

Effect of *Cuminum cyminum* Crude Extract on The Reproductive Capacity and Egg Conversion Ratio of The Ixodid Tick *Hyalomma anatolicum*

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المخلص

أجريت هذه الدراسة لتحديد أثر المستخلص الخام من نبات الشمار على خصوبة إناث قرادة الهيلوما أناتوليكيم المتغذية وأثر المعالجة المباشرة للبيض الناتج من إناث غير معالجة. أستخدم جهاز صنع محلياً ليتمكن تعرض القراد والبيض لأثر البخار الناتج من الزيت الأساسي الطيار وأستخدم التركيز 0.75% بناءً على نتائج تجرية سابقة. في الأناث المعالجة وجد تأثير غير معنوي في فترة قبل الفقس ($P \geq 1.0$) وكتلة البيض الناتج ($P \geq 0.5$) وأثر معنوي في فترة قبل الأباضة ($P \leq 0.04$) بينما كان الأثر عالي المعنوية في نسبة الفقس ($P \leq 0.001$). في البيض المعالج كان الأثر معنوياً في فترة قبل الفقس ($P \leq 0.01$) بينما كان التأثير في نسبة الفقس عالي معنوياً ($P \leq 0.001$).

Summary

This study was done to determine the effect of *Cuminum cyminum* crude extract on the reproductive performance of engorged female *Hyalomma anatolicum* ticks, and on directly treated eggs from untreated female ticks. A locally made device was used to enable exposure of ticks and eggs to the vapour of the volatile essential oil. The concentration used was 0.75% based on results of a previous study. Of treated female ticks, insignificant effect ($P \geq 1.0$) was reported on the pre-hatching period, and on the egg conversion rate ($P \geq 0.5$). Significant effect ($P \leq 0.04$) was reported on the pre-oviposition period, while on hatchability the effect was highly significant ($P \leq 0.001$). For directly treated eggs, significant effect ($P \leq 0.01$) was reported on the pre-hatching period, while the effect on hatchability was highly significant ($P \leq 0.001$).

Introduction

Ectoparasitic arthropods represent a group of organisms of major importance in veterinary medicine. They may impair animal health directly or indirectly through bites or their ability to transmit pathogens and associated diseases, some of which are zoonotic (Otranto *et al.*, 2009). Reducing tick abundance is an important but elusive goal. Chemical pesticides applied to ticks habitats can be effective but appear to have significant negative effects on nontargeted organisms (Richard *et al.*, 2006). Several potential approaches are available for controlling ectoparasites, including the use of chemical acaricides, vaccination or biological control (Otranto and Wall, 2008). Research on plant extracts for tick control has been developed into attempts of finding extracts with acaricidal properties that can be used in association with, or even as replacements

for, synthetic compounds (Balandrin *et al.*, 1985). Plant extracts have the advantage of being used in cattle farming or even replacing synthetic acaricides. They are associated with low environmental and food contamination (Borges *et al.*, 2011). This preliminary study is meant to assess the acaricidal properties of *Cuminum cyminum* crude extract on the reproductive performance of the engorged female *Hyalomma anatolicum* ticks, and on eggs from untreated female ticks.

Materials and Methods

Cumin (*Cuminum cyminum*), extracted according to the method described by Harborne (1984), was supplied by the Medicinal and Aromatic Plants Research Institute, National Council for Research, Sudan. The concentration of 0.75% was selected as an effective concentration based on a previous study

(Mohammed *et al.*, 2012). As the crude extract is volatile, the method of the packet test was substituted by a fumigation test using a special locally made device which enables better exposure of ticks to the effect of the oil. The device was formed of Perspex sample containers (6 cm height X 3.3 cm diameter) which were used to contain the ticks. Holes of 3 cm in diameter were made on the plastic caps of each container. One container was used to contain the ticks, while the crude extract was poured in the second one. Both were covered with cotton mesh cloth, closed tightly with their screw caps. To perform the test, the containers containing the ticks were placed inverted on those containing the extract solution and fixed together with a sealing tape.

Effect on reproductive capacity of female ticks

Male and female *H. anatolicum* ticks were fed on calves. Dropped engorged female ticks were collected. Ten engorged female ticks were individually weighed and divided into control and treatment groups of five female ticks each. They were treated for 24 hrs with *Cuminum cyminum* extract at 0.75% concentration and incubated at 27 °C, 75% RH until the end of oviposition. Pre-oviposition period, egg conversion rate (ECR), pre-hatching period and number of emerging larvae and unhatched eggs were determined. Estimated reproductive (ER) index was calculated following the formula of Drummond (1983) as the equation shown below:

$$ER = \frac{2000XY}{Z}$$

Where 2000= average number of eggs per gram (constant), X= Weight in gram of eggs produced, Y= Estimated percentage hatchability of eggs, and Z= Weight of experimental female in gram

Egg conversion ratio was also calculated according to Drummond (1983)

$$\text{Egg Conversion ratio} = \frac{\text{Produced egg mass weight}}{\text{Weight of engorged female tick}} \times 100$$

Effect of direct treatment on hatchability of eggs

Using a horse hair brush, a small mass of eggs was transferred into each of ten containers,

which were divided into two groups, each consists of five replicates. The first group was left as control, while the second was treated as aforementioned. Pre-hatching period, numbers of emerging larvae and unhatched eggs were counted and percent hatchability was determined.

