

Original Article***Rhipicephalus annulatus* (Acari: Ixodidae) Control by *Nigella sativa*, Thyme and Spinosad Preparations*****Shawky Mohamed Aboelhadid¹, Hesham A Mahran², Hazem M El-Hariri³, Khalid Mohamed Shokier⁴**¹Department of Parasitology, Faculty of Veterinary Medicine, Beni-Suef University, Egypt²Department of Hygiene, Management and Zoonoses, Faculty of Veterinary Medicine, Beni-Suef University, Egypt³Department of Community Medicine Research, National Research Center, Egypt⁴Animal Health Research, Beni-Suef Branch, Beni-Suef, Egypt

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Abstract**Background:** Several compounds obtained from plants have potential insecticidal, growth deterrent or repellent characteristics. The control of hard ticks by non-chemical substances was targeted in this study.**Methods:** The effect of 36 materials on in-vitro ticks was studied, including 2 absolute controls (water only or absolute ethyl alcohol only), 6 conventionally used spinosad preparations (aqueous solutions), 12 *Nigella sativa* (*N. sativa*) preparations (aqueous and alcoholic solutions), and 12 Thyme preparations (aqueous and alcoholic solutions). The engorged ticks were tested in-vitro for mortality and oviposition ability using the studied materials.**Results:** The final mortality after 48 hours of application in *N. sativa* aqueous preparations began from 10.0% concentration, 1.0% to 100% by concentration preparations 10%. In addition, *N. sativa* alcoholic preparations began from 50.0% concentration, 2% to 100% by concentration 5%. Meanwhile, Thyme aqueous and alcoholic preparations began from 70.0% concentration, 5% to 90% by concentration 10–20%. Additionally, spinosad aqueous preparations and both of control preparations (Water and Alcohol) resulted in no mortality. All differences were statistically significant. The oviposition was stopped in *N. sativa* (aqueous 10% and alcoholic 5%) and in spinosad (aqueous 25%). The aqueous dilution of the used matters killed *B. annulatus* larvae beginning from the concentration 5%.**Conclusion:** *Nigella sativa* alcohol 20% was the best of studied preparations being the lowest concentration (20%) that could achieve the highest lethal (100%) effect in shortest time (12 hours). Moreover, Thyme oil and spinosad could not kill 100% of adult but did on larvae.**Keywords:** *Rhipicephalus annulatus*, *Nigella sativa*, Thyme oils**Introduction**

Rhipicephalus annulatus (formerly *Boophilus annulatus*) represents one of the main risks for the cattle industry in Egypt. Tick control is more difficult due to the presence of resistant populations to all major families of acaricides (Rodriguez-Vivas et al. 2006). Chemical control is a common strategy used in daily life. Synthetic insecticides became the common used forefront of insect-controlling agents. However, the environmental threat that these chemicals pose, effects on non-

target organisms, and the resistance of insect to insecticides have all raised during the last five decades (Wattanachai and Tintanon 1999, Amer and Mehlhorn 2006). There is an increasing need to develop insecticides for controlling insect, which are more environmentally safe and also biodegradable and target-specific against the mosquitoes. Several compounds obtained from plants have potential insecticidal, growth deterrent or repellent characteristics (Isman 2006, Pavela 2008).

Essential oils, secondary metabolites of the plants, are odor components that can be extracted from plant tissue by water steam distillation or supercritical fluid extraction. Most of them are complex mixtures of mono- and sesquiterpenes and biologically related phenolic compounds. Essential oils and their volatile constituents are commonly used in the prevention and treatment of human illnesses. Various essential oils (garlic oil, onion oil, etc) have also been documented to exhibit acute toxic effects against insects (Aboelhadid et al. 2013).

Nigella sativa is an herbal plant grows in countries of the Mediterranean Sea area, Pakistan and India. The seed of it is known as black seed, HabbatuSawda, and Habatul Baraka “the Blessed Seed”. It is referred to the prophet Mohammed as having healing powers. The essential oil in its extract was shown to have antihelminthic effect, antinematodal and antischistosomal (Mahmoud et al. 2002). There is no available data about the application of this oil on ticks.

Thyme (*Thymus vulgaris* L), a perennial labiate, is endemic to the Mediterranean area. Its components and quality vary with geographical distribution, growing states, and stage and method of extraction (Stahl-Biskup and Sáez 2002). The biological activity of Thyme was found to have insecticide potential of obtained extracts using both organic solvents and hydro-distillation (Regnault-Roger et al. 1993, Isman et al. 2001, Knio et al. 2008). Some essential *T. vulgaris* chemotypes or the essential oils obtained from them had larvicidal and adulticidal efficiency. There is no previous work to investigate its effect on ticks.

