic acid and ferulic acid caused oxidative stress by the production of superoxide free radicals which have deleterious effects on the growth and development of the insect [48]. In another study, it was confirmed that the application of the phenolics to the insects particularly in ticks produced oxidative stress markers (lipid peroxide, hydrogen peroxide, and superoxides) that are involved in producing oxidative stress and hence act as anti-tick agents. Guneidy et al.[49] confirmed the effects of phenolics, flavonoids, and tannins on tick cuticles and their penetration into the cell membranes to create vacuum and dissociation.

RC_{50} and RC_{90} values were also calculated which showed a repellent effect after 4 and 8 h treatment. The repellent activities of *M. oleifera* may be due to the chemicals that produce a vapor barrier around the insects and prevent the insects from touching or settling on the skin. The repellent effect of plant extracts decreases with time, as results show that it is higher 4 h after treatment and lower 8h after treatment. Based on the evidence provided it is justified that phytochemicals are responsible for anti-tick activities.

The results of the current study confirmed that the ethyl acetate extract of *M. oleifera* seeds has acaricidal and repelling effects against the *R. microplus* ticks. However, animal trials are advised before endorsing the use of this *M. oleifera* extract in practical application. Further study and better techniques are also required to determine the exact chemical composition of *M. oleifera*. In addition, better and improved techniques may increase the yield and residual life of plant extracts.

Declarations

Availability of Data and Material: Data may be available on demand if the reader requires it.

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Declaration of Generative Artificial Intelligence (AI):

It is declared that all data and content in this article are original. No AI tool has been used for any diagram, table, or figure in the article.

Author Contribution: RZA, MMM: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Writing - Original Draft, Writing - Review & Editing. RZA, AMAK, MM: Investigation, Writing - Review & Editing.

References

1. Sultan S, Zeb J, Ayaz S, Rehman SU, Khan S, Hussain M, Senbill H, Husain S, Sparagano OA: Epidemiologic profile of hard ticks and molecular characterization of *Rhipicephalus microplus* infesting cattle in central part of Khyber Pakhtunkhwa, Pakistan. *Parasitol Res,* 121 (9): 2481-2493, 2022. DOI: 10.1007/s00436-022-07596-3

2. Zerek A, Erdem I, Yaman M, Altuğ ME, Orkun Ö: Ixodid ticks (Ixodoidea: Ixodidae) infesting wild animals in Hatay, Türkiye. *Kafkas Univ Vet Fak Derg,* 29 (6): 641-647, 2023. DOI: 10.9775/kvfd.2023.30132

3. Zeb J, Shams S, Ayaz S, Din IU, Khan A, Adil N, Ullah H, Raza A: Epidemiology of ticks and molecular characterization of *Rhipicephalus microplus* in cattle population in North-Western Pakistan. *Int J Acarol,* 46 (5): 335-343, 2020. DOI: 10.1080/01647954.2020.1775294

4. Diarra AZ, Kelly P, Davoust B, Parola P: Tick-borne diseases of humans and animals in West Africa. *Pathogens,* 12 (11): 1276, 2023. DOI: 10.3390/pathogens12111276

5. Ali A, Khan MA, Zahid H, Yaseen PM, Qayash Khan M, Nawab J, Ur Rehman Z, Ateeq M, Khan S, Ibrahim M: Seasonal dynamics, record of ticks infesting humans, wild and domestic animals and molecular phylogeny of *Rhipicephalus microplus* in Khyber Pakhtunkhwa Pakistan. *Front Physiol,* 10:793, 2019. DOI: 10.3389/fphys.2019.00793

6. Khan AMA, Wei CR, Asghar S, Saeed Z, Akram MS, Idrees H, Azhar R, Sohail M: Seroprevalence, distribution pattern and control of Crimean Congo Hemorrhagic fever (CCHF) with its risk factors in Pakistan and neighboring countries. **In,** Aguilar-Marcelino L, Zafar MA, Abbas RZ, Khan A (Eds): Zoonosis. Vol, 3, Unique Scientific Publishers, Faisalabad, Pakistan. 230-239, 2023.

