

Effect of *Allium sativum* and *Allium cepa* oils on different stages of *Boophilus annulatus*

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Abstract

The aim of this study is to investigate the acaricidal effect of *Allium sativum* (garlic) and *Allium cepa* (onion) oils on different stages of *Rhipicephalus (Boophilus) annulatus* hard tick. Semi-engorged and engorged *B. annulatus* females were collected from naturally infected cattle. A number of engorged ticks were incubated at 28 °C and 85% relative humidity to lay eggs, which were incubated in the same conditions for 14 days to obtain larvae. Garlic and onion oils were prepared by steam distillation. These oils were dissolved in ethanol, methanol and partially, in water. The oils were used in different concentrations; 20, 10, 5, 2 and 1%. These concentrations were applied on adult ticks through adult immersion test (AIT); on larvae by larval packet test and on eggs. The 20, 10 and 5% of garlic oil dilutions in ethanol and methanol killed all adult ticks and larvae within 24hrs. Similar results were obtained for garlic oil dissolved in water in 10 and 20%. The effect of the concentration 10% of garlic oil in any of the used diluents on embryonated eggs appeared as; no hatching, deformity in eggs such as shrinkage in the eggs' wall, becoming spindle in shape and changing of its color to dark brown. In case of onion oil, only the 20 and 10% concentrations with ethanol and methanol killed 70-85% of the adult ticks while all larvae were killed within 24hrs at those concentrations. The two concentrations of oil in water killed 70-80% of the treated ticks. Moreover, 10% aqueous solution of onion oil prevented hatching of embryonated eggs. From these results, we conclude that garlic and onion oils have acaricidal effect on all stages of *B. annulatus* at concentrations higher than 5% on all stages.

Key words: *Garlic oil, onion oil, Rhipicephalus (Boophilus) annulatus, acaricide, adult immersion test, larval packet test*

Introduction

Ixodid ticks constitute a major problem for cattle industry because ticks may cause severe blood loss and reduction in weight gain of infested cattle. Besides, they may transmit certain diseases such as bovine babesiosis, theileriosis and anaplasmosis. (Ribeiro *et al.*, 2008). Chemical acaricides have played a major role in controlling hard ticks. However, their intensive use has lead to development of resistant tick populations (Rodriguez-Vivas *et al.*, 2006). It is therefore necessary to look for alternative products or strategies for tick control. Plant extracts provide potential alternatives to currently used acaricides because encouraging results have been obtained by several researchers with some plants possessing toxicological

effects on *R. microplus* (Ribeiro *et al.*, 2008). The use of *Allium* plants including shallot, garlic and onion as medicinal plants predates written history (Harris *et al.*, 2001). Onion had antilishmanial effect (Saleheen *et al.* 2004); anthelmintic effect on *Trichenella spiralis* and *Schistosoma* (Abu El Ezz, 2005, Mantawy *et al.* 2011); antimicrobial and antifungal (Kocia-Tanackov *et al.*, 2009; Dimia *et al.*, 2008). Garlic was known as an antiparasitic agent and this activity has been attributed to allicin, the main constituent of garlic (Ankri and Mirelman, 1999). Birrenkott, *et al.* (2000) treated mite in hens by application of garlic in 10 % concentration. Garlic essential oils produced high mortality (90–100%) on 10-days old *R. microplus* tick larvae by the tested concentra-

tions (Martinez-Velazquez *et al.*, 2011). Larvicidal activities was reported against the filarial mosquito *Culex quinquefasciatus* (Kalu *et al.*, 2010). Insecticidal activity of garlic juice was studied on two dipterian pests *Delia radicum* and *Musca domestica* (Gareth *et al.* 2006).

So we aimed in this work to investigate the potency of *Allium sativum* and *Allium cepa* oils as acaricides against different stages of *B annulatus*.