Spinosad was first registered for agricultural/horticultural use in the late 1990s and by 1999; it was approved for use on over 100 crops in 24 different countries (Thompson et al. 2000). Being a natural product derived from fermentation of the microorganism *Saccharopolyspora spinosa* (Mertz and

Yao 1990), spinosad has several favorable characteristics for a pesticide. For example, Anastas et al. (1999) reported that spinosad will not bio-accumulate volatilize or persist in the environment and will degrade naturally when exposed to light. In addition, spinosad displays activity against a range of insect pests, especially those in the genera of Lepidoptera, Diptera and Thysanoptera, and to a lesser extent the Coleoptera and Orthoptera (Thompson et al. 2000). Spinosad gives highly efficacious control of many caterpillars of lepidopteran insects on numerous crops, including cotton, apples, sweet corn, potatoes, fruiting/leafy vegetables and tobacco, however, Spinosad is relatively non-toxic to most beneficial insects and mites (DeAmicis et al. 1997).

The present investigation aimed to evaluate the acaricidal effect of *N. sativa* and Thyme oils and also spinosad against adult and larvae of *R. annulatus* through using different dilutions and different solvents.

Materials and Methods

Sampling of *R. annulatus*

In summer 2012, engorged females ticks were carefully detached from naturally infested cattle according to Walker et al. (2003). The collected ticks were kept alive in a special glass tubes closed with hydrophobic cotton a strip of filter paper soaked in 15 % NaCl. In the laboratory of Parasitology, Faculty of Veterinary Medicine, Beni Suef University, the tubes were labeled, incubated at 28 °C and relative humidity of 85 % for further studies.

Procedure of biological studies

Each engorged female tick was put separately in glass tube contains a filter paper soaked in 15% NaCl, was put to give a relative humidity of 85–90% (BOD incubator, Felp^R), then the tubes were closed tightly with a cotton tampon and were labeled (ani-

mal host, date, tick species), and were kept in an incubator at the same conditions during the whole period of the experiments. The tubes were observed daily to record the duration of the preoviposition and oviposition periods, daily egg output then the eggs were placed in sterile disposable syringes, identified, plugged with cotton balls and were placed in the incubator under the previously mentioned conditions to observe the hatching. The pre-hatching and hatching periods were noticing and recording.

Oils extraction from plants

The used oils of *N. sativa* and Thyme were obtained by cold squeezing of the plant seeds. In this work, the oils were obtained from Royal herbal company.

Preparation of alcoholic and aqueous solutions of the used oils

Five concentrations of (1, 3, 5, 10 and 20%) were prepared for oils (*N. sativa* and thyme), were diluted by distilled water or ethyl alcohol as solvents. The control individuals were treated with ethyl or distilled water only.

Preparation of spinosad (Tracer^R) for applied on adult tick

This product was used in agriculture for control of insects. Different concentrations were prepared from this product according the label of the manufacturing (2 ul, 20 ul and 40 ul for 50 ml water). The higher concentrations 10%, 25 % and 50% were tested.

Application methods for treatment of ticks with the used oils and product:

Adult immersion test (AIT)

Semi and fully engorged females of *Rhipicephalus (Boophilus) annulatus*, *B. annulatus* were used in the present study were to adult immersion test (AIT) as described in Drummond et al. (1973) and Benavides et al. (1999), the engorged females

of *R. annulatus*, after being washed in a sieve with tap water, 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 oils, for 2 min. The control group was also composed of 10 engorged females that had been immersed in alcohol or distilled water for the same period. Ticks were then 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 hrs and 48hs post treatment.

Evaluation of the acaricidal efficiency of the used materials

The mortality rate of treated adults was reported by noticing the number of dead ticks for each concentration. The oviposition ability of the treated engorged females and the ability of these eggs to hatch were investigated. Dead ticks were diagnosed based on three criteria, signs of cuticle darkness and hemorrhagic skin lesions, leg movement, and halted Malpighian tube movement. The legs movement was tested with a paintbrush with ticks placed in an inverted position under a stereomicroscope lamp. The change of cuticle color and hemorrhagic skin lesions were demonstrated in dead ticks. Failure in egg laying was then directly observed by eye (Chungsamarnyart and Jansawan 2001).

