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Comparative in Vitro Study on the Efficacy of Some Commercial Insecticides against *Rhipicephalus (Boophilus) annulatus* Ticks

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ABSTRACT

Commercially available preparations including Butox® 50% (containing 5% deltamethrin), Diazinon 15% and Achook 0.15% (containing 0.15% Azadirachtin) were compared for their acaricidal activity against *Rhipicephalus (Boophilus) annulatus* engorged females obtained from naturally infested cattle from Rashid city, Behera Province, Egypt, and other developmental stages using immersion test. It was found that the highest mortality (86.6%) was achieved by using Diazinon 15% and Achook at concentrations of 4 ml/l and 0.15%, respectively. The complete inhibition of oviposition was observed when using Butox® 50% and diazinon 15% at all used dilutions while Achook caused drop in reproductive index (RI) of tested females reached 75% at concentration of 0.075%. On the other hand, the bioassays on eggs and larvae revealed the superior effect of Achook over Butox® 50% and Diazinon 15%; as the lowest hatchability (0%) of eggs and highest mortality (100%) within the first 24 hours post treatment on unfed larvae was achieved in Achook at both concentrations of 0.15% and 0.075% azadirachtin. Finally, it was concluded that Butox® 50%, Diazinon 15% and Achook 0.15% commercially available pesticides showed variation in percentages of adult tick and larval mortalities, inhibition of oviposition and hatching of laid eggs. Promising results of Achook which is a product for agricultural use and does not have recommended dose for veterinary application yet needs further investigations to be recommended.

Key words:

Comparison, Butox®, Diazinon, Achook, Acaricidal Activity

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1. INTRODUCTION:

Human demand for dairy and beef production is increased day after day as a result of increasing of population. Animal products provide one sixth of human food energy and more than one third of the protein on a global basis (Bradford, 1999). Also, it was estimated that the global average meat consumption is 100 g per person per day (McMichael et al., 2007). Unfortunately, cattle infestation by hard ticks is considered a major problem facing cattle farming. The threats of ticks' infestation include transmission of certain diseases, blood loss and lowering of animal production. Moreover, ticks are considered a biological threat to human health as they serve as invertebrate vector for many zoonotic diseases. The economic loss as a result of ticks' infestation is a pronounced problem as the global costs of ticks and tick- borne diseases in cattle (TTBDs) is between US$ 13.9 and US$ 18.7 billion annually (De Castro, 1997). These losses occur as a result of reduction of weight gain and milk production, deterioration of quality of animal coats and mortalities in severe infestation as well as the costs of eradication of ticks. Presently, tick control is focused on the repeated use of organophosphates and pyrethroid group of acaricides (Magadum et al., 2009). The use of pesticides in the world has increased five folds in the last 30 years (Nawab et al., 2003). However, the unreasonable use of these insecticides may lead to development of acaricide resistant ticks, environmental contamination, and even contamination of milk and meat products with insecticide residues (Graf et al., 2004). The present and future challenge is to get safe, cheap and environmentally friendly alternative pesticide. So, in the current study, the efficacy of two commercially available insecticides (Butox® 50% and Diazinon 15%) widely used in Egyptian veterinary field will be evaluated against the different developmental stages of *Rhipicephalus (Boophilus) annulatus* tick. At the same time, a comparative study will be conducted between their efficacy and the efficacy of Achook® insecticide (a product of agricultural application only) derived from neem (*Azadirachta indica*) seed oil extract (azadirachtine is the main active principle).

2. MATERIAL AND METHODS:

2.1. Tested insecticides:

2.1.1. Butox® 50%:

It is produced by the Arab Company for Chemical Industry, Egypt. It contains 5% deltamethrin active principle. It will be evaluated at two dilutions; 1 ml/l and 2 ml/l.

2.1.2. Diazinon 15%:

It is produced by Adwia Company, Egypt. It contains 150 g/l active principle. It will be evaluated at two dilutions; 4 ml/l and 8 ml/l.