Materials and Methods

Tick stages:

Adult ticks:

Semi-and fully-engorged female ticks (*Boophilus annulatus*) were collected from naturally infected cattle. Ticks were kept under controlled conditions ($28 \pm 1^\circ\text{C}$, 80% humidity and 12hs photoperiod) in a BOD (Biological Oxygen Demand).

Tick's eggs and larvae

The engorged female ticks were placed in clean glass tubes plugged with cotton wool and incubated at $28 \pm 1^\circ\text{C}$, 80% humidity and 12 h photoperiod in BOD. A part of the eggs were subjected to the optimum tested concentrations. The rest of eggs in each tube were transferred into a clean glass tube with cotton plug and dead female ticks were discarded. The eggs were kept under same incubating conditions until hatching into larvae and then starved in the incubator at 28°C for two weeks before use. These larvae were used in the study.

Preparation of *Allium sativum* and *Allium cepa* oils:

The plant bulbs were brought in the harvesting season (fresh) then the plant bulbs were subjected to steam distillation to obtain the oils.

Preparation of oils concentrations by different dissolvents:

Five concentrations were prepared (1, 2, 5, 10 and 20%). These dilutions were primed by water, absolute ethanol and methanol. The ready concentrations were applied on adult ticks, eggs and fed and unfed larvae.

Application methods:

Adult immersion test (AIT):

Semi and fully-engorged females of *Boophilus annulatus* were screened against the five concentrations of garlic oil using adult immersion test (AIT) as described by Benavides & Romero (1999). Briefly, the engorged females were washed in a sieve with tap water then were dried on soft absorbent paper. After that, 50 females were divided into five groups of 10 females each and were immersed in Petri dishes containing the above different concentrations of different dissolvents for 2 min. The control group composed of 10 engorged females that had been immersed in distilled water only, ethanol or methanol for the same period. Ticks were dried in absorbent paper and placed in an incubator at 28°C and 85 % relative humidity. The observations of oil effects on tick mortality were recorded at 6, 12, 24, 48 and 72hs post treatment. Dead ticks were confirmed by observing motility loss, pedal reflex and after exposing to light Benavides *et al.* (2001). The number of dead females was recorded, and the mortality percentages of females were calculated.

Application of oils on tick's eggs:-

Approximately, 200 *B annulatus* embryonated eggs were placed in glass tubes and immersed for 2 min in 10% Garlic oil aqueous solution. The solutions were decanted. The tubes closed with cotton plugs and incubated at 28°C & 85% relative humidity for 15 days, until the eggs started hatching. Eggs immersed in the same manner in water were used as control (Ribeiro *et al.* 2008).

Application of oils on *B annulatus* larvae by larval packet test (LPT):-

The different concentrations of the used oils in aqueous solution were screened against the unfed (15-day-old) larvae. The tested ticks by the larval packet test (LPT) proposed by Shaw (1966). A group of approximately 600 tick larvae, 120 larvae for each concentration and control, were distributed on a 14cm diameter of filter paper lying in a petri dish. Five milliliters of the diluted oil of each concentration was poured over the larvae. Another 14cm diameter filter paper was placed over the larvae and 5ml of the extract poured over it. After the immer-

sion period of 5min, the larvae were removed from the filter paper and placed in a folded filter paper (5x10cm) with the openings closed with a tape then incubated at 28°C & 85% RH. (Borges *et al.*, 2003). To calculate the mortality after incubation, the packets were opened and the number of live and dead larvae was recorded at 24, 48 and 72hs post application. All larvae that show no movement were considered dead Giglioti *et al.* (2011).

Evaluation of the acaricidal efficiency of the used garlic oil

The mortality rate of treated adults and larvae was reported by the number of deaths at every concentration. Duration of application and time of death were recorded. The oviposition ability of the treated engorged females and also the ability of these eggs to hatch were investigated. Dead ticks were diagnosed based on three criteria: signs of cuticle darkness with hemorrhagic skin lesions, leg movement and halted Malpighian tube movement. The main criterion to diagnose the death of ticks was the lack of Malpighian tube movement, which is very clear in *B. annulatus*. The second criterion was the lack of movement of legs tested with a paintbrush with ticks placed in an inverted position under a stereomicroscope lamp. The third criterion was a change of cuticle color with hemorrhagic skin lesions demonstrated by dead ticks. Failure in egg laying was then directly observed by eye and calculated. All groups, including treatments and controls, were checked every 6 h and data were recorded. Any changes in ticks such as egg laying were added to the data set (Chungsamarnyart and Jansawan, 2001).