The effects of treated eggs on reproductive capacity and hatchability were subjected to statistical analysis system (SAS) according to Ryan-Einot-Gabriel-Welsch Multiple Range Test (Day and Quin, 1989).

Results

Effect on fed female ticks

The effect of treatment on the reproductive capacity of *H. anatolicum* female ticks is shown in Table 1. Mean \pm SE and *P* values indicated significant difference on the pre-oviposition period ($P \leq 0.05$) and highly significant difference ($P \leq 0.001$) on per cent hatchability.

The estimated reproduction (ER) index of the control group ranged between 88143.8 and 116558.9 with a mean of 98828, while in the treated group it ranged between 4650.68 and 11067.8, with a mean of 6910.94, indicating an obvious effect of the material used on the reproductive capacity. Egg conversion ratio ranged between 23.2 and 65% with a mean of 49.6% in the control group. However, in the treated group, ECR ranged between 40.8% and 51.2% with a mean of 49.3% (Table 2).

Effect of direct treatment of eggs with *C. cyminum* crude essential oil

Treatment of eggs resulted in increased pre-hatching period and decreased per cent hatchability. The pre-hatching period of eggs of the control group ranged between 19 and 20 days, while that of the treated group ranged between 20 and 22 days. Statistically significant effect ($P \leq 0.016$) was detected on the pre-hatching period, while the effect on the per cent hatchability was highly significant ($P \leq 0.001$). The same findings were obtained when mean separation analysis was performed (Table 3).

Table 1: Mean \pm SE and *P* value of the effect of 0.75% *Cuminum cyminum* crude extract on the reproductive capacity of the tick *Hyalomma anatolicum*

Character	Mean \pm SE		<i>P</i> \geq F
	Control	Treated	
Pre- oviposition period	6.0 \pm 0.00a	6.6 \pm 0.24b	<i>P</i> \leq 0.04*
Pre-hatching period	22.4 \pm 0.24a	22.4 \pm 0.6a	<i>P</i> \geq 1.0ns
% hatch	0.995 \pm 0.0a	0.364 \pm 0.01b	<i>P</i> \leq 0.001***

Rows with the same letters are insignificantly different, **P* \leq 0.05, *** *P* \leq 0.001, ns: not significant

Table 2: Effect of *Cuminum cyminum* crude extract (0.75%) on the estimated reproduction and egg conversion ratio of *Hyalomma anatolicum* tick

Group	Tick No.	Tick wt (gm)	ER	Mean ER	ECR%	Mean ECR
control	1	0.176	88143.86		44.2	
	2	0.192	113436.66		57.14	
	3	0.211	46195.88		23.2	
	4	0.214	129805.00		65	
	5	0.222	116558.92	98828.06	58.5	49.61
treated	1	0.138	11067.82		49.9	
	2	0.176	4650.68		40.8	
	3	0.202	5118.81		51.2	
	4	0.209	8373.24		44.2	
	5	0.216	5344.17	6910.94	50.4	49.3

ER: Estimated reproduction Index; ECR: egg conversion ratio

Table 3: Effect of *Cuminum cyminum* crude extract (0.75%) on hatchability of *Hyalomma anatolicum* treated eggs

Character	Mean \pm SE		<i>P</i>
	Control	Treated	
Prehatching period	19.2 \pm 0.2a	20.8 \pm 0.49b	<i>P</i> \leq 0.016*
% hatchability	95.49 \pm 1.61a	62.992 \pm 2.35b	<i>P</i> \leq 0.001***

Rows with different letters are significantly different; *= *P* \leq 0.05, ***= *P* \leq 0.001

Discussion

No mortality was reported among treated fed female ticks as well as no significant difference was found in the pre-hatching period. On the other hand, significant effect was reported on the pre-oviposition period, while per cent hatchability was highly significantly affected. Similar results were reported by Mohammed and Mohammed (1994) and Roland *et al.* (2008) using the chemical acaricide Amitraz and by Osman (2010) using *Gueira senegalensis* and *Ambrosia maritime* extracts. In the current study, differences in pre-oviposition period, and pre-hatching period were observed, but they were statistically insignificant. Egg conversion ratio, in the control group, ranged between 23.2 and 65% with a mean of 49.6% compared to 50- 58.9% reported by El Ghali *et al.* (2003)

on the same tick species. However, in the treated group ECR ranged between 40.8% and 51.2 % with a mean of 47.3% and showed an inhibitory effect. Treated eggs obtained from untreated female ticks have almost produced the same pattern of results obtained from eggs of treated females ticks where decreased hatchability is the prominent finding. However, treatment of ticks was done after they were fully engorged and dropped. If they were treated before or during feeding, the effects might have been different. Estimated reproduction index is significantly effective when female ticks were treated with the crude extract. The same finding was reported by Osman (2010). Similar results were also reported when testing Amitraz, as spray, against *H. anatolicum* (Mohammed and Mohammed, 1994). Moreover, Rae (1987)

has found that Amitraz decreases the number of eggs laid by treated female ticks. He attributed the effect to inhibition of oviposition and maturation of eggs. Osman (2010) also reported decreased egg mass weight, per cent hatchability and estimated reproduction index, using high concentrations of *A. maritime* and *G. sengalensis* extracts. Despite the present promising results, more necessary data, including the chemical stability, safety, formulation and route of application as raised by Pirali-Kheirabadil and Teixeira (2011) is urgently needed.

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