Larval immersion test

The different concentrations of the used oils in aqueous solution were screened against the unfed (15 days old) larvae. The larval immersion technique was proposed by Shaw (1966). A group of approximately 600 larvae, 5 replicates in each one 120 larvae for each concentration and control, were distributed on a 14 cm diameter of filter paper lying in a Petri dish. Five milliliters of each concentration was poured over the larvae. Another 14 cm diameter filter paper was

placed over the larvae and 5 ml of the extract poured over it. After the immersion period of 5 min, the larvae were removed from the filter paper and were placed in a folded filter paper (5×10 cm) with the openings closed with a tape and then were incubated at 28 °C and 85% relative humidity (Borges et al. 2003).

Statistical analysis

The collected data were coded, tabulated, and statistically analyzed using SPSS program (Statistical Package for Social Sciences) software version 18.0. The data was independent categorical, descriptive statistics were done as number and percentage, while Inferential analyses were done using Chi square. The level of significance was taken at $P < 0.050$ is significant, otherwise is non-significant.

Results

The effect the used materials on adult *R. annulatus*

No lethal effect were noticed in ticks that were subjected to the 2 absolute control (water only and ethanol only) and the 6 conventionally used Tracer preparations (Aqueous solutions of different concentrations, 2 µl/50 ml, 20 µl/50 ml, 40µl/50 ml, 10%, 25% and 50%).

None of the studying materials produced any lethal effects 6 hours after application.

The highest lethal effect 12 hours after application was *N. sativa* aqu (50% and 100%), *N. sativa* alc (20%, 50% and 100%) this was significantly higher than *N. sativa* aqu 20% and *N. sativa* alc 10% that reached 90% death.

The final evaluation of different materials is 48 hours after application of each material. The most lethal materials (100% death) were *N. sativa* aqu (10%, 20%, 50% and 100%), *N. sativa* alc. (10%, 20%, 50% and 100%), this was significantly higher than the lesser lethal material (90% death) produced by Thyme aqu (10% and 20%) and Thyme alc (10% and 20%), they also significantly higher than the lesser lethal material (70% death) produced

by *N. sativa* aqu 5% and Thyme alc 5%), they also significantly higher than the lesser lethal material (50% death) produced by *N. sativa* alc 3%), they also significantly higher than the lesser lethal material (10% death) produced by *N. sativa* aqu 3%, the other materials produced no lethal effects (Table 1).

The effect on egg oviposition of *R. annulatus*

The oviposition of live ticks after treatment, the live ticks after application of *N. sativa* oil stopped egg oviposition in aqu (10%, 20%, 50% and 100%), *N. sativa* alc (5%, 10%, 20%, 50% and 100%). By following the ability of the produced eggs from treated live ticks, the eggs hatched in the expected period of normal eggs from untreated ticks (14 days).

The live ticks subjected to different concentrations of Thyme oils oviposited normal eggs just after application (24 h). These eggs hatched in the mean time after incubation.

At the lower concentrations of spinosad (Tracer^R) the ability of treated ticks to oviposition did not affect and the produced eggs were normal in quantity and time of oviposition. These produced eggs hatched in the expected time of the normal eggs from untreated ticks, while the oviposition activity of treated ticks stopped at the higher concentrations (25% and 50%) where the ticks became to shrinkage, contracted but not dead and it did not produced eggs (Table 3).

The effect on larvae of *R. annulatus*

No lethal effect was noticed in ticks that were subjected to the absolute control (water only) and *N. sativa* aqueous 1% and Thyme aqueous 1%. The lethal effect was significantly variable among different concentrations of aqueous concentrations of *N. sativa*, Thyme and Spinosad, it reached 100% in aqueous concentrations of *N. sativa* 5% and Spinosad 20µl/50ml while none of aqueous concentrations of Thyme showed that level of mortality (Table3).

