LARVICIDAL EFFECT OF *Cuminum cyminum* L. CRUDE EXTRACT ON THE LARVAE OF THE IXODID TICK *Hyalomma anatolicum*

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المستخلص

عنيت هذه الدراسة بتقييم اثر المستخلص المائي الخام لنبات الشمار على يرقة قرادة الهيالوما اناتوليكم (*Hyalomma anatolicum*) لمعرفة اثره كمبيد للقراد. ولقد أوضحت النتائج الأولية فعالية عالية لهذه المادة. عرضت يرقات القراد بعمر عشرة أيام للمادة تحت الدراسة بتراكيز متدرجة من 50 الى 78, % بأستخدام جهاز تم تركيبه محليا ليمكن تعريض القراد لأثر البخار الناتج من الزيت الطيار بينما عرضت مجموعة السيطرة لبخار الماء فقط. ولقد اوضحت النتائج نسبة نفوق 100%. وفى ما بعد أتخذ التركيز 78, % نموذجا يستخدم فى التجارب اللاحقة كتركيز عالى الفعالية. ولمعرفة اقل تركيز فعال تمت دراسة المادة على اليرقات بتراكيز تدنت حتى 00009, % حيث كانت نسبة النفوق 100% عند التركيز 02, % وعند التركيز 012, كانت نسبة النفوق 98,85% ولكنها تدنت عند التركيز التالى 006, % الى 29,6%. درس أيضا الزمن اللازم لاحداث الأثر حيث وجد أن نسبة النفوق تزداد من 32,61% عند الساعة الأولى الى 100% عند الساعة الرابعة حيث ان الوقت اللازم لنفوق 50% من العينة حوالى ساعة واربع وعشرين دقيقة بينما 100% من العينة تموت فى اقل من اربع ساعات. وبدراسة الأثر المتبقى فقد وجد أن نسبة نفوق اليرقات كانت 34%، 19,7%، 13,1% 6,7 %، 2,3 % و1,3% فى الساعة الأولى الى الساعة السادسة على التوالى.

Abstract

Cuminum cyminum crude water extract was assessed against ten days old larvae of *Hyalomma anatolicum*. Serial two fold dilutions starting from 50% and downwards to 0.78% were assessed using a locally made device to enable exposure of larvae to the effect of the volatile extract, while the control group was exposed to water vapour only. To determine the lower lethal concentration, larvae were exposed to serial dilutions down to 0.00009%. The results showed 100% mortality at 0.0121% dilution and above. The effective exposure time was tested using 0.78% concentration, where it was found to increase from 32.61% in the first hour to 100% in the fourth hour. The time to kill 50 % T_{50} was 1.24 hour, while T_{100} was 3.51 hours. In regards to the residual effect, larval mortality after overnight exposure was 34%, 19.7%, 13.1%, 6.7%, 2.3% and 1.3% in the first to the sixth hour, respectively.

Key words: *Hyalomma anatolicum*, larvae. *Cuminum cyminum*

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Introduction

Animal resources play an important role in Sudan not only by their share in national income, but basically by sustained food security. Increased productivity of this sector is a vital issue and all efforts were taken to improve productivity through upgrading programs and importation of high producing foreign breeds of cattle. Unfortunately both tools were faced by tick problems that stand against the programs to achieve the goals. Ticks constitute the most important livestock pests in Africa and are to be found in the entire 30 million square kilometers of the African continent (Punyua, 1992). Of all ecto-parasites of livestock they cause the greatest economic losses in livestock production (Gray, 1985; Latif *et al*., 1995; Achi *et al*., 2005). They transmit a greater variety of pathogens than any other arthropods, and can cause severe toxic conditions (Muriithi, 1984; Jongejan and Uilenberg, 2004). However, tick-borne diseases ranked high in terms of their impact on the livelihood of poor farming community's resources in developing countries (Minijauw and MacLeod, 2003). According to Osman (1976) it is quite evident that without control of ticks and the diseases they transmit, it seems impossible to increase livestock production by raising pure exotic breeds or cross breeding. On the other hand, Osman (1989) stated that upgrading of cattle in the way it is practiced in Sudan, lowers the innate tolerance of animals and make them succumb to tick borne diseases. Other than the direct economic losses and disease transmission to animals, ticks play an epidemiological key role in health issues in concern of outdoor activity (Boyard *et al*. 2005).