Statistical analysis

Data obtained were statistically computized using SPSS (2006).

Effect of garlic and onion oils concentrations on adult engorged females of *B. annulatus*.

The garlic oil was mixed with water. It began to affect engorged females beginning from the concentration 2% after 24hs and relatively increase the deaths number but all ticks did not die even after 72hs post treatment. When the oil concentration increased, the mortality rate increased. The living ticks after treatment at these concentrations gave eggs and hatched in the optimal time. The mortality rate in ticks reached 100% of treated ticks at concentration 10 and 20% after 24hrs and 48hrs respectively. For onion oil, there was no concentration resulted in mortality rate 100% but only 80% of treated ticks by 20% achieved after 72hs post treatment. The lowest concentration of onion oil which began to give mortality in ticks was 10% after 12hrs post treatment at rate of 70% deaths. Comparing results of garlic and onion oils; it was noticed that garlic affected ticks earlier than onion; also garlic achieved 100% death of ticks. On the other side, onion oil did not achieve 100% mortality even with the highest concentration 20% which killed 80% of the treated ticks. The living ticks (25%) gave scant number of eggs.

The dissolved *Allium sativum* oil in ethyl alcohol began to kill ticks from 2% concentration after 12hrs post treatment but mortality of all ticks began from 5% concentration after 72hs. Only 20% concentration achieved 100% mortality rate within 24hrs. While *Allium cepa* effect appeared from concentration 5% and cause 40% after 72hrs post treatment. The higher rate of mortality was achieved by the concentration 20% after 72hrs and gave 85% mortality rate. This 15% living ticks after 72hs laid few numbers of eggs. Comparing the garlic and onion oils effects, it was noticed that garlic achieved 100% at 5% concentration while that was not achieved by the onion oil even at higher concentrations and after 72hs post treatment (10 & 20%).

The dissolved *Allium cepa* in methanol began to affect ticks beginning from the concentration 5% after 24hs. Time and concentrations increased the mortality rates which reached its maximum at the highest concentration 20% after 72hs and reached to 85% only. The 15% living ticks after

Results

72hs laid few numbers of eggs. On the other side garlic oil achieved 100% mortality rate beginning from the concentration 5% after 72hs and also with 10 and 20% but at shorter time.

Effect of the used oils on eggs of *B annulatus*:

The results indicated that there was a significant effect of garlic and onion oils in aqueous solution 10% on embryonated *B annulatus* eggs. This effect was clearly observed at 15th day post treatment. Where no hatching occur and the deformity of the eggs were very clear. These deformities were shrinkage in the egg wall, became spindle in shape and its color changed from bright golden color to dark brown one (Fig. 1)

lution on unfed larvae of *B annulatus* by larval packet test (LPT):

The concentrations 1% of both oils had no effect on the larvae even though after 72hs. The larvicidal effects of the used oils began from 2% concentration and killed 50% and 16.6% after 72hs post treatment by garlic and onion oils respectively. One hundred percent of larvae died after 24hs post treatment at concentration of 5, 10 and 20% of *A. sativum*. While 100 % of larvae died after treatment at concentrations of 5% after 72hs, 10 and 20% after 24hs of treatment for *A. cepa*. On the other hand, all larvae in the control group remained alive in all periods post treatment by water alone (0 concentrations).