Table 1. Effect of different materials on Ticks viability

S	Material	6 hours	12 hours	24 hours	48 hours
1	Water only	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
2	Ethanol only	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
3	N. sativa aqu 1%	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
4	N. sativa aqu 3%	0 (0%)a	0 (0%)a	5 (10%)b	5 (10%)b
5	N. sativa aqu 5%	0 (0%)a	25 (50%)c	35 (70%)d	35 (70%)d
6	N. sativa aqu 10%	0 (0%)a	35 (70%)d	45 (90%)e	50 (100%)f
7	N. sativa aqu 20%	0 (0%)a	45 (90%)e	50 (100%)f	50 (100%)f
8	N. sativa aqu 50%	0 (0%)a	50 (100%)f	50 (100%)f	50 (100%)f
9	N. sativa aqu 100%	0 (0%)a	50 (100%)f	50 (100%)f	50 (100%)f
10	N. sativa alc 1%	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
11	N. sativa alc 3%	0 (0%)a	5 (10%)b	25 (50%)c	25 (50%)c
12	N. sativa alc 5%	0 (0%)a	25 (50%)c	35 (70%)d	50 (100%)f
13	N. sativa alc 10%	0 (0%)a	45 (90%)e	50 (100%)f	50 (100%)f
14	N. sativa alc 20%	0 (0%)a	50 (100%)f	50 (100%)f	50 (100%)f
15	N. sativa alc 50%	0 (0%)a	50 (100%)f	50 (100%)f	50 (100%)f
16	N. sativa alc 100%	0 (0%)a	50 (100%)f	50 (100%)f	50 (100%)f
17	Thyme aqu 1%	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
18	Thyme aqu 3%	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
19	Thyme aqu 5%	0 (0%)a	0 (0%)a	25 (50%)c	35 (70%)d
20	Thyme aqu 10%	0 (0%)a	0 (0%)a	40 (80%)d,e	45 (90%)e
21	Thyme aqu 20%	0 (0%)a	0 (0%)a	40 (80%)d,e	45 (90%)e
22	Thyme aqu 50%	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
23	Thyme aqu 100%	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
24	Thyme alc 1%	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
25	Thyme alc 3%	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
26	Thyme alc 5%	0 (0%)a	0 (0%)a	25 (50%)c	35 (70%)d
27	Thyme alc 10%	0 (0%)a	0 (0%)a	40 (80%)d,e	45 (90%)e
28	Thyme alc 20%	0 (0%)a	0 (0%)a	40 (80%)d,e	45 (90%)e
29	Thyme alc 50%	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
30	Thyme alc 100%	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
31	Spinosadaqu μ l/50ml	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
32	Spinosad aqu20 μ l/50ml	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
33	Spinosad aqu40 μ l/50ml	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
34	Spinosadaqu 10%	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
35	Spinosadaqu 25%	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
36	Spinosadaqu 50%	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a

alc: Alcoholic form, **aqu:** Aqueous form.

%: Percentage of died ticks in relation to studied sample (50) of each group,

a,b,c,d,e,f: Homogenous groups so that, similar letters are not-significantly different, while different letters are significantly different.

Table 2. Effect of different materials on Ticks oviposition ability

S	Material	Oviposition
1	Water only	Normal
2	Ethanol only	Normal
3	<i>N. sativa</i> aqu 1%	Normal
4	<i>N. sativa</i> aqu 3%	Normal
5	<i>N. sativa</i> aqu 5%	Normal
6	<i>N. sativa</i> aqu 10%	Stopped
7	<i>N. sativa</i> aqu 20%	Stopped
8	<i>N. sativa</i> aqu 50%	Stopped
9	<i>N. sativa</i> aqu 100%	Stopped
10	<i>N. sativa</i> alc 1%	Normal
11	<i>N. sativa</i> alc 3%	Normal
12	<i>N. sativa</i> alc 5%	Stopped
13	<i>N. sativa</i> alc 10%	Stopped
14	<i>N. sativa</i> alc 20%	Stopped
15	<i>N. sativa</i> alc 50%	Stopped
16	<i>N. sativa</i> alc 100%	Stopped
17	Thyme aqu 1%	Normal
18	Thyme aqu 3%	Normal
19	Thyme aqu 5%	Normal
20	Thyme aqu 10%	Normal
21	Thyme aqu 20%	Normal
22	Thyme aqu 50%	Normal
23	Thyme aqu 100%	Normal
24	Thyme alc 1%	Normal
25	Thyme alc 3%	Normal
26	Thyme alc 5%	Normal
27	Thyme alc 10%	Normal
28	Thyme alc 20%	Normal
29	Thyme alc 50%	Normal
30	Thyme alc 100%	Normal
31	Spinosadaqu 2µl/50ml	Normal
32	Spinosad aqu20µl/50ml	Normal
33	Spinosad aqu40µl/50ml	Normal
34	Spinosadaqu 10%	Normal
35	Spinosadaqu 25%	Stopped
36	Spinosadaqu 50%	Stopped

Normal means the quantity and time of egg deposition of untreated normal tick.

