

## EVALUATION EFFECTS OF DIFFERENT CONCENTRATIONS OF AZADIRACHTAINDICA(NEEM) SEEDS OIL AS A BOTANICAL PESTICIDE TO ELIMINATE THE PARASITE OF *Hyalomma spp* IN COWS

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### ABSTRACT

The current study was conducted on extracted *Azadirachtaindicaneem* seeds oil using the ethanol, it was measured effectiveness inhibitory of neem seeds oil extraction in different concentrations of 0.001 and 0.003 unit/ml compared with commercial neem oil, known (Azadirachtin) concentration of 0.002 unit/ml on the *Hayalomma Spp*. The study was conducted on six calves were selected randomly with different ages ranging from 4-6 months in the veterinary clinic in Baghdad / Diyala bridge area. The animals were divided into three group with 2 animals each, namely control group that treated with 0.002 neem oil (Azadirachtin 3.000 ppm) and treatment groups 1 and 2 were treated with neem oil with concentration of 0.0001 and 0.0003 respectively. The parasitic infestation of *Hayalomma Spp* was confirmed in the laboratory diagnosis and the presence of ticks in addition to the clinical symptoms of infected calves. The objective of this study was to evaluate the effects of different concentrations of seeds oil of AzadirachtaIndica(Neem) on Hyalomma Spp eliminate on the Cows. Results showed high efficiency of neem oil concentration 0.003 unit/ml as an anti-ticks, where there was a decline of the number of parasites since of the first day to the fourth day of the treatment that showed the mean number of parasites isolated 18.0, 15.8, 3.5 and 0.0 respectively, compared with concentration of 0.001 and control group with significantly reduced of the parasites number  $P < 0.004$  at 0.05. Also, the most important result showed that there was no side effects of the high concertation of 0.003 of neem oil to treat the infected animals. In

conclusion, the concentration 0.003 of neem showed the best extraction to eliminate the parasites without effects of animals' health status.

## INTRODUCTION

Ticks infestation is one of the most prevalent diseases among cows in Iraq are caused direct economic losses as a result of injury cows, anemia and cause paralysis of the arms and legs of young cows and rapid death. In spite of the Iraqi Ministry of Agriculture to prevent it that use of some chemical pesticides such as diazinon and deltamethrin which have negative impact on the human being causes of cancer, according to the recommendations of the International Organization of Health (WHO), it has been used these pesticides to the lack of environmentally friendly alternatives. Typically, many of the chemical pesticides used to combat ticks in Iraq livestock or fogging spray or dipping (1).

Neem *Azadirachta indica* plant belongs to the family Meliaceae a timber trees which is characterized by the high volume of root where its roots extend to large areas accidental. The advantage of the tree trunk measured the solid, dark brown, ranging in diameter between 75-150 cm with a cracked shell structure, clustered at the ends of branches and the leaf length up to 30 cm. Several floral inflorescence side length up to 20 cm, white, using parts of the neem plant are peels, leaves and seeds and juices (2). Began cultivation of neem in Iraq before a number of years successfully is extracted neem oil which is sour taste, almost 50% of the seed weight is oil, vitamin E. The researchers believe that the therapeutic property which is characterized by neem in the treatment of many skin diseases, back to the amino acids of medical significant (3).

The Neem oil effective against many pests because of its better for insects that might it causes an imbalance in growth hormones for insect Juvenile hormones for the existence of material Azadiractin resulting in a process of alienation larvae or nymphs and stop the growth and death of the insect in the end stop (4), it has been used neem oil repellent as an insect repellent in many scientific experiments (5). Aqueous extract of garlic has been used to combat ticks researchers and has proved highly effective (6), and it has been

recorded several types of ticks in Iraq, including *Hyalomma* Spp which was classified as a Kingdom of articulated legs (Arthropoda, class arachnids (Arachnida), order Acari form (Acariformes), within the family hard ticks (Ixodidae). *Hyalomma* tick that can survive in low humidity, harsh climatic conditions in which families are scarce and where less hiding places and environments have been diagnosed in Iraq livestock by the researchers, namely: *H. anatolicum*, *H. marginatum* and *H. asiaticum* (7). There are few studies have been reported about neem oil used as antiparasitic. Therefore, the objective of this study was to evaluate the effects of different concentrations of seeds oil of *Azadirachta Indica* (Neem) on *Hyalomma* Spp eliminate in the Cows.