2.1.3. Achook 0.15%:

It is produced by Egyptian Agricultural Development Company, Egypt. It contains 0.15% azadirachtin active principle. It is an insecticide product for agricultural use only and does not have a recommended dose for veterinary application. It will be evaluated at two concentrations; 0.15 and 0.075%.

2.2. Ticks used for in vitro test:

Engorged females of *Rhipicephalus (Boophilus) annulatus* were collected from naturally infested cattle reared in a farm Rashid city, Behera province. They were identified according to their external morphology in the Department of Animal Parasitology, Faculty of Veterinary Medicine, Alexandria University. Eggs were obtained by incubation of some engorged females separately in test tubes at 27 ± 1°C for 14 days and the tubes were supplied by strips of filter paper impregnated with NaCl solution of 15% concentration and plugged with wet cotton that was moistened daily by tap water to keep the needed RH% for incubation. The obtained eggs were incubated under the same conditions for 21 days till hatching to obtain larvae.

2.3. In vitro evaluation of insecticides against different stages of *R. annulatus*:

2.3.1. Adult Immersion Test (AIT), (Drummond et al., 1973):

A group of fifteen engorged females was used to evaluate the efficacy of each selected dilution of the investigated insecticides. Each group was divided into three replicates; each replicate contained five engorged females. Additionally, a control group of untreated fifteen engorged females was kept. The engorged females were immersed into different dilutions of insecticides for a period of one minute (the usual dipping period for animals) then the insecticide solution was discarded and the engorged females were keptin Petridishes padded with filter paper (9 cm in diameter) for 15 minutes for dryness then each female was weighed separately and placed in a test tube to be incubated for 14 days under the same previously mentioned conditions. Finally, the following parameters were calculated:

A. Mortality rate:

Mortality of engorged females was recorded on daily basis. Dead females were confirmed by absolute loss of motility. Mortality rate was calculated according to formula of Abbott, (1925) that was recommended by FAO (2004).

$$\text{Mortality \%} = \frac{\text{No.of females} - \text{No.of dead females}}{\text{No.of females}}\, x\, 100.$$

B. Reproductive index (RI) = egg mass/engorged tick weight

C. Inhibition of oviposition (IO %) (Drummond et al., 1967):

$$\textbf{IO \%} = \frac{\text{RI (control)} - \text{RI (treated)}}{\text{RI (control)}}\, x\, 100.$$

2.3.2. Eggs Immersion Test:

A group of 600 eggs of *R. annulatus* were used to estimate the efficacy of selected insecticides. Each group was divided into three replicates; each replicate contained 200 eggs. Additionally, a control group of untreated eggs was kept. Evaluated dilutions of insecticides were applied to eggs in plastic vials for one minute and then eggs were placed on filter paper for dryness before they were placed in glass test tubes for incubation under the same previously mentioned conditions. For control group, eggs were immersed in distilled water for one minute. Hatchability % and hatching inhibition % (HI %) were calculated according to the equations of Rodriguez and Cob, (2005).

$$\textbf{Hatchability \%} = \frac{\text{No.of hatched larvae}}{\text{No.of eggs}}\, x\, 100.$$

$$\textbf{HI\%} = \frac{\text{hatchability of control} - \text{hatchability of treated}}{\text{hatchability of control}}\, x\, 100.$$

2.3.3. Larval Immersion Test (Shaw, 1966):

A group of thirty unfed larvae of *R. annulatus* were used to estimate the efficacy of selected insecticides. Each group was divided into three replicates; each replicate contained 10 larvae. Additionally, a control group of untreated larvae was kept. Larvae of *R. annulatus* tick were placed in Petridish on a filter paper. 10 ml of insecticides solution were added to the larvae as follow; 6 ml were poured on the larvae then they were covered by another filter paper and the rest 4 ml of the insecticides solution were poured on the added filter paper. One minute contact time is allowed then larvae were transferred to a new Petridish padded with filter paper to allow dryness. Finally, larvae were transferred into test tubes plugged with a piece of wet cotton and incubated at 27 ± 1°C. Concerning the control group, larvae were immersed in distilled water for one minute. Larvae were noticed for detection of mortality over 3 days. Mortality of larvae was estimated according to the equation of Abbott, (1925).

$$\text{Mortality \%} = \frac{\text{No.of larvae} - \text{No.of dead larvae}}{\text{No.of larvae}} x\ 100$$

2.4. Statistical analysis:

The recorded data were analyzed by analysis of variance (ANOVA) using **SAS,(2002)** software, the significance between different treated groups were obtained by Duncan test. All frequencies of mortality and hatchability were analyzed by Chi^2 test to assess the significance between different treated groups.