Tick control in Sudan depends mainly on the chemical acaricides. According to Wharton (1967) the indiscriminate use of these chemicals leads to development of resistance. Acaricides are also expensive in term of money and cause environmental pollution (Drummond, 1982). Therefore, alternative approach to be incorporated into integrated tick management package is highly needed. Sudan is rich in herbal plants of medicinal use, and this branch of folklore is widely practiced with good reputation. Muna *et al*. (2003) tested Neem extract against the soft tick *Argas persicus*, while Osman (2010) tested *Ambrosia maritima* and *Guiera senegalenisis* against the hard tick *Hyalomma anatolicum* with promising results. This study was conducted with the aim of assessing the acaricidal activity of the crude extract of *Cuminum cyminum* to be incorporated into tick control programs reducing reliance on chemical acaricides. The overall objectives is to enhance the animal health by controlling ticks and consequently control and prevention of tick-borne diseases through better utilization of environmentally friendly approaches.

Materials and Methods

Larvae: Larvae of *Hyalomma anatolicum* were supplied by the Department of Entomology and Ticks, Veterinary Research Institute, Soba, Khartoum. They were supplied when needed and at the age required.

Cumin extract: Cumin (*Cuminum cyminum*) was purchased from the local market, and sent for extraction to the Medicinal and Aromatic Plants Research Institute, National Council for Research. Distillation was carried out according

to the method described by Harborne (1984). An amount of 450 gm of the dried Cumin was introduced into a 2000 ml rounded bottom capacity flask. A volume of 1000 ml of distilled water was added, and the Clevenger (lighter than water) was received while condenser attached to the top of the flask. The system was heated at 100ºC for 4 hours untill the volume of the oil at the top of the receiver was constant. Oil was pipetted and dehydrated over sodium sulphate anhydrous, and kept in a dark container in a refrigerator untill used.

The device used: As the essential oil is volatile, the packet test method was substituted by another contact test using a special device locally made to enable better exposure of larvae to the effect of the volatile extract. The device (fig,1) was formed of perplex sample containers (6 cm height X 3.3 cm diameter) with holes of 3 cm diameter 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 a cotton cloth, closed tightly with screw caps. To perform the test, the containers with the ticks were immediately placed inverted on those with the extract solution and fixed together with a sealing tape.

Fig.1: The device used to expose the ticks to the effect of the crude extract

Experiment 1: Larvicidal effect of *Cuminum cyminum* on unfed larvae (%mortality)

Ten days old larvae of *H. anatolicum* were used in this experiment. A horse tail brush was used to transfer a batch of larvae to each of three containers. Serial dilutions of the emulsified essential oil were prepared starting from 50% and downwards to 0.78%. Immediately the larvae were treated by placing the containers containing the ticks inverted over those containing the crude extract. Containers were then well fixed together using sealing tapes. Three replicates were made of each concentration as well as three replicates containing water only as a control group. The containers were

then introduced into desiccators containing concentrated sodium chloride solution (Na Cl) to give 75% RH and kept in a well ventilated room for 24 hours before being inspected for dead and alive larvae. To determine the lowest effective concentration, double fold serial concentrations down to 0.000095% were tested.

Experiment 2.Time to death of larvae

Larvae were treated with the same concentrations and the method described above. Six groups, three replicates each were used for treatment. After placing the containers with the larvae over those containing the essential oil they were left for one hour before counting the number of dead and alive larvae of the first group. Then the rest of the groups were inspected for dead or alive at one hour intervals. The control group was exposed to water only. Mean per cent mortality of each group was calculated.

Experiment 3: The residual effect of *Cuminum cyminum* on unfed larvae

Three ml emulsified solution of 0.78 % *Cuminum cyminum* were placed into each of 18 containers, well shaken and discarded. The containers were then divided into six groups three each and left open to evaporate. After one hour, the first group of larvae was transferred into the first three containers, and introduced into a desiccator. Treatment of the other five groups commenced in the same way at one hour intervals. The desiccators were kept overnight in well ventilated room before being inspected for dead and alive larvae to determine the length of period that the substance is effective.

Statistical analysis:

Data from the replicates was pooled and the log dose-response was assessed by probit analysis (Busvine, 1957), then 50% and 99% response value (LC_{50} and LC_{99}) were assessed. Mortality data and the corresponding exposure time were subjected to a linear analysis program (Finney, 1971) to assess time needed to score a certain response.

Results

All concentrations starting from 50% and downwards to 0.78% resulted in 100% mortality of larvae. More dilutions showed 100% mortality at 0.012. Table 1 shows raw and corrected mortality, where high concentration used caused high larval mortality indicating a significant positive correlation (r= 0. 9072). Since the twelve higher dilutions caused 100% mortality, the first eleven were neglected and represented by the lower one and then data of the lowest nine concentrations was transferred into log dose -probit to draw the linear regression line. The steep slope of dose-mortality curve revealed homogenous response and the calculated LC_{50} and LC_{99} values which were 0.004008% and 0.039638%, respectively, expressed the high toxicity of *C. cyminum* extract or susceptibility of *H. anatolicum* larvae (Fig.2).