Effect of Garlic and onion oils in aqueous so-

Table (1) Effect of different concentration of garlic onion oils in aqueous solution on adult female ticks (n=10) (Mean $\pm$ SE)

Concentrations %		Number of dead ticks post treatment				
		6h	12h	24h	48h	72h
0	Garlic oil	0	0	0	0	0
	Onion oil	0	0	0	0	0
1	Garlic oil	0	0	0	0	0
	Onion oil	0	0	0	0	0
2	Garlic oil	0	0	4.6 $\pm$ 0.3*	5.3 $\pm$ 0.3*	5.6 $\pm$ 0.3*
	Onion oil	0	0	0	0	0
5	Garlic oil	0	0	5.3 $\pm$ 0.3*	7.6 $\pm$ 0.3*	7.3 $\pm$ 0.3*
	Onion oil	0	0	0	0	0
10	Garlic oil	0	3 $\pm$ 0.5*	9.3 $\pm$ 0.3*	10 $\pm$ 0*	-
	Onion oil	0	0.6 $\pm$ 0.3	1.6 $\pm$ 0.3	3 $\pm$ 0.5	5.6 $\pm$ 0.8
20	Garlic oil	0	5.3 $\pm$ 0.3*	10 $\pm$ 0*	-	-
	Onion oil	0	1.6 $\pm$ 0.3	5.6 $\pm$ 0.3	7.3 $\pm$ 0.3	8 $\pm$ 0.5

(*) $P < 0.05$ is significant between garlic and onion within the same row.

Table (2) Effect of different concentration of garlic onion oils in ethyl alcohol on adult female ticks (n=10) (Mean $\pm$ SE)

Concentrations %		Number of dead ticks post treatment				
		6h	12h	24h	48h	72h
0	Garlic oil	0	0	0	0	0
	Onion oil	0	0	0	0	0
1	Garlic oil	0	0	0	0	0
	Onion oil	0	0	0	0	0
2	Garlic oil	0	1.6 $\pm$ 0.3	4 $\pm$ 0.5*	4.6 $\pm$ 0.3*	4.6 $\pm$ 0.3*
	Onion oil	0	0	0	0	0
5	Garlic oil	0	2.3 $\pm$ 0.3	7.6 $\pm$ 0.3	10*	
	Onion oil	0	0	3.3 $\pm$ 0.3	4.3 $\pm$ 0.3	4.6 $\pm$ 0.3
10	Garlic oil	0	4.6 $\pm$ 0.3	10*	-	-
	Onion oil	0	3.6 $\pm$ 0.3	4.6 $\pm$ 0.3	6.6 $\pm$ 0.3	7.6 $\pm$ 0.3
20	Garlic oil	0	5.6 $\pm$ 0.3*	10*	10	10
	Onion oil	0	1.3 $\pm$ 0.3	4.3 $\pm$ 0.3	7.6 $\pm$ 0.3	8.6 $\pm$ 0.3

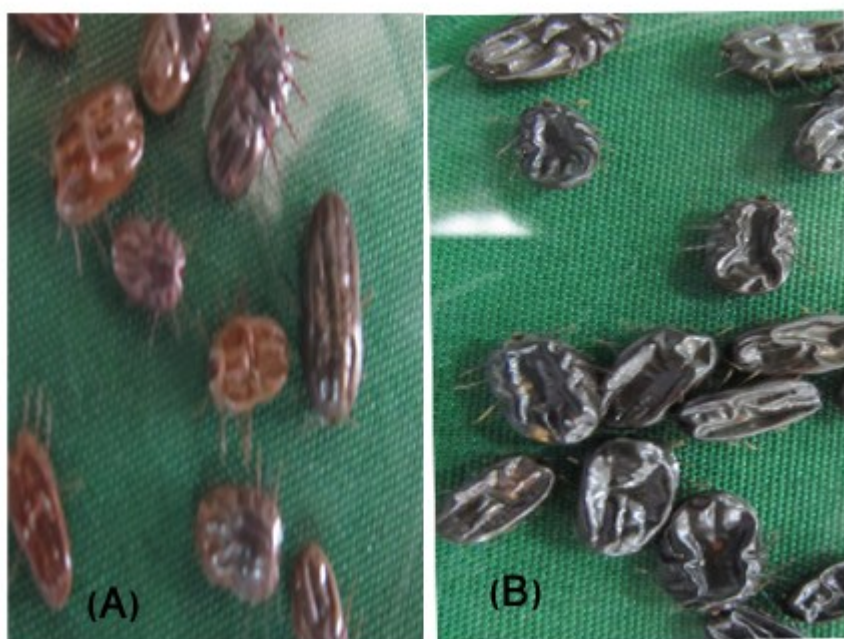
Table (3) Effect of different concentration of garlic onion oils in methyl alcohol on adult female ticks (n=10) (Mean $\pm$ SE)