## **MATERIALS AND METHODS**

The neem seeds plant was obtained in this study from local market in Baghdad have been classified in the general authority for examination and certification of seeds department of botany, seeds were washed several times to remove the dirt and impurities, and then dried in the oven at a temperature of 50<sup>0</sup>C. The pure seeds were grind electric grinder to powder form. Then was extracted neem oil from the seed powder using solvent ethanol as a rate of 1:5 for three hours, and centrifuged and then filtrated and evaporated to get a pure of solvent oil, then oil was weight to calculate the oil concentration in solution (2).

For the treatment, it was selected the commercial neem oil (Azadirachtin 3.000 ppm) for comparison, It is an Indian original and was available in Iraq. Animals were selected randomly by veterinarian in veterinary clinic in Diyala/Baghdad bridge area. There were six infected calves with ticks in age between 4-6 months that diagnosed clinically and confirm in the laboratory examination. Animals were examined and confirmed their health status in general and there is no disease or other injuries that might affected the results of the research, the animals were divided into three group with 2 animals each, namely control group treated with 0.002 neem oil (Azadirachtin 3.000 ppm) and treatment groups 1 and 2 were treated with neem oil with concentration of 0.0001 and 0.0003 respectively.

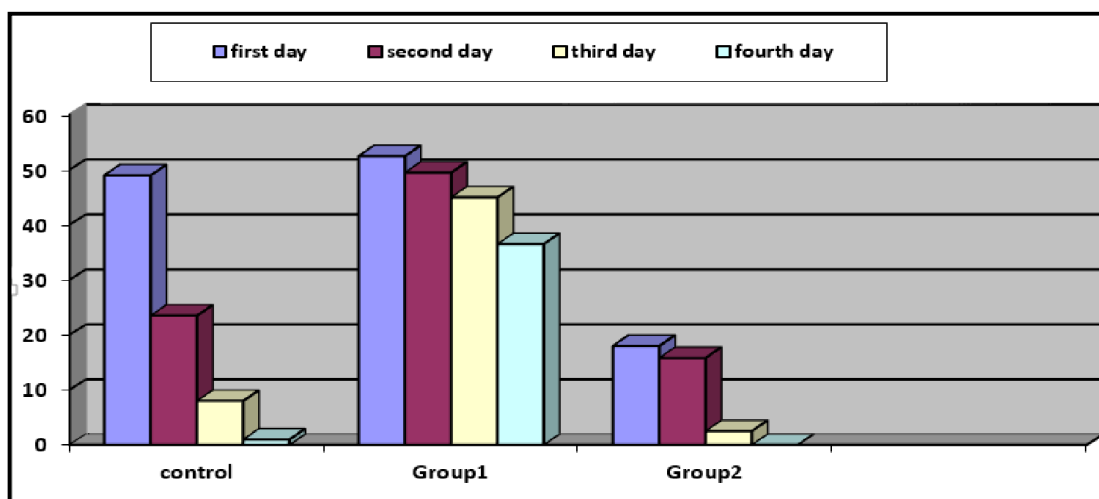
The treatment procedure was prepared into different concentrations of neem seed extractions of 0.003 and 0.001 unit/ml and also the commercial oil neem oil 0.002 (Azadirachtin 3000 ppm) 0.002 unit concentration unit/ml. The working solutions were prepared using 5ml from each concentration and mixed with 5 liters water daily for four days and mixed until get a white color emulsion, then put the emulsion in special spraying machine that to spray the emulsion over each animal's body except the head. It took four days duration of the experiment and the alive ticks' number on animals were calculated before the spraying trial for every day, T test was used for statistical analysis and results showed  $P < 0.05$  that consider significantly.

