

Research Article

In Vitro Acaricidal Effect Of Neem Leaves (*Azadirachta Indica*) and *Citrullus Colocynthis* Extracts against the Camel Ticks, *Hyalomma Dromedarii* (Acari:Ixodidae)

Osman M. Mahran¹, Ahmed A. Wahba² and Mogda K. Mansour ^{2*}

¹Department of Parasitology, Animal Health Research Institute, ARC, Giza, Egypt

²Department of Biochemistry, Animal Health Research Institute, ARC, Giza, Egypt

*Corresponding author: Mansour KM, Department of Biochemistry, Animal Health Research Institute, ARC, Giza, Egypt, E-mail: hingabuh77@gmail.com

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Abstract

The present study aimed to screening the acaricidal potential of methanolic extract of neem leaves and different concentrations of Citrullus colocynthis extract against adult females, eggs hatchability and larvae of camel ticks, Hyalomma dromedarii (H. dromedarii). in vitro using immersion method. Acaricidal activity of each plant (timemortality and failure hatchability experiment) was evaluated at three different exposure times 10, 20 and 30 minutes at 1st, 2nd, 3rd 7th and 15th day post treatment (DPT). The results of control on engorged female H. dromedarii ticks with methanolic extract of neem leaves showed that it had an acaricide activity, induced a high increase in mortality rates from 1st to 15th DPT (up to 100%), with some changes in morphology. Also, there was a high effect of methanolic extract of neem leaves on hatchability of H. dromedarii eggs (100%) from 1st up to 15th DPT. For the newly hatched larvae the present study demonstrated that methanolic extract of neem leaves induced 100% mortality on the newly hatched larvae of H. dromedarii tick at 1st and 2nd DPT. On the other hand, Citrullus colocynthis extract induced moderate or lowest acaricidal efficacy on adult H. dromedarii ticks, eggs hatchability and newly hatched larvae. Also, variation had been recorded in acaricidal effect between the different concentrations of Citrullus colocynthis, where the methanolic extracts of whole fruits, seeds and surface fruits resulted in moderate acaricidal effects on adults, larval mortality and eggs hatchability respectively. On the other hand, water extract concentrations had low acricidal effect. This study implied that the acaricidal effect of methanolic extract of neem leaves was found to be more effective and could be economically used for controlling H. dromedarii ticks than Citrullus colocynthis extract.

Keywords Camel; Acaricidal activity; Neem leaves extract; *Citrullus Colocynthis* extract; *Hyalomma dromedarii*

Introduction

The camel ticks, Hyalomma dromedarii is one of the most important ectoparasites of camels and is widely distributed in tropical and subtropical regions including Egypt. Hyalomma dromedarii causes huge economic loss in camel production by reducing weight gain, milk production and causing tick worry, blood loss, tick toxicosis and tick paralysis. Furthermore, ticks involved in transmission of some bacterial, viral, protozoal and rickettsial diseases [1]. Keeping in view the impact of ticks and tick borne diseases on the individual and national economics, the developing world should focus on ticks control on priority basis [2]. In present days synthetic chemicals are used to control tick infestations. By its wider applications, ticks developed resistance to most of the acaricides [3]. Also, it causes problems of potential residues of synthetic acaricides in milk, meat and other animal products [4]. Thus, there is an urgent need for alternative parasitic control strategies to overcome the drawbacks associated with the use of synthetic acaricides [5]. However, the use of non-chemical control methods including the use of plant-borne products containing acaricidal compounds had also been proposed by Pavela et al., [6] to curtail the environmental and economic impact of synthetic acaricides. Natural products offer a cheap alternative to synthetic acaricides [7]. One of the commonly cited advantages that may result from the use of botanicals for tick control is their degradability [8]. Therefore, the aim

of this study was to evaluate the acricidal efficacy of neem leaves and *Citrullus colocynthis* extracts on engorged adult females, eggs hatchability and larvae of *Hyalomma dromedarii* ticks.

Materials and Methods

Study design:

This investigation employed an experimental study design in a laboratory based in vitro acaricidal activity test of methanolic extract of neem leaves and different concentrations of *Citrullus colocynthis* using in vitro immersion method (IM) described by Drummond et al., [9].