7. Martins KR, Garcia MV, Bonatte-Junior P, Duarte PO, de Higa LOS, Csordas BG, Barros JC, Andreotti R: Correlation between *Rhipicephalus*

microplus ticks and *Anaplasma marginale* infection in various cattle breeds in Brazil. *Exp Appl Acarol,* 81 (4): 585-598, 2020. DOI: 10.1007/s10493-020-00514-1

8. De León AAP, Mitchell Iii RD, Watson DW: Ectoparasites of cattle. *Vet Clin North Am Food Anim Pract,* 36 (1): 173-185, 2020. DOI: 10.1016/j.cvfa.2019.12.004

9. Selles SMA, Kouidri M, González MG, González J, Sánchez M, González-Coloma A, Sanchis J, Elhachimi L, Olmeda AS, Tercero JM: Acaricidal and repellent effects of essential oils against ticks: A review. *Pathogens,* 10 (11): 1379, 2021. DOI: 10.3390/pathogens10111379

10. Rahman A, Kashif M, Nasir A, Idrees A, Jamil M, Ehsan M, Sana MA: A review of tick and tick control strategies in Pakistan. *Pakistan J Med Health Sci,* 16, 652-655, 2022. DOI: 10.53350/pjmhs22161652

11. Abbas RZ, Zaman MA, Colwell DD, Gilleard J, Iqbal Z: Acaricide resistance in cattle ticks and approaches to its management: The state of play. *Vet Parasitol,* 203 (1-2): 6-20, 2014. DOI: 10.1016/j.vetpar.2014.03.006

12. Cossio-Bayugar R, Martinez-Ibañez F, Aguilar-Diaz H, Miranda-Miranda E: Relationship between acaricide resistance and acetyl-cholinesterase gene polymorphisms in the cattle tick *Rhipicephalus microplus. Parasite,* 31:3 2024. DOI: 10.1051/parasite/2024003

13. Rani L, Thapa K, Kanojia N, Sharma N, Singh S, Grewal AS, Srivastav AL, Kaushal J: An extensive review on the consequences of chemical pesticides on human health and environment. *J Clean Prod,* 283:124657, 2021. https://doi.org/10.1016/j.jclepro.2020.124657

14. Nath S, Mandal S, Pal S, Jadhao S, Ottalwar N, Sanyal P: Impact and management of acaricide resistance-pertaining to sustainable control of ticks. *Int J Livest Res,* 8:46, 2018.

15. Mehnaz S, Abbas RZ, Kanchev K, Rafique MN, Aslam MA, Bilal M, Ather AS, Zahid A, Batool T: Natural control perspectives of *Dermanyssus gallinae* in poultry. *Int J Agri Biosci,* 12 (3): 136-142, 2023. DOI: 10.47278/journal.ijab/2023.056

16. Akbar M, Aleem K, Sandhu K, Shamoon F, Fatima T, Ehsan M, Shaukat F: A mini-review on insect pests of wheat and their management strategies. *Int J Agri Biosci,* 12 (2): 110-115, 2023. DOI: 10.47278/journal.ijab/2023.052

17. Al-Hoshani N, Zaman MA, Syaad KMA, Salman M, Rehman TU, Olmeda AS: Assessment of repellency and acaricidal potential of *Nigella sativa* essential oil using *Rhipicephalus microplus* ticks. *Pak Vet J,* 43 (3): 606-610, 2023. DOI: 10.29261/pakvetj/2023.054

18. Khan MK, Imran M: Parasite control practices used by horse owners in Punjab, Pakistan. *Int J Agri Biosci,* 12 (4): 257-261, 2023. DOI: 10.47278/journal.ijab/2023.073

19. Salman M, Abbas RZ, Israr M, Abbas A, Mehmood K, Khan MK, Hussain R, Saleemi MK, Shah S: Repellent and acaricidal activity of essential oils and their components against *Rhipicephalus* ticks in cattle. *Vet Parasitol,* 283:109178, 2020. DOI: 10.1016/j.vetpar.2020.109178

20. Demir S, Karaalp C, Tabanca N, Bernier UR, Linthicum KJ: Evaluation of the repellent activity of 13 *Achillea* L. species from türkiye against the virus vector *Aedes aegypti* (L.) mosquitoes. *Kafkas Univ Vet Fak Derg,* 29 (1): 33-40, 2023. DOI: 10.9775/kvfd.2022.28409

21. Ahmad S, Humak F, Ahmad M, Altaf H, Qamar W, Hussain A, Ashraf U, Abbas RZ, Siddique A, Ashraf T: Phytochemicals as alternative anthelmintics against poultry parasites: A review. *Agrobiol Rec,* 12, 34-45, 2023. DOI: 10.47278/journal.abr/2023

22. Akter T, Rahman MA, Moni A, Apu MAI, Fariha A, Hannan MA, Uddin MJ: Prospects for the protective potential of *Moringa oleifera* against kidney diseases. *Plants,* 10 (12):2818, 2021. DOI: 10.3390/plants10122818