## **RESULTS**

The results of this study showed highly effectiveness of treatment with neem oil concentration of 0.003 unit/ml as an anti-external parasites (ticks). There was efficiency of neem oil concentration 0.003 unit/ml as an anti-ticks, where there was a decline of the number of parasites since the first day to the fourth day of the treatment that showed the mean number of parasites isolated 18.0, 15.8, 3.5 and 0.0 respectively, compared with concentration 0.001 and control groups with significantly reduced of the parasites number  $P < 0.004$  at 0.05. The most important result showed that there was no side effects of the high concentration 0.003 of neem oil to treat the infected animals. On the other hand, the focus on the concentrations of 0.001/ml showed less reduced the average of the number of parasites at 52.5 to 36.5 from the first day to the fourth day respectively, compared with control group that declined the parasites number with concentration of 0.002 unit/ml since the first day 49.0 to 1 in the fourth day of the treatment (Table 1 and Figure 1).

**Table 1: showing the number of ticks per day of each animal group treated in different concentration of neem oil**

Treatment / Days	Mean number of ticks in the control group treated with 0.002 neem oil N= 2 animals	Mean number of ticks in the treatment groups in different concentrations of neem oil (Unit/ml)	
		Group1 treated with 0.001neem oil N=2 animals	Group2 treated with 0.003 neem N=2 animals
First day	49.0	52.5	18.0
Second day	23.5	49.5	15.8
Third day	8.0	45.0	3.5
Fourth day	1.0	36.5	0.0

**Figure 1: showing the number of ticks per day of each animal group treated in different concentration of neem oil**

## DISCUSSION

Results of this study showed that the effectiveness of neem oil and the extract concentration of 0.003 units/ml more concentrations of the effectiveness and impact on the eradication of the external parasites completely on the fourth day of the treatment period compared with treatment group with neem concentration of 0.001 and control group. The first day after the transaction focus and gave the fourth full day as a result of the elimination of the parasites. The results of this study were agree with the results obtained by experiments conducted previously on infected cows (8), other researchers

also reported through the study of the effectiveness of the neem oil using against different species of ticks that affected the cattle(9).

The reason for the effectiveness of neem to the presence of material Azadirachtin effective in the elimination of insects and parasites, which is the active ingredient in the herb president neem oil, which is Tetranortriterpenoid (10). The use Azadirachtin as insect repellents as a non-toxic to humans and pets such as birds, cows and all kinds of cattle so they do not pose a threat to the environment because they decompose quickly. However, using the neem in the soil may stay some time and the plant absorbs the Azadirachtin that also works on plant protection from insect pests that feed it, and even when using very low the concentration of parts per million (ppm). In other experiments found that the larvae or nymphs, which had been treated with Azadirachtin were die, of which 70% within three to 15 days, where Azadirachtin effect on an ecdysone as a hormonal system that works on the alienation of larvae and nymphs to complete their life cycle and turn into a full insects. Unlikely, pesticides using industrial chemical as insecticides that affect the digestive system or the nervous directly and environmentally hazardous to human health, the impact of Azadirachtin be on the hormonal system of insects and insect thus not be able to configure his immunity in the future (11).In conclusion, the concentration 0.003 of neem showed the best extraction to eliminate the parasites without effects of animals' health status.

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## تقييم تأثير التراكيز المختلفة للزيت المستخلص من بذور نبات *Azadirachta indica* (النيم) كمبيد نباتي للقضاء على الطفيلي *Hyalomma Spp* في الأبقار

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### الخلاصة

أجريت الدراسة الحالية على الزيت المستخلص من بذور نبات (النيم) *Azadirachta indica* باستخدام الإيثانول، وقد تم قياس الفعالية التثبيطية لزيت بذور النيم المستخلصة بتركيزات مختلفة من 0.001 و 0.003 وحدة / مل مقارنة مع زيت النيم (التجاري)، كمبيد نباتي للقضاء على طفيلي *Hyalomma Spp* في الأبقار وتركيز 0.002 وحدة / مل المعروف (في الأبقار).

أجريت الدراسة على ستة أبقار تم اختيارها بشكل عشوائي بمختلف الأعمار التي تتراوح بين 4-6 أشهر بمساعدة كادر إحدى العيادات البيطرية في منطقة جسر بغداد / ديالى. تم تقسيم الحيوانات إلى ثلاث مجموعات بواقع حيوانين لكل مجموعة وهما مجموعة السيطرة التي عولجت باستعمال زيت النيم *Azadirachta indica* التجاري بتركيز 0.002 وحدة / مل (3000 جزء في المليون) ومجموعتي العلاج 1 و 2 التي تم معاملتها بتركيز زيت النيم المستخلص 0.001 و 0.003 وحدة / مل على التوالي.