Concentrations %		Number of dead ticks post treatment				
		6h	12h	24h	48h	72h
0	Garlic oil	0	0	0	0	0
	Onion oil	0	0	0	0	0
1	Garlic oil	0	0	0	0	0
	Onion oil	0	0	0	0	0
2	Garlic oil	0	1.3 $\pm$ 0.3	3.6 $\pm$ 0.3	4.6 $\pm$ 0.3*	6 $\pm$ 0.5*
	Onion oil	0	0	0	0	0
5	Garlic oil	0	2.3 $\pm$ 0.3	6.6 $\pm$ 0.3*	9 $\pm$ 0.5*	10*
	Onion oil	0	0	0.6 $\pm$ 0.3	4 $\pm$ 0.5	5.3 $\pm$ 0.5
10	Garlic oil	0	2.3 $\pm$ 0.5	9.6 $\pm$ 0.3*	10	-
	Onion oil	0	2 $\pm$ 0.5	4.6 $\pm$ 0.3	8 $\pm$ 0.5	8 $\pm$ 0.5
20	Garlic oil	0	4 $\pm$ 0.5	10*	-	-
	Onion oil	0	3 $\pm$ 0.5	5.3 $\pm$ 0.5	7 $\pm$ 0.5	8.6 $\pm$ 0.3

(*) P < 0.05 is significant between garlic and onion within the same row.

Table (4): Percent of larval deaths (per 120) in garlic / onion oil dissolved in aqueous solution.

Concentration (%)		Number of dead larvae post treatment		
		24hs	48hs	72hs
0	Garlic	0	0	0
	Onion	0	0	0
1	Garlic	0	0	
	Onion	0		
2	Garlic	0	41%	50%
	Onion	0	16.6	16.6
5	Garlic	100%	-	
	Onion	50%	70%	100%
10	Garlic	100%	-	
	Onion	100%	-	
20	Garlic	100%	-	
	Onion	100%	-	

***B. annulatus* engorged females treated with**

A-Garlic oil (cuticle darker in color) after 3 days of treatment

B-Onion oil (cuticle shrinkage 3 days post treatment)