3. RESULTS:

Although, statistical analysis of results of the efficacy of tested insecticides on engorged females of *R. (B.) annulatus* showed that there were non-significant differences between dilutions of the same insecticide and between dilutions of the different insecticides, it showed high significant difference between the three tested insecticides and the control group (Table, 1). The recorded results clarified that Butox® 50% and diazinon 15% insecticides were highly effective in lowering the reproductive index and completely inhibiting the egg laying by adult females at the two investigated dilutions. Concerning Achook insecticide, the recorded results showed significant differences between concentration of 0.15% and 0.075% as the lower concentration (0.075%) achieved lower reproductive index of tested females and higher oviposition inhibition (75%), (Tables, 2 and 3). Moreover, the presented data in Tables (4), (5) and (6) showed that Achook was the most potent insecticide affecting the developmental stages (eggs and unfed larvae) of *R. (B.) annulatus* when compared to Butox® 50% and diazinon 15% as it achieved 100 % hatching inhibition of eggs and 100 % mortality in unfed larvae within 24 hours at both used concentrations.

Table (1): Efficacy of insecticides on mortality of engorged females of *R. annulatus*

Days PT	Butox® 50%		Diazinon 15%		Achook 0.15%		Control
	1ml/l	2 ml/l	4 ml/l	8 ml/l	0.15%	0.075%	
	Deaths % of ticks post treatment (15 female ticks in three replicates)						
1	0	0	0	0	0	20	0
2	0	0	0	0	0	0	0
3	0	0	0	0	26.66	6.66	0
4	0	0	0	0	0	0	0
5	6.66	0	0	0	0	6.66	0
6	13.33	0	0	0	0	0	0
7	0	0	6.66	6.66	0	0	0
8	0	0	20	13.33	13.33	0	0
9	0	6.66	13.33	13.33	13.33	0	0
10	0	0	6.66	6.66	0	6.66	0
11	6.66	6.66	6.66	6.66	6.66	0	0
12	26.66	6.66	20	13.33	0	20	0
13	6.66	0	6.66	6.66	6.66	6.66	0
14	13.33	20	6.66	0	20	0	0
Efficacy %	73.33	40	86.66	66.66	86.66	66.66	0
Chi^2	3.39 NS		1.68 NS		1.68 NS		29.58*** (P<0.0001)

3.81 NS

PT: post treatment.

Table (2): Efficacy of insecticides on reproductive index of engorged females of *R. annulatus*

Insecticide	Dilutions	Female weight (g)	Egg mass weight (g)	Reproductive index (RI)
Butox® 50%	1ml/l	0.067 ± 0.006^{a}	0.000 ± 0.000^{b}	0.000 ± 0.000^{b}
	2 ml/l	0.073 ± 0.004^{a}	0.000 ± 0.000^{b}	0.000 ± 0.000^{b}
	Control	0.068 ± 0.006^{a}	0.021 ± 0.004^{a}	0.303 ± 0.045^{a}
Diazinon 15%	4 ml/l	0.067 ± 0.003^{a}	0.000 ± 0.000^{b}	0.000 ± 0.000^{b}
	8 ml/l	0.053 ± 0.003^{b}	0.000 ± 0.000^{b}	0.000 ± 0.000^{b}
	Control	0.068 ± 0.006^{a}	0.021 ± 0.004^{a}	0.303 ± 0.045^{a}
Achook 0.15%	0.075%	0.037 ± 0.003^{b}	0.004 ± 0.001^{c}	0.099 ± 0.031^{c}
	0.15%	0.081 ± 0.006^{a}	0.016 ± 0.003^{b}	0.213 ± 0.042^{b}
	Control	0.066 ± 0.007^{a}	0.028 ± 0.004^{a}	0.397 ± 0.040^{a}

Means bearing different letters within the same column within the same insecticide are significant at ($P<0.05$).