Table, 1. Corrected mortality of the lower lethal concentrations of *Cuminum cyminum* water extracted essential oil on larvae of *Hyalomma anatolicum*

Concentration	Mean Mortality	Corrected Mortality
Control	0.79	0
0.000095367431328125	0.77	0
0.00019073486265625	1.99	1.21
0.0003814697253125	2.96	2.19
0.000762939450625	2.71	1.94
0.00152587890125	4.85	4.09
0.0030517578125	4.58	3.85
0.006103515625	23.72	23.11
0.01220703125	99.62	99.61
0.0244140625	100	100

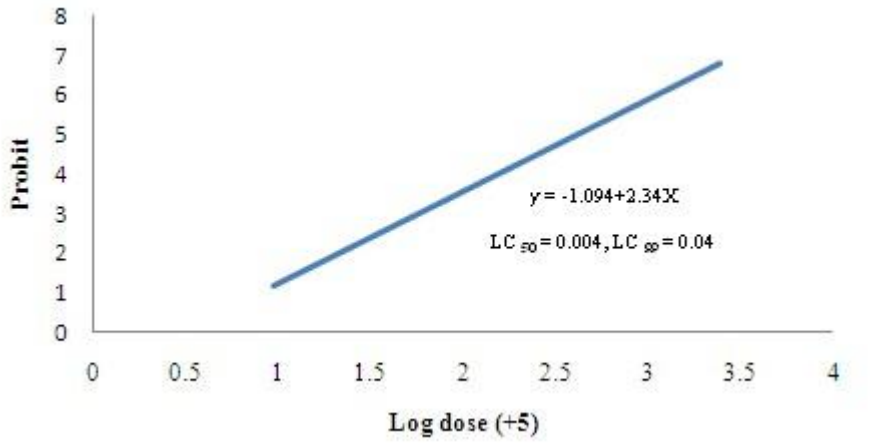

Fig. 2. Log dose probit linear regression of response of *Hyalomma anatolicum* larvae to serial dilutions of *Cuminum cyminum* crude extract.

Mean mortality of larvae exposed to 0.78% *C. cyminum* of the crude extract ranged between 32.61% in the first hour to 100% in the fourth to six hours. Liner regression line shown in fig (3) shows the positive correlation. T_{50} was 1.24 hours, while T_{100} was 3.51 hour. Applying a linear analysis program, the time needed to score 50% or 100% mortality of the exposed larvae were 1h: 14m; 24s and 3h: 30m: 36s, respectively.

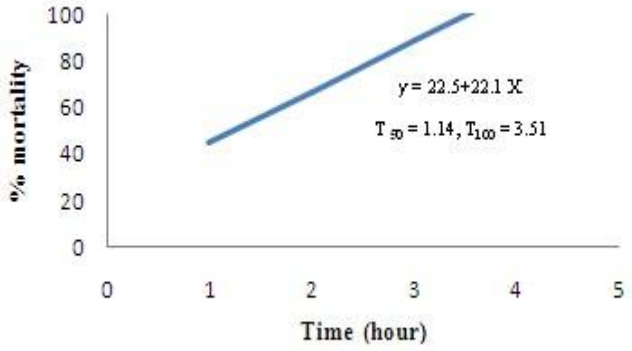

Fig. 3 Effect of exposure time to 0.78% *Cuminum cyminum* crude extract on *Hyalomma anatolicum* larvae.

The residual effect of 0.78% *C. cyminum* crude extract decreased sharply with time. Using a linear analysis program the residual life span was 5h: 30m. Mean mortality during the first hour was 34% only and declined to 1.298% in the 6th hour. The negative correlation is shown in figure 4.

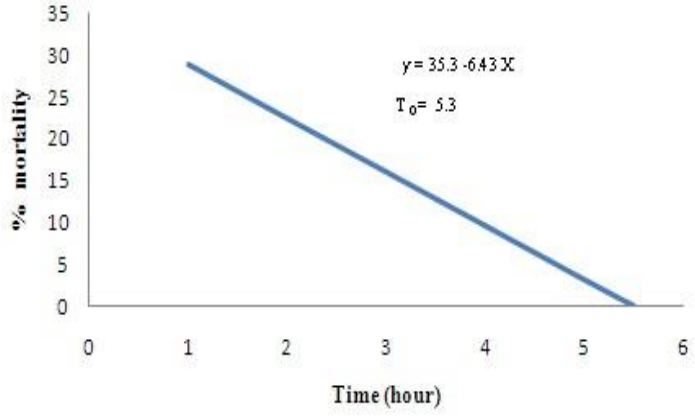

Fig. 4 Residual effect of 0.78% *Cuminum cyminum* crude extract on larvae of the ixodid tick *Hyalomma anatolicum*.