Plant preparation and extraction

Neem extract, Kumar et al. [10]: Neem leaves plant were washing and cutting into small pieces, spread out on paper sheets, dried at room temperature for one week and crushed using a pestle and mortar. About 100 gm the solvent (80 ml of methanol) was added to a round bottom flask of a soxhlet extractor. The crushed plant materials were loaded into the thimble and placed inside the soxhlet extractor. The process runs for a total of 16 hours. The extract was taken out, filtered over anhydrous sodium sulphate to remove traces of alcohol and evaporator to concentrate until use. *Citrullus colocynthis*, **Tabassum et al.** [11]: *Citrullus colocynthis* plants were dried in dry and cold place at room temperature to prepare methanolic aqueous extracts .Dried powdered plant was extracted with methanol-aqueous (70:30) solvent for about 72 h. After filtration, new solvents were added and the procedure was repeated for three times. Crude extract was obtained after the evaporation of solvent in evaporator to concentrate until use in different concentration.

Collection of H. dromedarii ticks

Engorged females of H. dromedarii ticks with body lengths greater than 5 mm were collected from the skin of thigh, abdomen, perennial area, ear and forelegs of naturally infested camels (5-15 years old) from El-Basteen and El-Warrak slaughter houses, Giza governorate. Ticks were examined under stereomicroscope and identified according to taxonomic key described by Kaiser [12]. No information on prior parasitic treatment was obtained .Engorged ticks were placed in cardboard containers with perforated lids to allow ventilation and then transported to the laboratory. Some of the ticks were used immediately and other ticks were maintained in an incubator at 27-28°C and 70-80 % RH to allow egg laying and egg hatching.

Evaluation of acaricidal effect of methanolic extract of neem leaves on adult H. dromedarii ticks

The anti-acaricidal activity of methanolic extract of neem leaves against engorged H. dromedarii ticks was determined in vitro using immersion method (IM). The design was completely randomized with four groups, each one contained 10 engorged female ticks. Ticks in the 1st, 2nd and 3rd groups were placed in a mesh cloth piece and immersed in methanolic extract of neem leaves for 10, 20, and 30 minutes respectively. The 4th group left without treatment as a control one. Test solution was decanted and ticks were dried on filter paper. All groups were placed in sterile Petri dishes and incubated at 27-28°C and 70-80 % RH. The number of alive and dead ticks counted during the whole time of test post treatment. Ticks were considered alive if they exhibited normal behavior when pressed upon or physically stimulated with wooden dowels. Ticks which were incapable of movement, maintaining normal posture, leg coordination and ability to right themselves or any signs of life were considered moribund or dead according to Khater and Ramadan [13]. At the end of lying period (15 day), the acaricidal efficacy of each treatment was calculated using the following equation recorded by Wang et al., [14].

$$AE = \frac{(B-T)}{B} \times 100$$

AE: The acaricidal efficacy

B: The mean number of surviving ticks in the untreated control

T: The mean number of surviving ticks in treated group.

Morphological changes of *H. dromedarii* ticks were determined following exposure of adults to methanolic extract of neem leaves.

Evaluation of acaricidal effect of methanolic extract of neem leaves on H. dromedarii tick eggs hatchability

Following oviposition, four groups of 12 day-old eggs were weighed each of 1.0 gm. Eggs in the 1st, 2nd, and 3rd groups were placed in filter paper envelopes and immersed in methanolic extract of neem leaves for 10, 20 and 30 minutes respectively. The 4th group left as a control one. Test solution was decanted and eggs were dried on filter paper. All groups were placed into glass tubes and maintained in the incubator at 27-28°C and 70-80% RH. Hatchability of eggs was recorded during the whole time of test. The tubes containing larvae and/or eggs were placed in a temperature controlled chamber at 40 °C for 24 h to kill the hatched larvae to facilitate the counting. The hatchability for each sample was determined according to the equation recorded by Bicalhoet et al., [15].