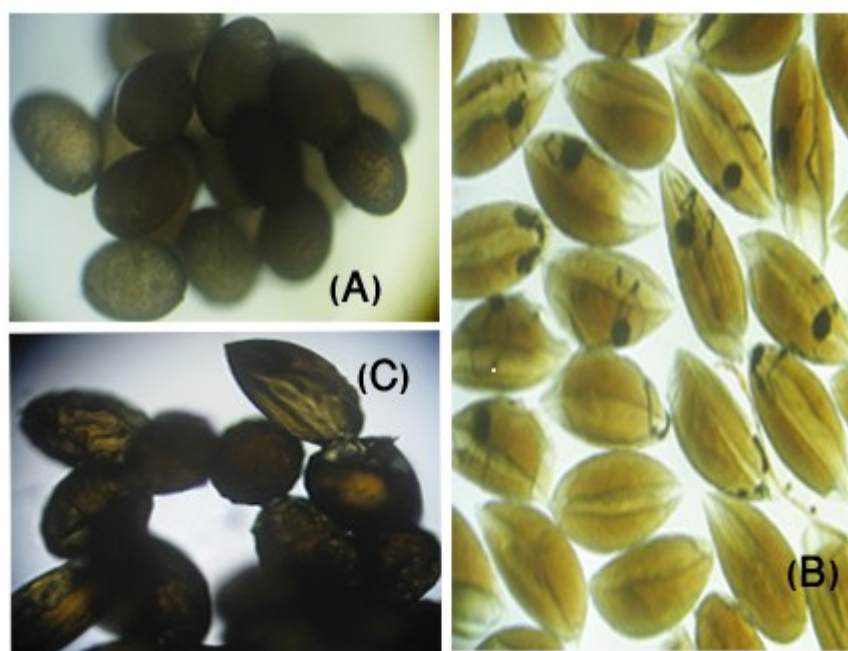


Fig. 1. Eggs of *B. annulatus* treated with

- A-Control one treated by distilled water
- B- Effect of 10% garlic oil
- C-Effect of 10% onion oil

Discussion

The present findings revealed that plant garlic or onion oils could be an alternative tick control method with similar benefits to chemical acaricides.

The study revealed that garlic oil has remarkable acaricidal effect on ticks in vitro. Ten percent concentration aqueous solution, 5% ethanolic and methanolic concentrations of garlic oil caused 100% mortality of adult engorged females *B.annulatus* through 72hs post treatment using the adult immersion method for 2min. Application of garlic oil in aqueous 10% on eggs of *B. annulatus* for 2min and incubation of eggs in 28°C and 85% relative humidity, through 15days no hatching occurred even to 30days. Beside, the eggs appeared with deformities (wrinkling with corrugations in the wall, became spindle in shape and change in color). In addition, the same concentration 10% caused 100% mortality on 15days old larvae of *B. annulatus* through 24hrs post treatment using the larval packet test. In comparable with the control groups either of adults or larvae remained alive without any mortality

and the non treated eggs hatched normally after 12days at the same conditions.

The efficacy of garlic oil as an antiparasitic drug and its insecticidal properties are well documented by **Park *et al.* (2006)**, **Peyghan *et al.*, (2008)**, **Noor El Deen and Mohamed (2009)**. **Birrenkott *et al.* (2000)** reported that repeated topical applications of garlic juice (10%) on hens heavily infested with mite *Ornithonyssus sylvarium* significantly reduced the level of infestation, mainly because of a repellent effect and not direct acaricidal effects. Another study showed that a dichloromethane extract obtained from *A. sativum* bulbs was toxic for adult *Hyalomma marginatum rufipes* and *Rhipicephalus pulchellus* ticks, in a contact toxicity bioassay (**Nchu *et al.* 2005**); in which dichloromethane extract of *A. sativum* demonstrated a high acaricidal bioactivity against *H. m. rufipes* with 100% of ticks killed in less than one hour, and toxicity persisted to the second day. Furthermore, **Maurer *et al.* (2009)** found that pure garlic juice (10 and 100%) inhibited reproduction and significantly reduced survival of female *Dermanyssus gallinae*

mites. **Costa and Furlong (2011)** reported that Enxofre-*Allium sativum* can reduce the intensity of the *R. (B.) microplus* infestation. **Martinez-Velazquez *et al.* (2011)** found that garlic essential oil produced 90% mortality on 10-d-old *R. microplus* larvae, in a concentration as low as 2.5% essential oil. The Organosulfur compounds in garlic such as diallyl disulfide and diallyl trisulfide possess remarkable toxicity on insects (**Kimbaris *et al.* 2009**). **Martinez-Velazquez *et al.* (2011)** suggested that garlic essential oils could be useful as new agents to control tick infestations. Other studies on Garlic, **Madsen *et al.* (2000)** stated that, garlic extract (200 mg L⁻¹) eradicated *Trichodina* infection in eels, **Noor El Deen and Mohamed (2009)** recorded that 800 mg kg⁻¹ diet eliminated all *Trichodina* species in Tilapia, **Abd El-Galil and Aboelhadid (2012)** showed that 2-, 2.5- and 3-ppt garlic oil for 4 hrs treated 100% of the diseased fries with trichodinosis and gyroductylosis, **Kalu *et al.* (2010)** stated that ethanol extract of *Allium sativum* (garlic bulb) exhibited larvicidal activity against the filarial mosquito *Culex quinquefasciatus*. The mode of action of aqueous garlic extract exerts its effect by the oxidation of thiol groups present in the essential proteins, causing inactivation of enzymes and subsequent microbial growth inhibition (**Ghannoum, 1988**).