Table (3): Efficacy of insecticides on the oviposition of *R annulatus* engorged females

Insecticide	Dilutions	Reproductive index (RI)	Control group RI.	Oviposition inhibition (OI %)
Butox® 50%	1 ml/l	0	0.3	100
	2 ml/l	0	0.3	100
Diazinon 0.15%	4 ml/l	0	0.3	100
	8 ml/l	0	0.3	100
Achook 0.15%	0.075 %	0.1	0.4	75
	0.15 %	0.2	0.4	50

Table (4): Efficacy of insecticides on Hatchability % of *R. annulatus* egg

Insecticide	Dilutions	Hatchability %	Chi-square	
Butox® 50%	1 ml/l	0.666	416.31***	
	2 ml/l	0.833	(P<0.0001)	249.40***
Control	--------	33.5		(P<0.0001)
Diazinon 0.15%	4 ml/l	17.33		
	8 ml/l	5.333	50.91*** (P<0.0001)	
Control	--------	17.5		
Achook 0.15%	0.075 %	0		
	0.15 %	0	475.47*** (P<0.0001)	
Control	--------	35		

Table (5): Efficacy of insecticides on the hatching inhibition of *R. annulatus* egg

Insecticides	Dilutions	Hatchability %	Control group hatchability %	Hatching inhibition (HI %)
Butox® 50%	1 ml/l	0.66	33.5	98
	2 ml/l	0.83	33.5	97.5
Diazinon 15%	4 ml/l	17.3	17.5	1.14
	8 ml/l	5.3	17.5	69.7
Achook 0.15%	0.075 %	0	35	100
	0.15 %	0	35	100

Table (6): Efficacy of insecticides on mortality of larvae of *R. annulatus*

Insecticides	Dilutions	Number of larvae	No of dead larvae			Total mortality after 3 days PT	Mortality %	Chi^2
			1st day PT	2nd day PT	3rd day PT			
Butox 50%	1 ml/l	30	14	7	7	28	93.3	17.55**
	2 ml/l	30	21	4	4	29	93.3	(P<0.001)
Diazinon 0.15%	4 ml/l	30	23	2	5	30	100	30.39***
	8 ml/l	30	30	0	0	30	100	(P<0.0001)
Achook 0.15%	0.075 %	30	30	0	0	30	100	30.39***
	0.15 %	30	30	0	0	30	100	(P<0.0001)
Control		30	3	8	6	17	56.6	
Chi^2	60.80***(P<0.0001)							

4. DISCUSSION:

Presently, tick control is achieved by the repeated use of organophosphates and pyrethroid group of acaricides (Magadum et al., 2009). Organophosphorus compounds have replaced organochlorine compounds because of the persistence and accumulation of the latter in the environment (Bavcon et al., 2003) but now synthetic pyrethroids are becoming predominant insecticides for agricultural and urban applications (Holmes, et al., 2008). The continued usage of the same compound for controlling certain insect for long period resulted in developing resistance against the used compound. Many publications documented the resistance of hard tick species to Diazinon; Li et al., (2003), Miller et al., (2008), Fernández-Salas et al., (2012) and Alonso-Díaz et al., (2013) and deltamethrin; Brito et al., (2011), Sharma et al., (2012) and Shyma et al., (2015).So, the current trend in the world now is the use of natural products to overcome the drawbacks of currently used chemicals. In the last decades, plant extracts were widely used against phytophagus pests and mosquitoes (Balandrin et al., 1995). The effect of neem extract on hard ticks was investigated by Rice, (1993), Magadum et al., 2009, Broglio-Micheletti et al., (2010) and Garcia, (2012) and their results revealed a considerable efficacy against ticks.