 $Hatchability \% = \frac{No.of hatched eggs}{No.of unhatched eggs + No.of larvae} X 100$

Evaluation of acaricidal effect of methanolic extract of neem leaves on *H. dromedarii* tick larvae

For the assays with the larvae, the larval packet test (LPT) was used [16]. The larvae were obtained only from engorged female ticks that weighed between 160 and 300 mg, because this is considered the optimal weight range for obtaining viable eggs [17]. Each engorged female tick was placed in a clean glass tube plugged with cotton wool and incubated at 27-28 °C and 70-80 % RH The eggs laid in each tube were transferred into a clean tube with cotton plug. The eggs were kept under the same incubating conditions until they hatched into larvae. About 400 larvae were used, with ages between 14 and 21 days. The larvae were divided into four replicates. The larvae in 1st, 2nd and 3rd replicates, were put in a filter paper enveloped (2 cm×2 cm) and immersed in test solutions at different times (10, 20 and 30 min.). The 4th group left without treatment as a control, the treated groups and control were incubated in the same conditions described above. The readings were recorded with magnifying glass during the whole period of test separating alive from dead larvae. All larvae that showed no movement were considered dead. Mortality rate was calculated according to the following equation

 $Mortality rate = \frac{No.dead \ larvae}{No.of \ dead \ larvae + No.of \ alive \ larvae} X \ 100$

Evaluation of Citrullus colocynthis on adult H. dromedarii ticks

Nine concentrations from *Citrullus colocynthis* were evaluated against adult *H. dromedarii* ticks. The concentrations were from seeds (20% methanolic extract and 20% water extract), from surface (10%, 20% methanolic extract and 20% water extract) and from the whole fruit (20%, 40% methanolic extract and 20% and 40% water extract). All treatments were tested in three replicates (10, 20 and 30 minutes), where the control replicate left without treatment. For the bioassays with engorged females, the adult immersion test was used as described by Drummond et al., [9].

Evaluation of acaricidal effect of Citrullus colocynthis concentrations on H. dromedarii tick eggs hatchability

Following oviposition, nine lots of 0.001g of 12 day-old eggs were weighed and using three replicates for each extract concentration test .Control group was left without treatment. Eggs in the 1st, 2nd, and 3rd groups were placed in filter paper enveloped and was immersed in *citrullus colocynthis* extract concentrations for 10, 20 and 30 minutes respectively. Test solution was decanted and eggs were dried in filter paper. All treated replicates and controls were placed into glass tubes and maintained in the incubator at 28-28°C and

RH 70-80% the tubes containing larvae and/or eggs were left in a temperature controlled chamber at 40°C for 24 h to kill the hatched larvae and thus facilitate the counting according to Giglioti et al., [18]. The hatchability for each sample was determined according to the equation recorded by Bicalhoet et al., [15].

Hatchability
$$\% = \frac{\text{No.of hatched eggs}}{\text{No.of unhatched eggs} + \text{No.of larvae}} X 100$$

Evaluation of acaricide efficacy of Citrullus colocynthis concentration on H. dromedarii tick larvae

For the assays with larvae, the larval packet test (LPT) was used [16]. The larvae were obtained only from engorged female ticks that weighed between 160 and 300 mg, because this is considered the optimal weight range for obtaining viable eggs [17]. Each engorged female tick was placed in a clean plastic tube plugged with cotton wool and incubated at 27 - 28°C and RH 70 - 80%, to promote oviposition .The eggs laid in each tube were transferred into a clean tube with cotton plug and dead female ticks discarded. The eggs were kept under the same incubating conditions until they hatched and then the larvae starved in the incubator at 29°C for two weeks before use. About 400 larvae were used in each extract concentration test. The larvae were divided into four groups. Larvae in the 1st, 2nd and 3rd groups placed between two pieces of filter paper $(2 \times 2 \text{ cm})$ and immersed in the test solutions at different times, previously described for the assays with the engorged females and then closed to form packets, where the 4th group leaved without treatment as a control one. The treated packets and control were incubated in the same conditions described above. The readings were made with magnifying glass after incubation, separating live from dead larvae. All larvae that showed no movement were considered dead.

Results

Effect of methanolic extract of neem leaves on adult H. dromedarii ticks

The mortality rate of methanolic extract (ME) of neem leaves on adult *H. dromedarii* ticks was increased by increasing time (Table 1). It ranged from 10-25%, 30-40%, 45-55%, 60-85% and 90%-100% at 1st, 2nd, 3rd, 7th and 15th DPT respectively in 10 to 30 min. immersion time versus 1 % in the control group at the termination of experiment.

Effect of methanolic extract of neem leaves on H. dromedarii tick eggs hatchability

The hatching rate was determined by counting the number of larvae and the remaining unhatched eggs in representative samples. The results indicated that there was high effect of ME of neem leaves on hatchability of *H. dromedarii* eggs. This effect was observed from the 1st up to 15th DPT, where there was no hatching of eggs in 10 to 30 min. immersion time. In control group the hatchability increased from 0% at 1st DPT to 98% at 15th DPT (Table 2).