وقد تم تأكيد الإصابة بطفيلي *Hyalomma Spp* في التشخيص المختبري للقراد بالإضافة إلى الأعراض السريرية للأبقار المصابة. وكان الهدف من هذه الدراسة هو تقييم آثار التراكيز المختلفة من زيت بذور نبات النيم للقضاء على الطفيلي *Hyalomma Spp* في الأبقار. وأظهرت النتائج هذه الدراسة كفاءة عالية لزيت النيم بتركيز 0.003 وحدة / مل للقضاء على طفيلي القراد حيث كان هناك انخفاض واضح في عدد الطفيليات منذ اليوم الأول إلى اليوم الرابع من العلاج الذي أظهر متوسط عدد الطفيليات معزولة 15.8، 18.0، 3.5 و 0.0 على التوالي، مع انخفاض كبير في معدل عدد الطفيليات عند  $P < 0.004$  في 0.05 أيضاً مقارنة مع تركيز 0.001 والمجموعة السيطرة وكذلك أظهرت نتائج هذه الدراسة عدم وجود أي آثار جانبية من استعمال زيت النيم بتركيز 0.003 لعلاج الحيوانات المصابة. في الختام، يعتبر زيت النيم بتركيز 0.003 أفضل تركيز مستخلص لزيت النيم للقضاء على الطفيليات دون أي آثار جانبية.

### REFERENCE

1. Abdel-Shafy, S. (2000). Microbiological and control studies on ticks infesting farm animals and poultry. Ph.D. Cairo University, p. 209.
2. Helson, B.; Lyons, B.; de Groot, P. (1999). Evaluation of neem EC formulations containing azadirachtin for forest insect pest management in Canada. Pp. 79-89 in RP Singh, RC Saxena (Eds.), *Azadirachta indica* A. Juss. International. Neem Conference, Gatton, Australia, Feb. (1996). Oxford & IBH Publishing Co. PVT. Ltd. New Delhi.
3. Nielsen. SS. (1998). Food Analysis. An Aspen Publication. Maryland. U.S.A.

4. Abudulai, MB. Merle Shepard, and Paula Levin Mitchell. (2004). Effects of neem (Azadirachta indica A. Juss.) on predators of Nezaraviridula (L.) (Hemiptera: Heteroptera: Pentatomidae). J Agr Urban Entomol, 21(1): 9–13.
5. U.S. Environmental Protection Agency. (2012). Cold Pressed Neem Oil, Biopesticides Registration Action Document. P. 1-22.
6. - داود، خيرى عبد الله، المحنة، ثناء اسماعيل، جاسم، غيداء عباس (2011). امكانية استعمال المستخلص المائي ، 1 ، العدد 10 للثوم ومبيد السايبرميثرين في مكافحة القراد. مجلة القادسية لعلوم الطب البيطري ، المجلد 42-47. رقم الصفحات.
7. Hasson. RH, AlZubaidi, HH. (2014). Cattle and buffaloes tick's infestation in Wasit province districts, Iraq. Kufa J. Vet. Med.Sci, 5 (1): 31-40
8. Abdel Al-Shafy, S. and 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 (Ixodoidea: Ixodidae). J. Vet.Parasitolo, 106, 89-96.
9. Makeri H K, Maikai, VA, Nok, JA. (2007). Effect of tropical application of neem seed (Azadirachta indica) extract on sheep infested with Amblyomma variegatum. Afri. J. Biotechnol, 6(20): 2324-2327.
10. Fasina OO, Ajibola, OO.(1989). Mechanical Expression of Oil from Conophor Nut. J.Agr. Engine, 44:275-287.
11. Khraisha, YH. (2000). Retorting of oil shale followed by solvent extraction of spent shale: Khosla, P. Sangeeta, B. Singh, J. and Srivastava, RK. (2000). Antinociceptive activity of Azadirachta indica (neem) in Rats. Indian J. of Pharmacol. 32: 372-374.