The obtained results in the current study revealed that diazinon 15% at recommended dilution by manufacturer (4 ml/l) and Achook (0.15% azadirachtin) achieved the highest efficacy (86.6%) against *R. (B.) annulatus* engorged females, while Butox® 50% (1 ml/l) achieved (73.3%) efficacy. These results were in agreement with that of Fernandes, (2001) who treated *B. micropilus* ticks with 25 and 50 ppm of deltamethrin and found that the mean death rates were 76.3 and 87.5 %, respectively after one day of treatment. However, mortalities of engorged females in the current study began at 4th day post treatment, Brito et al., (2011) who found that the mean efficacy of the deltamethrin was 67.10±4.70 % when evaluated using AIT for controlling *R. microplus* and Shyma et al., (2015) who concluded that higher concentration of deltamethrin would be required for causing significant mortality. Although, result in the current study revealed lower efficacy (40%) when double dose of deltamethrin was used (2 ml/l). On the other hand, the obtained results disagreed with Mehlhorn et al., (2010) who noticed a superior efficacy of deltamethrin as acaricide against ticks and recommended the use of Butox® at intervals of 3 weeks, Fernández-Salas et al., (2012) who recorded mortalities of 94.2 % in the *R. microplus* population at the discriminated dose of deltamethrin. Moreover, El-Bahy, et al., (2015) recorded 100 % efficacy of Butox® 50% on the adult *B. annulatus* ticks at 3 hours post exposure. In addition, Ramadan, (2009) found that dipping of chicken with deltamethrin 0.05% caused prolonged complete (100%) reduction in the number of ticks till the end of the 3rd week post dipping. These variations between results of the current study and other studies may be attributed to the developed resistance of deltamethrin and the species of investigated ticks.