Effect of methanolic extract of neem leaves on H. dromedarii larvae

Table (3) showed that the methanolic extract of neem leaves induced high mortality rates of newly hatched larvae at the 1st up to 2nd DPT. The mortality rates increased as the time of application increased. It reached 60 - 85% and 90 - 100% at 1st and 2nd DPT respectively in times of 10 to 30 min. immersion time in comparison to 8% only in control group at termination of experiment.

Effect of nine concentrations of *Citrullus colocynthis* extract on adult *H. dromedarii ticks*

Table (4) showed that, mortality rate was 0% at 1st till 3rd DPT. It was 6% and 25% at 7th and 15th DPT respectively in 30 min. immersion time in 10% in mrethanolic extract (ME) of *Citrullus colocynthis* seeds. In case of water extract (WE) of seeds, it reached 5% at 15th DPT in 30 min. immersion time. In case of 10% ME of surface concentrations of Citrullus colocynthis, the mortality rate was 4% in 30 min. immersion times, while it was 3% and 20% at 7th and 15th DPT in the same immersion time.

In case of 20% WE, mortality rates reached 1% and 8% at 7th and 15th DPT in 30 min. immersion time. In case of whole fruit (seeds and surface) of *Citrullus colocynthis* concentrations, the mortality rates were 8% and 30% at 7th and 15th DPT respectively in case of 20% ME in 30 min. immersion times, while the mortalities were 5% and 40% in case of 40% ME of whole fruit in the same immersion time. In case of 20 and 40% WE of whole fruit, the mortalities were 8% and 10% respectively at 15th DPT in 30 min. immersion time. In control group the mortality rate was 0% till 7th DPT and reached 1% only at the termination of experiment (15th DPT).

Effect of nine concentrations of Citrullus colocynthis on hatchability of H. dromedarii tick eggs

Table (5) showed that the hatching rates reached % - 15%, 20%- 40% and 50% - 75% at 3rd, 7th and 15th DPT respectively in case of 10% ME of *Citrullus colocynthis* seeds in 10 to 30 min. immersion time. In case of 20% WE of seeds, mortality rate was 80% at 15th DPT in 30 min. immersion time.

In case of 10% and 20% ME of surface concentration, the hatchability rates were 70% and 60% in 30 min. immersion time at 15th DPT. In 20% WE, the hatchability rate was 80% at 15th DPT in the same immersion time. In case of 20% ME of whole fruit, the hatchability rate was 50% at 15th DPT in 30 min. immersion time, while it was 5% and 40% at 7th and 15th DPT with 40% ME of whole fruit at the same immersion time.

In case of 20% and 40% WE of whole fruit, the hatchability rates were 70% and 80% respectively at 15th DPT in 30 min. immersion time. In control group, the mortality rate reached 95 % at the termination of experiment.

Effect of nine concentrations of *Citrullus colocynthis* on newly hatched *H. dromedarii* larvae

Table (6) showed that the hatching rates reached 1% - 4%, 5% - 10% and 22% - 40% at 3rd, 7th and 15th DPT respectively in case of 10% ME of *Citrullus colocynthis* seeds in 10 to 30 min. immersion time. In case of 20% WE of seeds, mortality rate was 25% at 15th DPT in 30 min. immersion time.

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In case of 10% and 20% ME of Citrullus colocynthis surface concentration, the hatchability rates were 35% and 45% respectively at 15th DPT in 30 min. immersion time, while, in case of 20 % WE, the hatchability rate was 20% at 15th DPT in the same immersion time.

time. In control group, the mortality rate was 6% at the termination of experiment.

Morphological changes of H. dromedarii ticks

In case of 20% ME of whole fruit, the hatchability rate was 48% at 15th DPT in 30 min. immersion time, while they were 5% and 55% in case of 40% ME of whole fruit at 7th and 15th DPT respectively at the same immersion time.

In case of 20% and 40% WE of whole fruit, the hatchability rates were 25% and 30% respectively at 15th DPT in 30 min. immersion

In the present study, no morphological changes were observed in control group, but, on the other hand, morphological changes in dromedarii ticks were observed after treatment with methanolic extract of neem leaves. As an external shrinkage and a slight reduction in the hardness of dorsal shield (scutum).