All ticks that were subjected to each material gave the same response (normal/ stopped) oviposition

Table 3. Effect of different concentrations of in aqueous solution on tick larvae after 24 h (n=100 larvae in 5 replicates)

S	Material	24 hours
1	Water only	0 (0%)a
2	<i>N. sativa</i> aqu 1%	0 (0%)a
3	<i>N. sativa</i> aqu 3%	338 (67.6%)d
4	<i>N. sativa</i> aqu 5%	500 (100%)g
5	<i>N. sativa</i> aqu 10%	500 (100%)g
6	<i>N. sativa</i> aqu 20%	500 (100%)g
7	<i>N. sativa</i> aqu 50%	500 (100%)g
8	<i>N. sativa</i> aqu 100%	500 (100%)g
9	Thyme aqu 1%	0 (0%)a
10	Thyme aqu 3%	145 (29.0%)b
11	Thyme aqu 5%	251 (50.2%)c
12	Thyme aqu 10%	342 (68.4%)d
13	Thyme aqu 20%	417 (83.4%)e
14	Thyme aqu 50%	426 (85.2%)e, f
15	Thyme aqu 100%	435 (87.0%)e, f
16	Spinosadaqu 2µl/50ml	446 (89.2%)f
17	Spinosad aqu20µl/50ml	500 (100%)g
18	Spinosad aqu40µl/50ml	500 (100%)g
19	Spinosadaqu 10%	500 (100%)g
20	Spinosadaqu 25%	500 (100%)g
21	Spinosadaqu 50%	500 (100%)g

aqu: Aqueous form

N: The total number of died larvae

%: Percentage of died ticks in relation to studied sample (500) of each group,

a,b,c,d,e,f,g: Homogenous groups so that, similar letters are not-significantly different, while different letters are significantly different.

Discussion

Most plants contain compounds that they use in preventing attack from phytophagous (plant eating) insects. These compounds fall into several categories, including repellents, feeding deterrents, toxins, and growth regulators. It can be grouped into five major groups, nitrogen compounds (primarily alkaloids, terpenoids, phenolics, proteinase inhibitor, and growth regulators. Although the primary functions of these compounds are defense against phytophagous insects, many are also effective against mosquitoes and other

biting Diptera, especially those volatile components released because of herbivory (Pichersky and Gershenzon 2002)

These properties encourage the researchers to investigate the effect of *N. sativa* oil on *R. annulatus*. The present results investigated the ability of *N. sativa* oil on the adult females of *R. annulatus*. Both alcoholic and aqueous or solution of *N. sativa* killed all ticks after 12 h or 24 post-treatment from the concentration 5% and 10%. The same results were obtained from the higher concentrations. Regarding the oviposition of live ticks after treatment, the live ticks after application of oils were found to oviposit the normal eggs and in the normal manner. By following the ability of the produced eggs from treated live ticks, the eggs hatched in the expected period of normal eggs from untreated ticks.

The *N. sativa* oil (NSO) had many activities, antihelminthics, anticestodal effects in children (Akhtar and Riffat 1991), antischistosomal (Mahmoud et al. 2002), *Trichenella spiralis* (Abu El Ezz 2005), *Aspiculuris tetraptera* and *Hymenolepis nana* (Ayaz et al. 2007), These changes were correlated mainly with the ability of NSO to improve liver function and the immunological system of infected mice and to its antioxidant effects (Mahmoud et al. 2002). The protection is also due to the ability of NSO and TQ to reduce the cytogenetic damage induced by schistosomiasis infection (Aboul-Ela 2002). The *N. sativa* aqueous extract could be useful in the treatment of *Blastocystis hominis* (El Wakil 2007). Thymoquinone, the most abundant constituent of black seed essential oil, is the active principle responsible for many of the seed's beneficial effects (Muhtasib et al. 2006).

The seed has diverse chemical composition. They contain amino acids, proteins, carbohydrates, fixed and volatile oils (Khan 1999). Many of the pharmacological activities have been attributed to quinine and thymoquinone (TQ), constituents in the seed.

Mahfouz and El-Dakhkhny (1960) were isolate of 'nigellone' possess antihistaminic properties. El-Fatraty (1975) reported the isolation of thymohydroquinone (THQ) from *N. sativa* seed volatile oil. The medicinal potential of black seed (*N. sativa*) referred to the presence of 135 nigellimine and nigellicine (ur-Rahman et al. 1985), saponins and crude fiber as well as minerals such as calcium, iron, sodium and potassium. Other constituents of the volatile oil include thymol (Aboutabl et al. 1986).