23. Padayachee B, Baijnath H: An updated comprehensive review of the medicinal, phytochemical, and pharmacological properties of *Moringa oleifera. S Afr J Botany,* 129, 304-316, 2020. DOI: 10.1016/j.sajb.2019.08.021

24. Singh S, Dubey S, Rana N: Phytochemistry and pharmacological profile of drumstick tree *"Moringa oleifera* Lam": An overview. *Curr Res Nutr Food Sci,* 19 (5): 529-548, 2023. DOI: 10.2174/1573401319666221226144613

25. Walker JB, Keirans JE, Horak IG: The Genus Rhipicephalus (Acari, Ixodidae): A Guide to the Brown Ticks of the World. Cambridge University Press, 2000.

26. Estrada-Peña A, Bouattour A, Camicas JL, Walker AR: Ticks of Domestic Animals in the Mediterranean Region. University of Zaragoza, Spain, 131, 2004.

27. Stendel W: The relevance of different test methods for the evaluation of tick-controlling substances. *J S Afr Vet Assoc,* 51 (3): 147-152, 1980. DOI: 10520/AJA00382809_3176

28. Islam S, Talukder S, Ferdous J, Hasan MM, Sarker YA, Sachi S, Alim MA, Sikder MH: *In-vitro* efficacy of verenda *(Ricinus communis)* leaves extract against ticks in cattle. *Bangl J Vet Med,* 16 (1): 81-86, 2018. DOI: 10.3329/bjvm.v16i1.37380

29. Sabatini GA, Kemp DH, Hughes S, Nari A, Hansen J: Tests to determine LC50 and discriminating doses for macrocyclic lactones against the cattle tick, *Boophilus microplus. Vet Parasitol,* 95 (1): 53-62, 2001. DOI: 10.1016/S0304-4017(00)00406-4

30. Fao R: Management and integrated parasite control in ruminants guidelines, module 1. Ticks: Acaricide resistance: Diagnosis, management and prevention, food and agriculture organization. Animal Production and Health Division, Rome, Italy, 2004.

31. Carroll JF, Solberg VB, Klun JA, Kramer M, Debboun M: Comparative activity of deet and AI3-37220 repellents against the ticks *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) in laboratory bioassays. *J Med Entomol,* 41 (2): 249-254, 2004. DOI: 10.1603/0022-2585-41.2.249

32. Khan AMA, Asrar R, Shrafat H, Qamar MH, Ahmad S, Kauser M, Aleem MT: Rationale to develop mRNA-based vaccines for *Trypanosoma brucei* (a review). *Continental Vet J,* 3(1):26-35, 2023.

33. Saleh M, Ramadan M, Elmadawy R, Morsi M, El-Akabawy L: The efficacy of alcoholic extracts of *Morus macroura* (mulberries), *Lepidium sativum* (garden cress seeds) and diclazuril against *E. stiedae* in experimentally infected rabbits. *Int J Vet Sci,* 12 (6): 869-878, 2023. DOI: 10.47278/journal.ijvs/2023.049

34. Ghazy TA, Sayed GM, Farghaly DS, Arafa MI, Abou-El-Nour BM, Sadek AM: *In vitro* antiprotozoal effect of alcoholic extract of hemolymph of *Galleria mellonella* larva against *Trichomonas gallinae. Int J Vety Sci,* 12 (3): 302-308, 2023. DOI: 10.47278/journal.ijvs/2022.192

35. Rashid MHU, Mehwish WH, Ahmad S, Ali L, Ahmad N, Ali M, Fazal H: Unraveling the combinational approach for the antibacterial efficacy against infectious pathogens using the herbal extracts of the leaves of *Dodonaea viscosa* and fruits of *Rubus fruticosus. Agrobiol Rec,* 16, 57-66. 2024. DOI: 10.47278/journal.abr/2024.012

36. Naseer MU, Sindhu ZuD, Iqbal Z, Aslam B 2: *In vitro* efficacy of *Areca catechu* against cypermethrin resistant *Rhipicephalus microplus* and its phytochemical analysis. *Pak Vet J,* 42 (3): 414-418. DOI: 10.29261/pakvetj/2022.053

37. Eltaly RI, Baz MM, Radwan IT, Yousif M, Abosalem HS, Selim A, Taie HAA, Farag AAG, Khater HF: Novel acaricidal activity of *Vitex castus* and *Zingiber officinale* extracts against the camel tick, *Hyalomma dromedarii. Int J Vet Sci,* 12 (2): 255-259, 2023. DOI: 10.47278/journal.ijvs/2022.184