DPT	1st			2nd			3rd			7th			15th		
Immersion time (min.)	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Mortality (%)	10	15	25	30	35	40	45	50	55	60	70	85	90	95	100
Control (%)	0			0			0			0			1		
DPT: Days post treatment															
	10 15 25														

Table 1: Effect of methanolic extract of neem leaves on adult H. dromedarii ticks.

DPT	1st			2nd			3rd			7th			15th		
Immersion time (min.)	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Hatchability (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control (%)	0			10			25			50			98		
DPT: Days post treatment									1						

Table 2: Effect of methanolic extract of neem leaves on eggs hatchability of H. dromedarii ticks.

DPT	1st	1st			2nd					7th			15th		
Immersion time (min.)	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Mortality (%)	60	75	85	90	95	100	-	-	-	-	-	-	-	-	-
Control (%)	0			0			0			0			8		
DPT ⁻ Days post treatment															

'I: Days post treatment

Table 3: Mortality rates of methanolic extract of neem leaves on newly hatched larvae of H. dromedarii ticks.

DPT		1st			2nd			3rd			7th			15th		
Immersion time (min.)		10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Seeds	10 % ME	0	0	0	0	0	0	0	0	0	0	0	6	10	15	25
	20 % WE	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5
Surface	10 % ME	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4
	20 % ME	0	0	0	0	0	0	0	0	0	0	0	3	5	15	20
	20 % WE	0	0	0	0	0	0	0	0	0	0	0	1	4	6	8
Seeds & surface	20 % ME	0	0	0	0	0	0	0	0	0	0	1	8	10	12	30

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	40 % ME	0	0	0	0	0	0	0	0	0	2	3	5	10	20	40
	20 % WE	0	0	0	0	0	0	0	0	0	0	0	0	1	2	8
	40 % WE	0	0	0	0	0	0	0	0	0	0	0	0	1	2	10
Control %		0			0			0			0			1		
ME: Methanolic extract WE: Water extract	1	-		1				1								

Table 4: Mortality rates of nine concentrations of Citrullus colocynthis on adult H. dromedarii ticks.
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DPT		1st			2nd			3rd			7th			15th		
Immersion time (min.)		10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Seeds	10 % ME	0	0	0	0	0	0	8	10	15	20	35	40	50	65	75
	20 % WE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	80
Surface	10 % ME	0	0	0	0	0	0	0	0	0	0	0	0	0	2	70
	20 % ME	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60
	20 % WE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	80
Seeds & surface	20 % ME	0	0	0	0	0	0	0	0	0	0	0	0	0	12	50
	40 % ME	0	0	0	0	0	0	0	0	0	0	3	5	10	20	40
	20 % WE	0	0	0	0	0	0	0	0	0	0	0	0	0	2	70
	40 % WE	0	0	0	0	0	0	0	0	0	0	0	0	0	2	80
		0			2			20			50			95		

Table 5: Effect of nine concentrations of Citrullus colocynthis on eggs hatchability of H. dromedarii ticks.

A further reduction in the hardness of scutum was observed after increasing time of treatment with methanolic extract of neem leaves as and also a complete shrinkage of the whole body).but in case of *Citrullus colocynthis* concentration extract little morphological changes were observed only in methanol extract concentration.

DPT		1st			2nd			3rd			7th			15th		
Immersion time (min.)		10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Seeds	10 % ME	0	0	0	0	0	0	1	2	4	5	8	10	22	30	40
	20 % WE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25
Surface	10 % ME	0	0	0	0	0	0	0	0	0	0	0	0	0	2	35
	20 % ME	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45
	20 % WE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20
Seeds & surface	20 % ME	0	0	0	0	0	0	0	0	0	0	0	0	0	12	48
	40 % ME	0	0	0	0	0	0	0	0	0	0	3	5	10	20	55
	20 % WE	0	0	0	0	0	0	0	0	0	0	0	0	5	8	25

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	40 % WE	0	0	0	0	0	0	0	0	0	0	0	0	0	10	30
Control %		0												6		
ME: Methanolic extract			_				-	-	-				-			
NE: Water extract																
		1		-									,			

Table 6: Mortality rates of nine concentrations of Citrullus colocynthis on newly hatched larvae of H. dromedarii ticks.