Discussion

In the current study the crude extract of cumin (*Cuminm cyminum)* was assessed against unfed larvae of the tick *Hyalomma anatolicum*. This tick was selected due to its wide distribution and economic importance in Sudan. It is a known vector of bovine tropical theileriosis, maligmnant theieleriosis of sheep and goats (FAO, 1983), and equine babesiosis (Abdoon, 1986) due to *Babesia equi*, now considered as *Theileria equi* (Ikadai *et al.*, 2006).

As the extracted essential oil used in this study is volatile, the recommended packet test seemed to be unsuitable as the time required for the wetted filter papers to dry may affect the potency. So a device which is described

in the materials and methods was used to enable better exposure of ticks to the effect of the essential oil. The study however, showed a potent larvicidal candidate. On the unfed larvae at a concentration as low as 0.02% it caused 100 % mortality, compared to the results of Muna *et al*. (2008) using Neem extract on larvae of *H. anatolicum*, and Martinez *et al*. (2011) on *R.(B.) microplus* using *C. cyminum* crude extract, where 100 % mortality of larvae was achieved at 3.125% and 1.25 % concentrations, respectively. However, in both studies mentioned above the larval packet test was used, which might be the cause of higher concentrations required. This finding also might indicate that the local device used in the current study is efficient and more suitable.

In an intensive study, Osman (2010), assessing the acaricidal effects of ethanol and petroleum ether extracts of *Ambrosia martima* and *Guiera sengalensis* on larvae of *H. anatolicum* using packet and dipping tests, she reached the higher mortality rate of 96% and 100% at 15% using ethanol extract by packet and dipping test, respectively. On the other hand, petroleum ether extracts using the packet test caused 91.99% larval mortality at 15% concentration. The lower mortality and the high concentration used, compared to the current study may be attributed either to the high potency of Cumin or the efficiency of the device used. However, this discrepancy can be elevated by the most recent work on *C. cyminum* carried out by Martinez *et al*. (2011) who reported 100% mortality of *R. B. microplus* larvae at 1.25% using the larval packet test.

In general worldwide meager data is available on the acaricidal effect of *Cuminum cyminum*. To our knowledge, the work of Muna *et al.* (2008), Martinez *et al*. (2011) and Mohammed *et al*. (2012) is the only available data. In this study the questioned raised by Martinez *et al*. (2011) was answered by determining the lower lethal concentration which is a promising finding where concentration as low as 0.02% caused 100 % larval mortality, whereas 0.012 caused 99.6% mortality. Thereafter, the mortality decreased by increased dilution to be almost the same of that of the control at 0.00009 %

Linear regression lines and dose probit showed shift to the right indicating a positive correlation between the mortality and the concentration used. The same correlation was evident with mortality and time of exposure. However, it is not necessarily that the length of exposure time is the absolute factor, because it could be speculated that the lethal effect is encountered during the first moments of exposure but some reactions, events or circumstances within the tick's organ should commence before death ensues. Inversely, negative correlation was noticed when studying the residual effect. It is absolutely logic as volatile oils evaporate and their effects wane by time. To this extent the cause of death is not known. Martinez *et al*. (2011) mentioned that, the acaricidal activity of the cumin essential oil is perhaps attributed to the high level of cuminaldehyde (22.03%). γ –terpinene (15.69% and 2-caren-10-al (12.89%). However, specific research is warranted to determine mode of action and the real cause of death.

According to Chouldhry and Atta ur Rhman (2005) research and development efforts should be focused on the preparation of effective and save products from herbal plants. The current study aiming at assessing the acaricidal

activity of Cumin (*C. cyminum*) proved *in vitro* acaricidal effect which could add to the existing literature as promising results were obtained. Being available, affordable and easily extracted, in addition to the encouraging results obtained, Cumin could play an important role as a safe and environmentally friendly candidate plant to be incorporated into tick control program. Of the limiting factors, this plant is of high consumption as it used as spices in human food. So the amount needed to satisfy the control purpose may, one day reach a critical point beyond which it could not be supplied or its use other than food may be restricted. To overcome this difficulty the use should be as limited as possible and in corporation with other effective alternatives. Nevertheless, formulation and method of application warrant a thorough research to be fully identified.

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