38. Baz MM, Alfagham AT, Al-Shuraym LA, Moharam AF: Efficacy and comparative toxicity of phytochemical compounds extracted from aromatic perennial trees and herbs against vector-borne *Culex pipiens* (Diptera: Culicidae) and *Hyalomma dromedarii* (acari: Ixodidae) as green insecticides. *Pak Vet J,* 44 (1): 55-62, 2024. DOI: 10.29261/pakvetj/2024.144

39. Zaman MA, Iqbal Z, Abbas RZ, Khan MN, Muhammad G, Younus M, Ahmed S: *In vitro* and *in vivo* acaricidal activity of a herbal extract. *Vet Parasitol,* 186 (3-4): 431-436, 2012. DOI: 10.1016/j.vetpar.2011.11.018

40. Radwan IT, Eltaly RI, Baz MM, Yousif M, Selim A, Taie HAA, Manaa EA, Khater HF: Novel acaricidal and growth-regulating activity of *Aloe vera* and *Rheum rhabarbarum* extracts and their oil/water nanoemulsions against the camel tick, *Hyalomma dromedarii. Sci Rep,* 13 (1): 16802, 2023. DOI: 10.1038/s41598-023-43776-6

41. Alborzi A, Larki S, Jamshidian Ghale Sefidi J, Khodadad H: Anti-tick effect of aqueous, aqueous-alcoholic and alcoholic extracts of *Moringa olifera* on hatching eggs process and the growth of *Hyalomma anatolicum anatolicum* larvae by immersion test method. *Vet Res Biol Prod,* 36 (2): 22-31, 2023. DOI: 10.22092/vj.2022.358543.1969

42. Khater HF, Bazh EKA, Gawad SA, Selim A, Taie HAA, Radwan IT, Moustafa SA, Megahed AA, Baz MM, Kandeel SA: Acaricidal efficacy of thirty-five Egyptian plants against the camel tick, *Hyalomma dromedarii. Acta Parasitol,* 69, 1231-1243, 2024. DOI: 10.1007/s11686-024-00801-6

43. Bustos-Baena AS, Bravo-Ramos JL, Romero-Salas D, Sánchez-Montes S, Ortiz-Carbajal LA, Sánchez-Otero MG: *In vitro* and *In silico* studies of the acaricidal and anticholinesterase activities of *Randia aculeata* seeds against the southern cattle tick *Rhipicephalus (Boophilus) microplus. Rev Bras Parasitol Vet,* 33 (2):e001524, 2024.

44. Zhang X, Ran W, Li X, Zhang J, Ye M, Lin S, Liu M, Sun X: Exogenous application of gallic acid induces the direct defense of tea plant against *Ectropis obliqua* Caterpillars. *Front Plant Sci,* 13:833489, 2022. DOI: 10.3389/fpls.2022.833489

45. Baz MM, Eltaly RI, Debboun M, Selim A, Radwan IT, Ahmed N, Khater HF: The contact/fumigant adulticidal effect of Egyptian oils against the house fly, *Musca domestica* (Diptera: Muscidae). *Int J Vety Sci,* 12 (2): 192-198, 2023. DOI: 10.47278/journal.ijvs/2022.180

46. Ratajac R, Pavlićević A, Petrović J, Stojanov I, Orčić D, Štrbac F, Simin N: *In vitro* evaluation of acaricidal efficacy of selected essential oils against *Dermanyssus gallinae. Pak Vet J*, 2023. DOI: 10.29261/pakvetj/2023.123

47. Ravindran R, Chithra ND, Deepa PE, Ajithkumar KG, Chandrasekhar L, Sreelekha K, Nair SN, Juliet S, Ghosh S: *In vitro* effects of caffeic acid, nortriptyline, precocene I and quercetin against *Rhipicephalus annulatus* (Acari: Ixodidae). *Experimental and Applied Acarology,* 71 (2): 183-193, 2017. DOI: 10.1007/s10493-017-0105-2

48. Kruk J, Aboul-Enein BH, Duchnik E, Marchlewicz M: Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise. *J Physiol Sci,* 72 (1): 19, 2022. DOI: 10.1186/s12576-022-00845-1

49. Guneidy RA, Amer MA, Hakim AEE, Abdel-Shafy S, Allam SA: Effect of polyphenols extracted from *Punica granatum* and *Acacia saligna* plants on glutathione S-transferase of the cattle tick *Rhipicephalus (Boophilus) annulatus* (Acari: Ixodidae). *J Parasit Dis,* 45, 524-538, 2021. DOI: 10.1007/s12639-020-01323-4