Discussion

In the past decades, the control of ticks faced some major issues, such as the rapid development of resistance in targeted vectors and non-target effects on human health and the environment, due to the employ of synthetic acaricides and repellents. Plant products are a rich source of bioactive organic chemicals and offer an advantage over synthetic pesticides as these are less toxic, less prone to development of resistance and easily biodegradable. This study was performed to evaluate acaricidal activity of methanolic extract of neem leaves and different concentrations of Citrullus colocynthis extract against camel ticks (Hyalomma dromedarii). The present study showed that methanolic extract of neem leaves exhibited high acaricidal effect on adult ticks of Hyalomma dromedarii from the 1st day of application and continued up to 15th DPT resulting in 100 % mortality. Similar effects were observed by Abdel-Shafy and Zayed [19] and Landau et al., [20]. Likewise, high mortality rates on Amblyomma variegtum was observed at 100 %, 80 % and 20 % concentrations of nemm oil extract as recorded by Tamirat et al., [21]. In the present study no early hatching rate of larvae from the embryonated eggs of Hyalomma dromedarii ticks after treatment with methanolic extract of neem leaves from the 1st day of application up to 15th DPT. Similar results with very high effect of neem seeds extract on oviposition were recorded by Giglioti et al., [18], Rahul et al., [22] and Parte et al., [23]. The present study indicated that methanolic extract of neem leaves has high mortality rates against unfed larvae from 1st up to 2nd DPT (reached 100 %). Choudhury [24] and Dief Alla et al., (2009) recorded similar results of high mortality rates (100 %) on 3rd and 7th DPT with neem extract in controlling unfed larvae. Ismail et al., [25] reported that 40 % concentration of neem oil extract was very much effective against Rhipicephalus pulchellus larvae. Also, Choudhury [26] and Avinash et al., [27] recorded 100 % mortality of R. sanguinus larvae at 8, 6, 4, and 2 hrs after treatment with neem seeds oil at concentrations of 20%, 40%, 60% and 80% respectively. The mortality rates also increased by increasing time of application. Similar observations of the effect of neem oil extract on tick Amblyomma variegatum were studied by Ndumu et al., [28]. They observed that two main factors influenced the efficacy of neem extracts; time of exposure and quantity of oil in the extracts. The greater the exposure time to the oil and the higher the quantity of oil in the extract, the more effective the products were. Also, Ribeiro et al., [29] reported that variation had been found between different concentrations of herbal extracts and time taken for exerting toxic effects on ticks. In the present study, the time of exposure of female ticks, eggs and larvae to the extracts was differ, as 30 minutes was highly effective in comparison to 10 and 20 minutes of treatment.

In the present work, nine concentrations of Citrullus colocynthis extracts were assessed for their acaricidal activity against *Hyalomma dromedarii* ticks by estimating the percentage of adults and larval mortalities and eggs hatchability. The results showed that all nine

concentrations of Citrullus colocynthis extracts had moderate or lower acaricidal effects against adult, larval and embryonated H. dromedarii eggs. The present results were more or less similar with Shafiq et al., [30,31] whose recorded that lowest acaricidal efficacy of Citrullus colocynthis extract against Rhipicephalus microplus. On the other hand, Jeboury and Khalidy [32] recorded that the hot water extract of Citrullus colocynthis fruits against hard ticks causing paralysis at 6 minutes and dying in 26 minutes. Methanol extract concentrations resulted in moderate acaricidal effects on adult ticks and larval mortality and eggs hatchability, while water extract concentrations have low level of acricidal effect. Balan et al., [33] studied different solvent extracts from 67 plants against cattle ticks Rhipicephalus (Boophilus) microplus, who showed that ethanol extracts are the most commonly tested to control R. (B.) microplus, followed by methanol ones. The present study showed that the extract of whole plant of Citrullus colocynthis was more effective than seeds and surface. Abdul Rahuman et al., [34] recorded the same observation. The present study revealed that methanolic extract of neem leaves was highly effective in controlling H. dromedarii ticks in comparison to different concentrations Citrullus colocynthis extracts. Teka and Edilu [35,36] found that the crude extracts of neem plant had promising acaricidal properties and warrant further investigations.

Conclusion

The present study concluded that the effect of methanolic extract of neem leaves against *Hyalomma dromedarii* camel ticks are considered promising when compared with the effects of *Citrullus colocynthis* extract. Neem is a cheap natural source of insecticide that can play a high role in reducing the indiscriminate use of synthetic chemicals which are potentially dangerous to man and the ecosystem.