Among the plant derived lectins, mannose-binding lectins isolated from garlic bulb and leaf (**Smeets, *et al.*, 1997**) have gained attention in recent decades. These are toxic to both chewing (lepidopteran) and sap-sucking (homopteran) type insect pests and considered to be safe in principle due to the edible source of origin (**Sadeghi *et al.*, 2008** and **Mondal *et al.*, 2011**).

Onion killed ticks by 50-80% in aqueous solution. Also the onion solutions in ethanol or methanol achieved similar results by killing 70-86% of treated ticks at the higher concentrations. Moreover ovicidal affect was achieved at concentration 10 % in the aqueous solution of onion oil. In addition, the larvicidal effect was recorded on unfed larvae at concentration 5 and more (10 and 20%). Onions has an anti-

parasitic activity for many helminthes and protozoa such as, *Trichinella spiralis* and *leishmania* (**Saleheen and Yasinzai, 2004**).

The present study concludes that, the application of garlic and onion oils in control of ticks has a promising result for the field application. But the garlic is more potent than onion. In which, the garlic oil had a powerful acaricidal effect on all stages of *R. (B.) annulatus* in the laboratory at aqueous concentration 10 % and 5% in mathanolic or ethanolic solutions due to its effect on adult, eggs and larvae. In addition, garlic has a wide safe margin, available, easy to apply on animals and cheaper so it is a good indicator to be used in the field control of hard ticks. For their practical use, however, further studies are necessary to elucidate their mode of action, side effects, and formulation development to improve their efficacy and stability. Moreover, toxicity studies and in vivo efficacy studies (i.e. field trials) using formulated product are clearly essential, and the economic feasibility of the products has to be demonstrated.

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تأثير زيت الثوم والبصل على المراحل المختلفة من قراد البوفيلس انيولاتس شوقي محمد أبو الحديد – أسماء أحمد كامل – وليد محمود عرفة – خالد أحمد محمد شقير

الملخص العربي

تم في هذا البحث دراسة تأثير زيت الثوم و البصل على المراحل المختلفة من قراد البوفيلس انيولاتس بعد الحصول عليهما عن طريق التقطير بالبخار ثم تخفيف هذه الزيوت في الايثانول والميثانول وجزئيا في الماء بتركيزات (1,2,5,10,20%) تم تطبيق هذه التركيزات على القراد واليرقات والبيض . اناث القراض تم تجميعها من على حيوانات مصابة طبيعيا وحضنت عند 28°م، ورطوبة 85% حتى تضع البيض وتم تحضين البيض بنفس الطريقة لمدة 14 يوم للحصول على اليرقات وقد اظهرت النتائج ان زيت الثوم المذاب في الايثانول والميثانول تركيز (5,10,20%) قتل كل القراد واليرقات في 24 ساعة نفس النتائج مع زيت الثوم المذاب في الماء تركيز (10,20%) كما وجد انه يمنع فقس البيض مع تشوهات في جدار البيض و تغيير في لون البيض الى البني الداكن بينما زيت البصل تركيز (10,20%) في جميع المذبيات كان تأثيره 70:85% على القراد البالغ بينما قتل جميع اليرقات في 24 ساعة ومنع فقس البيض من خلال النتائج نستنتج ان زيت الثوم والبصل لها تأثير قاتل على جميع مراحل قراد البوفيلس انيولاتا بتركيز اعلى من 0.5%