From these constituents of *N. sativa* oil may be explaining the acaricidal effect of the oil on adult ticks. These components may be cause saphocation of the ticks due to immersion in the solutions.

It was noticed that the crude Thyme oil (100%) or 50%, 1% and 3% diluted oil by water or alcohol did not kill any ticks. The solvent (water or alcohol) has no effect on the ability of Thyme oil to cause death of the treated ticks. The tick deaths reached to 90% of treated ticks at concentrations of 10% and 20% in 48hs PA. The live ticks from these concentrations oviposited normal eggs just after application by short time (24 h) but the same quantity. These eggs hatched in the mean time after incubation.

The insecticidal effect was ensured through the direct toxicity on adult insects and by inhibiting reproduction. Therefore, one can profit using the essential oils of *T. vulgaris* and *T. serpyllumin* additionas an effective fumigant against a wide group of agricultural pests that damages its host plant. The oils have a toxic effect on adultinsects and inhibit the reproduction through ovidical and larvicidal effects (Regnaultroger and Hamraoui, 1994). This insecticidal action is also produced by othercomponents of the species such as non-volatile phenols, non-proteinic amino acids, andflavonoids (Regnaultroger and Hamraoui 1995). Thymol was shown to be more potent than Thyme oil as a deterrent factor for reducinegg lying by the mite. Mor-

tality percentage reached 100% with both materials used, however, at low concentrations the effect again was more pronounced applying thymol than applying Thyme oil (El-Gengaihi et al. 1996).

Thymus is a multiple constituent's plant its composition groups can be categorized into the following groups: essential oil, glycosides, flavonoids, flavonoid glycosides and phenolic acids. Most of them are complex mixtures of mono- and sesquiterpenes and biologically related phenolic compounds. Various essential oils have also been documented to exhibit acute toxic effects against internal and external parasites.

Using spinosadin in this study revealed that the low concentrations had no effect on adult *R. annulatus*. The higher concentrations (25 and 50%) led to the adult engorged female tick still alive but did not able to give eggs.

Spinosad is primarily a stomach poison with some contact activity and is particularly active against Lepidoptera and Diptera. It is a neurotoxin with a novel mode of action involving the nicotinic acetylcholine receptor and apparently the GABA receptors as well (Salgado 1998). The mode of action of the spinosyns through neural functions disruption, most likely via an alteration of nicotinic receptor function (Salgado and Saar 2004). Ploch et al. (2007) demonstrated that symptomological assessment of insects treated with spinosad and spinosyn-A has suggested that the most likely mode of action of these insecticides is via a neural mechanism. These studies reported a possibility of a secondary physiological action of spinosyns. The primary interaction was associated with activation of an α -BGT sensitive current, thus suggesting a nicotinic mode of action (Crouse et al. 2007). In addition, in some specific instances, a high-potency GABAergic response was also noted (Watson 2001). The results presented here demonstrate that spinosyn-A does not appear to interact with known nicotinic or GABA target sites.

The weak affinity of spinosyn-A for displacement of the voltage-gated calcium channel radioligand, [3 H] VPM is suggestive of an interaction of spinosyns with this channel. Spinosyn J and the C17 pseudoaglycone of spinosyn-A were both less potent than spinosyn-A in the [3 H] VPM assay, which is consistent with their reduced biological activity against several insect species (Salgado 2005). In general, the high non-specific binding of these ligands makes them suboptimal for studying membrane bound receptors. Recently, however, the utility of one spinosyn ligand, [3 H] 5, 6-dihydrospinosyn-A for cloned insect D_{a6} receptors has been demonstrated (Orr et al. 2006). Davey et al. (2001) reported that spraying of 0.05% and 0.15% spinosad on tick-infested cattle achieved 86% and 87% control, respectively, of larval *R. annulatus* at time of treatment. Besides, Cetin et al. (2009) who demonstrated that spinosad provides a complete control of larval *Argus persicus* and *R. turanicus* in a short exposure time.

Conclusion

Nigella sativa has an acaricidal effect than the other used materials. *Nigella sativa* at 20% was the best of studied preparations being the lowest concentration (20%) that could achieve highest lethal (100%) effect in shortest time (12 h). The Thyme oil is of limited effect. Spinosad did not affect adult ticks at the lower concentrations but at the higher ones, it stopped the egg oviposition.

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