References

- 1. Jongejan F, Uilenberg G (2004) The global importance of ticks. Parasitology, 129: 3-14.
- Miller RJ, Davey RB, George JE (2007) First report of permethrin resistant Boophilus microplus (Acari: Ixodidae) collected within the United States. J. Med. Entomol. 44: 308-315.
- Ghosh S, Rawat AKS, Srivastava S, Rastogi S, Sharma AK, et al. (2013) Identification of plant extract for ecofriendly development of phytoacaricides for controlling chemical resistant tick infestations in animals. Biochem. Pharmacol, 2: 226.
- Tarbin J, Bygrave J, Bigwood A, Harday T, Rose D, et al. (2006) The effect of cooking on veterinary drug residues in food: Nicarbazin (Dinitrocarbanilide component). Food. Add. Contamin., 22: 1126 -1131.
- Abbas ARZ, Abbas JA, Khan Z, Iqbal MMH, Bhatti ZUD, et al. (2014) Integrated strategies for the control and prevention of dengue vectors with particular reference to Aedes aegypti. Pak. Vet. J 34: 1-10.
- 6. Pavela R, Canale A, Mehlhorn H , Benelli G (2016) Application of ethnobotanical repellents and acaricides in prevention: control and management of livestock ticks: a Review. Res. Vet. Sci., 109: 1-9.

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- 7. Habeeb SM (2010) Ethno-veterinary and medical knowledge of crude plant extracts and its methods of application (traditional and modern) for tick control. World Appl. Sci. J. 11: 1047-1054.
- Ribeiro V, Santos J, Martins J, Schripsema J, Ionara R, et al (2011) Acaricidal properties of the essential oil and precocene II obtained from Caleaserrata (Asteraceae) on the cattle tick Rhipicephalus (Boophilus) microplus (Acari: Ixodidae). J. Vet. Parasitol., 179: 195-198.
- Drummond RO, Ernst SE, Trevino JL, Gladney WJ, Graham OH (1973) Boophilus annulatus and Boophilus microplus: laboratory tests for insecticides. J. Econ. Entomol. 66: 130-133.
- Kumar A, Singh S, Mahour K, Vihan VS (2011) In vitro and in vivo acaricidal activity of some indigenous plants under organized and farmer flock. Pharmacologyonline 3: 361-369
- Tabassum SM, Iqbal Z, Jabbar A, Sindhu ZD, Chattha AI (2008) Efficacy of crude neem seed kernel extracts against natural infestation of Sarcoptes scabiei var. ovis. J. Ethnopharmacol. 115: 284–287.
- Kaiser MN (2000) Ethiopia, Report on tick taxonomy and biology, AG: DP/ETH/83/023 Consultant report. Food and Agricultural Organization of the United Nations. P 92.
- Khater H.F, Ramadan MY (2007) The acaricidal effects of peracetic acid against Boophilus annulatus and Argas persicus. Acta. Sci. Vet. 35: 29-40.
- 14. Wang YN, Shi GL, Zhao LL (2009)Acaricidal activity of Juglans regia leaves extracts on Tetranychus viennensis and Tetranychus cinnabarinus (Acaris: Tetranychidae). J. Econ. Entomol. 100: 1298-1303.
- 15. Bicalhoet KA, Ferreira F, Borges LMF, Ribeiro MFB (2001) In vitro evaluation of the effects of some acaricides on life stages of Rhipicephalus sanguineus (Acari: Ixodidae). Arq. Bras. Med. Vet. Zootec.53: 548-552.
- 16. FAO (2004) Resistance management and integrated parasite control in ruminants Guidelines. Module 1. Ticks: Acaricide resistance: diagnosis, management and: Prevention. FAO, Animal Production and Health Division, Rome, pp: 25-77.
- Bennet GF. (1974) Oviposition of Boophilus microplus (Canestrini) (Acari: Ixodidae): I. Influence of ticks size on egg production. Acarologia, 16: 52-61.
- 18. Giglioti R, Forim MR, Oliveira HN, Chagas ACS, Ferrezinid J, et al. (2011) In vitro acaricidal activity of neem (Azadirachta indica) seed extracts with known azadirachtin concentrations against Rhipicephalus microplus Vet. Parasit. 181: 309-315.
- Shafy SA, Zayed AA. (2002) In vitro acaricidal effect of plant extract of neem seed oil (Azadirachta indica) on egg, immature and adult stages of Hyalomma anatolicum excavatum. Vet Parasitol. 106:89-96.
- 20. Landau SY, Provenza FD, Gardner DR, Pfister JA, Knoppel EL, et al (2009) Neem-tree (Azadirachta indica Juss.) extract as a feed additive against the American dog tick (Dermacentor variabilis) in sheep (Ovis aries). Vet. Parasit. 165: 311-317.
- 21. Tamirat S, Basu AK, Getachew T, Bersisa K (2014) Study on the acaricidal effects of Azadirachta indica and Phytolacca dodecandra on Amblyomma ticks in Ethiopia. Ethiop. Vet. J., 18 : 1-14.
- 22. Rahul S, Ghosh S, Mandal DB, Azhahianambi P, Singhal PS, et al. (2008) Efficacy of Azadirachta indica extracts against Boophilus microplus. Parasit. Res., 104: 149-153.