Concerning Diazinon 15% efficacy on adult ticks, El-Bahy et al., (2015) recorded that diazinon 15% (1 ml/l) reached (44%) efficacy on the adult *B. annulatus* within the first hour and became 100 % at 6 hours post treatment. This result disagreed with the result of diazinon 15% (4ml/l) in the current study as mortalities of adult ticks started at 7th day post treatment and the highest efficacy (86.6%) was achieved at 14th day post treatment (the end of the experiment). The resistance of hard ticks to diazinon insecticide was also recorded by Li et al., (2003), Miller et al., (2008), Fernández-Salas et al., (2012) and Alonso-Díaz et al., (2013). The rational explanation of this variation in results may be due to the continuous use of the same insecticide for long period that lead to resistance development by ticks. Achook insecticide (Azadirachtin 0.15%) also achieved its highest efficacy (86.6%) at concentration of 0.15% on the engorged females at the 14th days post treatment. This result was in harmony with that of Rice, (1993) who mentioned that a monthly spray of ethanolic azadirachtin (3000 ppm) was effective in controlling the cattle tick, *Boophilus microplus* and slightly higher than results obtained by Maske and Bhilegaonkar, (1995) who reported 70% efficacy of herbal preparation containing extracts of *Cedrus deodara*, *Azadirachta indica* and *Embelic* against Ixodid ticks and Broglio-Micheletti et al., (2010) who analyzed the efficacies of a hexane neem extract and a 2% oil concentration against *R. (B.) microplus* in vitro and found that neem extract achieved 73.2% efficacy while 2% oil recorded 65% efficacy. Also, Kaaya and Saxena, (1998) observed that undiluted neem oil containing (0.08 %) azadirachtin (active principle) was effective against *R. appendiculatus, A. variega tum,* and *B. decoloratus*. Finally, Kumar et al., (2016) found that Zerokeet (a commercial product in liquid form containing extracts of *Cedrus deodara, Pongamia glabra, Azadiracta indica, Eucalyptus globulus and Acoruscalamus*) showed an efficacy of 41.8–75.4 % using recommended dilution (1:2) against field ticks and resistant tick lines. In contrast, Khan et al., (2001) evaluated the acaricidal efficacy in naturally infested birds with *Argus persicus* and found that *Azadirachta indica* (20%) extract achieved 34.63 % efficacy and *Azadirachta indica* (40%) extract reached 51.89% efficacy which were lower than the results obtained in this study. As the same, Garcia, (2012) assessed the efficacy of commercial neem oil (*Azadirachta indica*) using AIT on adult *R. (B.) microplus* and *A. miniatus* at concentrations of 5, 10, 20 and 40 %. His results showed efficacies on *R. (B.) microplus* of 3, 16, 34 and 46%, respectively. While on *A. miniatus* efficacies reached 96, 100, 100 and 100. This explain that the efficacy of the same insecticide may be different due to different species of ticks what was confirmed by Al-Rajhy et al., (2003) who found out that the LC_{50} for both azadirachtin and neem oil against adult camel tick, *Hyalomma dromedarii*, either by contact or by dipping, was >5000 mg/L. Results also showed that both Butox® 50% and Diazinon 15% insecticides at both used dilutions for each one were very effective in lowering the reproductive index to (0) and completely inhibit the egg laying by adult females. These results were slightly higher than results obtained by Ravindran et al., (2014) who observed 93.37% and 91.7% inhibition of fecundity at the manufacturer recommended dosage of two commercial preparations of cypermethrin and Trevino, (1976) who recorded 86.7 % oviposition achieved by diazinon (250 µg/ml). Also, Eshetuet al., (2013) who studied the in vitro efficacy of diazinon 0.06% against *Rhipicephalus pulchellus* and *Amblyomma gemma* using modified adult immersion test and found that after 7 days incubation of females, Diazinon 0.06% cause control of oviposition by 88.85% and 71.41% in *A. gemma* and *R. pulchellus*, respectively. The results of the current study also confirmed by Ahanger et al., (2015) who observed that the increase in concentration of the deltamethrin could not cause mortality in all the exposed ticks but the egg laying capacity or the efficacy of conversion of live weight into egg mass decreased among the surviving ticks. On the other hand, these results were disagreed with results obtained by Kumar et al., (2016) who tested Butox® 0.003 % Vet (containing 1.25% deltamethrin) against field ticks and resistant tick lines and found that the product led to 1.5–7.7 % inhibition of oviposition in resistant tick lines. Achook insecticide at concentration of (0.075% azadirachtin) achieved lower reproductive index of tested females and higher oviposition inhibition (75%). Also, Kalakumar et al., (2000) reported significant effect of commercially available neem oil and custard oil on egg-laying capacity of *H. anatolicum*, *Rhipicephalus haemophysaloides*, and *B. microplus*, Srivastava et al., (2008) recorded a significant reduction of the reproductive index (RI) of the ticks treated with 8% concentration of neem extract in comparison to the ticks of control group. The higher concentration (0.15% azadirachtin) achieved only 50% oviposition inhibition. Similar result was obtained by Williams, (1993) who tested the efficacy of extracts from the *Azadirachta indica* plants on egg laying in the *Boophilus microplus* tick and achieved 50% inhibition of egg laying by a dose of 0.46 pg crude ethanol extract per tick. Moreover he noticed that the oocytes of the treated ticks were about three to six times smaller and lighter in color than those of the controls. A significant portion (60%) of the egg masses produced were malformed (sickle shaped) when ticks were treated with 1.15 pg of the extract. Arthur, (1962) suggested that The malformation of tick eggs may be due to the action of neem extracts on the activity of Gene's organ in ticks which produces an extracellular lipid-protein complex that is important in the water retention capacity of eggs. Sazena, (1993) and Williams and Mansingh, (1996) also concluded that neem extract and azadirachtin seem to disrupt oviposition of insects and act as chemosterilant agents. Moreover, Areaf, (2002) carried out an investigation on *Labidura riparia* and found that azadirachtin interrupted the female ovarian development in a dose-dependent manner. On contrast, the results obtained by Kaaya and Saxena, (2000) revealed lower values of oviposition inhibition (30 – 45 %) of neem oil applied directly on ticks attached to animals (rabbits and cattle). The eggs sensitivity was extremely varied to the different tested insecticides; the highest hatchability % of eggs was recorded in diazinon groups especially group treated by 4 ml/l dilution that was translated in turn to 1.14% hatching inhibition. While Butox® 50% led to 0.66% and 0.83% hatchability for dilutions of 1 ml/l and 2 ml/l, respectively. That reflected hatching inhibition values of 98% and 97.5%, respectively. The lowest hatchability (0%) was achieved in Achook treated groups at both concentrations 0.15% and 0.075% azadirachtin which led to the highest recorded hatching inhibition (100%) among the tested groups. These results confirmed that the azadirachtin insecticide is more efficient on developmental stages of insects than on adult stages. This also was mentioned by Sazena, (1993), Williams and Mansingh, (1996) and Al-Rajhi et al., (2000). Also, this result was approximately near to results obtained by Williams, (1993) who recorded 80% hatching failure of *Boophilus microplus* eggs when he used the extracts from the *Azadirachta indica* plants at dose of 0.46 pg crude ethanol extract per tick. While Kaaya and Saxena,(2000) obtained very lower results than the current study as they found that neem oil applied directly on ticks attached to animals (rabbits and cattle) was succeeded to reduce egg hatchability by (47–55 %) and molting of the larvae (22 – 93 %), and nymphs (98 %). They also noticed that most of the eggs were sterilized when exposed directly to various concentrations of the oil. Also, Abdel-Shafy and Zayed (2002) found that Neem Azal F (5% Azadirachtin containing insecticide) at 1.6% and 3.2% significantly increased the hatching rate of *Hyalomma anatolicum excavatum* eggs during the first 7 days post-treatment giving incompletely developed and dead larvae, while in this study no signs of hatching was noticed at all. Larvae of *R. (B.) annulatus* were highly affected by tested insecticides than adult ticks. Azadirachtin proved its superiority over deltamethrin and diazinon on its effect of developmental stages of insects for the second time. As it led to 100% mortality within the first 24 hours post treatment at both concentrations 0.15% and 0.075%. This result is similar to results obtained by Kaaya and Saxena, (2000) who also found that feeding neem seed powder mixed with rabbit pellets (100:400 g) to goats daily for three weeks completely inhibited attachment and development of *B. decoloratus* larvae. Also with Garcia, (2012) who recorded 100% efficacy of commercial neem oil (*Azadirachta indica*) on *A. miniatus* larvae using the larval packet test (LPT). On contrast, Al-Rajhy et al. (2003) concluded that azadirachtin showed some effects on larvae of camel tick, *Hyalomma dromedarii*. Moreover, he found that at 2500 mg/l, azadirachtin caused prolonged period required for molting to nymphal stage in comparison with the control group, 24–31 and 15–21 days, respectively. Diazinon insecticide also at both dilutions (4 ml/l and 8 ml/l) recorded 100% efficacy on *R. (B.) annulatus* larvae at the 3rd day post treatment which was in agreement with Trevino, (1976) who found that diazinon at concentration of 250 µg/ml caused 100% larval mortality after 3 days of *B. microplus*. While Butox® 50 insecticide reached 93.3% efficacy at both dilutions (1 ml/l and 2 ml/l) also after 3 days post treatment and this result was in harmony with results obtained by Fernandes et al., (2001) who found that the lethal capacity of deltamethrin against hard tick larvae of *R. sanguineus* after 30 hours was low, and the mean mortality percentages were 34, 40.2, 46, and 57.6 % after treatment with 0.5, 1, 2, and 3 ml/l, respectively. While disagreed with Cetin et al., (2009)who exposed larvae of *A. persicus* for 15 min to ceramic tiles treated at field application rates with deltamethrin (Impotek DMT_EW, 0.01 g ai/m^2) and they found that the deltamethrin achieved complete mortality at 1 hour post treatment. This difference may return to the difference in morphological structure between soft and hard ticks.

Finally, it was concluded that commercial formulations of Butox® 50%, Diazinon 15% and Achook 0.15% were compared *in vitro* for their acaricidal activity against different stages of *Rhipicephalus (Boophilus) annulatus* using immersion test. Diaziono and Achook achieved the same efficacy on engorged females, while oviposition was completely inhibited by application of Butox® 50 and Diazinon 15% insecticides, in addition Achook proved its efficiency over Butox® 50 and Diazinon 15% on the tick developmental stages (eggs and unfed larvae). Promising results of Achook which is a product for agricultural use and does not have recommended dose for veterinary application yet needs further investigations to be recommended.

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