- Parte SG, Patil RD, Patil MA, Patel NS, Chavan JA (2014) Utilization of herbals for the managements of cattle ticks. Intl. J. Curr. Microbiol. Appl. Sci, 3: 228-232.
- Choudhury MK (2009) Toxicity of Neem Seed Oil against the Larvae of Boophilus decoloratus, A one-host tick in cattle. Indian J. Pharm. Sci., 71: 562-563.
- 25. Ismail MH, Chitapa K, Solomon G (2002) Toxic effect of the Ethiopian neem oil on larvae of cattle tick, Rhipicephalus pulchellus. Gerstaeker Kesetsart J. Natural Sci. 36: 198-22.
- Choudhury MK (2001) Toxicity of neem seed oil against the larvae of Rhipicephalus sanguineus a three host tick in dog. J. Parasitic Dis. 25: 46-47.
- Avinash B, Suprajal N, Prasad TNV, Santhi CP. (2017) Evaluation of acaricidal activity of Azadirachta indica extracts against Rhipicephalus (Boophilus) microplus and its GC-MS analysis. Internat J Sci, Environment and Technol. 6 : 980-992.
- Ndumu PA, George JB, Choudhury MK (1999) Short communication: toxicity of neem seed oil (Azadiracta indica) against the larvae of Amblyomma variegatum a three-host tick in cattle. Phytother. Res., 13: 532–534.
- Ribeiro VLS, Toigo E, Bordignon SAL, Goncalves K, Poser GV (2007) Properties of extracts from the aerial parts of Hypericum polyanthemum on the cattle tick Boophilus microplus. Vet Parasitol, 147: 199-203.
- Shafiq U, Muhammad NiK, Muhammad SS, Ghulam M (2013) Comparative Anthelmintic Efficacy of Curcuma longa, Citrullus colocynthis and Peganum harmala. Global Veterinaria, 11: 560-567.
- Shafiq U, Muhammad NK, Muhammad SS, Zafar I,Ghulam M (2015) Comparative efficacies of Curcuma longa, Citrullus colocynthis and Peganumharmala against Rhipicephalus microplus through modified larval immersion test. Int. J. Agric. Biol., 17: 216-220.
- 32. Jeboury GAAL, Khalidy KAAL (2008) Study of effect of hot water extract of fruits of Citrullus colocynthis on hard ticks. AL-Qadisyia. Med. Vet. J. 71: 68-71.
- 33. Balan B, Baskaralingam V, Periyannan R, Narayanan MP, Palaniappan R, et al., (2017) Exploitation of chemical, herbal and nanoformulated acaricides to control the cattle tick, Rhipicephalus (Boophilus) microplus. A review. Vet. Parasit. 244:102-110.
- Rahuman AA, Venkatesan P ,Geetha G. (2008) Mosquito larvicidal activity of oleic and linoleic acids isolated from Citrullus colocynthis (Linn.) Schrad. Parasitol. Res. 103:1383-1390.
- 35. Teka F, Edilu A (2016): In vitro acaricidal activity of crude extracts of Schinus molle (L.) leaves against field population of Bophilus decoloratus and Rhipicephalus pulchellus ticks. African J. of Pharmacy and Pharmacology. 10: 772-777.
- Alla HAlRD, Azzam MAl, Hamdy IH, Salah MK (2009) Acaricidal effects of cardiac glycosides, azadirachtin and neem oil against the camel tick, Hyalomma dromedarii (Acari: Ixodidae)